Investigation of an in-feed reduction of tylosin on the prevalence and severity of liver abscesses, antimicrobial resistant enterococci and productivity in feedlot cattle

by

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ABSTRACT

The primary objective of this work was to assess alternative feeding strategies on liver abscesses, growth performance, carcass traits, and immune responses in beef cattle as well as to characterize antimicrobial resistance in enterococci isolated from cattle feces. In the first study, cattle (n = 90) were randomly assigned to 6 treatments (n = 15/treatment): 1) control, 2) low (12 g *Saccharomyces cerevisiae* fermentation product (SCFP) ·steer^{-1}·d^{-1}), 3) medium (15 g SCFP·steer^{-1}·d^{-1}), 4) high SCFP (18 g SCFP·steer^{-1}·d^{-1}), 5) encapsulated SCFP (**eXPC**; 7 g XPC·steer^{-1}·d^{-1}), and 6) antibiotics (**ANT**; 330 mg monensin + 110 mg tylosin·steer^{-1}·d^{-1}). In the second study, cattle (n = 7576; ~ 253 animals/pen, 10 replicate pens per treatment) were randomized to 3 treatments: tylosin phosphate (11 ppm) in-feed 1) for the first 125 days on feed (DOF) (**FIRST-78%),** 2) for DOF 41 to 161 (**LAST-75%)**, or 3) the entire feeding period (**CON**; day 0 to 161). Increasing SCFP tended ($P < 0.09$) to linearly increase feed efficiency. Average daily gain (**ADG**) tended ($P < 0.10$) to be greater in steers supplemented with eXPC than control. The percentage of erythromycin resistant (Ery^{R}) and erythromycin + tetracycline resistant enterococci was greater ($P < 0.05$) with ANT than control, SCFP and eXPC, while tetracycline resistant enterococci was not affected. Isolates were most frequently resistant to tylosin (86%), erythromycin (84%) and doxycycline (31%). Macrolide resistant isolates harbored primarily *erm*(B), *msr*C and tetracycline resistant isolates *tet*(L), *tet*(M), *tet*(O) genes. Cattle administered tylosin for a shorter duration had a greater risk of severe liver abscesses compared to controls; but there was no difference in risk of total liver abscesses, growth performance, carcass traits, morbidity or mortality. The proportion of Ery^{R} enterococci increased in all treatments over the feeding period. These studies support the potential to feed SCFP, eXPC or tylosin for a reduced duration as an alternative to the continuous administration of tylosin. This

study emphasizes the importance of continued research into feeding regimens that promote antimicrobial stewardship, while maintaining the productivity and health of feedlot cattle.

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DEDICATION

To my aunt Elviera Mearon, who introduced me to the University of Manitoba and let me nap on her office floor between classes in my undergrad.

ORGANIZATION OF THESIS

The following thesis is divided into four chapters and includes two manuscripts located in chapters two and three.

The first chapter is the literature review which includes the background information of liver abscesses in feedlot cattle, liver abscess management strategies, antimicrobial resistance and the future of the use of the antimicrobial, tylosin in feedlot cattle.

The second chapter is the manuscript from one experiment which includes the use of *Saccharomyces cerevisiae* fermentation product as an antibiotic alternative in beef cattle.

The third chapter is the manuscript from the second experiment which includes a shorter duration tylosin feeding regimen as an alternative strategy to continuous tylosin administration in feedlot cattle.

Chapter four states the overall conclusions arising from the thesis.

CONTRIBUTIONS OF AUTHORS

Contributions to the manuscript in Chapter two:

Yang W.Z., McAllister T.A., and Yoon I. made the experimental design. Shen Y.Z., Davedow T. and Saleem A.M. carried out all the sample collection and laboratory analysis. Shen Y.Z. carried out statistical analysis. The manuscript was written by Shen Y.Z. and Davedow T., revised by Yang W.Z., McAllister T.A., Yoon. I. and Narvaez-Bravo C., and Davedow T. made all the figures. The work of Davedow T. focused on the antimicrobial resistance in enterococci, and Shen Y.Z. focused on the animal performance, carcass traits and immune response in cattle.

Contributions to the manuscript in Chapter three:

McAllister T.A., Zaheer Z., Gow S., Booker C.W., Hannon S., and Klima C., conceived the project idea and devised a plan. Davedow T. coordinated laboratory level study implementation and conduced laboratory bench work with support from Sanderson H. McAllister T., Zaheer R. and Narvaez-Bravo C., were involved in planning and supervising the work. Bras A., and Klima C. coordinated feedlot-level study implementation and collected and delivered samples to the lab. Rodas-Gonzalez A. performed the statistical analysis on the bacterial data. Bras A. compiled animal health and performance data. Booker C.W. and Hannon S. helped to verify final animal health and performance data and were involved in results interpretation. Davedow T. wrote the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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CHAPTER ONE: Introduction

Liver abscesses in feedlot cattle are caused by high grain diets and can result in lower weight gain, feed efficiency and represent a major economic loss to the Canadian beef industry. Liver condemnation due to abscesses is on the rise and costing the Canadian beef industry nearly 60 million dollars annually. *Fusobacterium necrophorum* and *Trueperella pyogenes* are the top two bacterial species linked to liver abscesses in North America, although there are a number of other bacteria that have been recovered from feedlot cattle liver abscesses. There is still little information known about the agents that initiate infection and progress or persist in infected tissue.

Antimicrobials are frequently included in-feed for beef cattle diets for the therapeutic treatment and prevention of infections that can be caused by bacteria. Tylosin and erythromycin are classified as macrolide antimicrobials; tylosin is commonly used to reduce the incidence of liver abscesses in beef cattle, whereas erythromycin is used to treat infections in humans. However, recent concerns over the use of antimicrobials in livestock production and the development of antimicrobial resistant bacteria that cause infectious disease in humans have lead producers to seek new interventions that can help to reduce the use of antimicrobials, and therefore the risk of antimicrobial resistant bacteria entering the food chain.

1.1 Liver Abscesses in Feedlot Cattle

1.1.1 History and Definition

The terms ‘liver abscess’ or ‘hepatic abscess’ in cattle describes a liver disease in which thick, pus-filled capsules develop in the liver lobes, ranging in size from a pinpoint to over 15 cm in diameter (Nagaraja and Chengappa, 1998). In addition, the term “rumenitis-liver abscess complex” is often used to support the evidence of the relationship between ruminal lesions and

liver abscesses in feedlot cattle. This condition was first described by Smith (1944), and later confirmed by Jensen et al. (1954). Therefore, over the last 70 years, ruminal pathology and its link to liver abscess development has been extensively studied.

Liver abscesses are caused by aggressive grain-feeding programs in which high rates of starch fermentation promote rapid production of organic acids (lactic acid and volatile fatty acids) and considerable fluctuation in ruminal pH (Nagaraja and Lechtenberg, 2007). Dunlop and Hammond (1965) suggested the term 'D-lactic acidosis' to describe this syndrome brought about by excessive ingestion of readily available carbohydrates (Elam, 1976). When the ruminal pH falls below 5.5 the condition is classified as subacute acidosis, whereas acute acidosis is characterized by prolonged bouts of ruminal pH below 5.0 and is of primary concern (Nagaraja and Lechtenberg, 2007). Other common names for acidosis include: overeating, acute impaction, grain engorgement, founder, and overloading.

The association between acidosis and rumenitis was first reported by Ahrens (1967) when experimental manipulation of the rumen by adding lactic acid solution resulted in superficial damage to the rumen epithelium. This epithelial damage may serve as sites of bacterial invasion and the establishment of lesions in the ruminal wall and the development of rumenitis (Ahrens, 1967).

1.1.2 Grading System

A grading system based on size and number of abscesses was created to distinguish from severe (A+), moderate (A), slight (A-), and zero (0) incidence of liver abscesses (Brown et al., 1975). A liver with no abscesses present is designated as "0", whereas an "A-" score is categorized as a liver with only 1 or 2 small abscesses and inactive scars, an "A" liver has 1 or 2

large or several small abscesses, and finally, an "A+" liver has multiple large abscesses (Brown et al., 1975). As mentioned earlier, A+ liver abscesses have the greatest negative impact on animal performance, whereas categories A and A- livers have not been found to have a measureable impact on performance (Nagaraja and Chengappa, 1998).

Other categories include when an active or previously active liver abscess has caused the liver to adhere to the gastrointestinal tract and/or diaphragm (A+AD) or if a liver abscess has ruptured (A+OP) (Brown and Lawrence, 2010). If a liver abscess ruptures, this can result in partial or complete condemnation of the carcass and there is also potential for the production line to be contaminated, resulting in additional labour costs associated with clean up and disinfection (Nagaraja and Lechtenberg, 2007).

1.1.3 Factors Influencing Liver Abscesses

1.1.3.1 Influence from Diet

When cattle consume feed, the organic matter is catabolized into smaller compounds and energy is released in a process called fermentation (Owens and Basalan, 2016). Starch within grains is hydrolyzed to glucose which is fermented to volatile fatty acids. When the rate of fermentation is rapid, volatile fatty acids can accumulate, lowering the ruminal pH and causing acidosis, which can lead to ruminal damage and rumenitis and subsequently the formation of liver abscesses (Owens and Basalan, 2016).

While high-grain diets are a predisposing factor to acidosis, they are efficient for achieving the highest fat deposition during finishing. Typically, grains make up 80-90% of finishing diets with 5-15% of roughage, often in the form of silage, which helps slow the rate of

ruminal fermentation and reduce the incidence of digestive upsets (Owens, 1987; Stock et al., 1987).

The choice of grain type and extent of grain processing are key factors when formulating beef cattle diets because of the effect of starch fermentation. Barley is considered an ideal grain for feedlot cattle in Alberta as it grows well under the drier conditions on the prairies. Corn is widely used in feedlot cattle diets in Eastern Canada and throughout the United States. Compared to corn, barley has more protein, lower starch content, but a higher rate of starch digestion which allows the simultaneous release of energy and nitrogen for greater nutrient uptake (Nikkhah, 2012). In addition, compared to corn and wheat, barley is not used as much for food or feed for humans and non-ruminants, respectively (Nikkhah, 2012). Wheat is considered the worst grain when it comes to the development of acidosis because of the higher starch content and higher rate of starch fermentation compared to barley grain (Offner et al., 2003). The ruminal degradation rate of starch in barley grain is greater than starch in corn (Offner et al., 2003), which makes the latter the least predisposing grain to acidosis.

Higher amounts of roughage in the diet provides a good source of neutral detergent fibre (NDF) which promotes a more stable ruminal fermentation and better buffering through saliva production, and therefore leads to lower incidence and severity of liver abscesses (Gill et al., 1979; Zinn and Plascencia, 1996; Nagaraja and Chengappa, 1998). Utley and colleagues (1973) found that when cattle were fed a finishing diet containing 20% whole, ground or pelleted peanut hulls, incidence of liver abscesses increased from 3.7, to 56, and 59%, respectively. In a study where sunflower seeds fully replaced barley silage as the roughage source, there were no negative effects on the incidence or severity of liver abscesses, feed intake or performance of feedlot cattle (Gibb et al., 2004). This is despite the particle size of sunflower seeds being less

than barley silage. Cattle fed dry hay versus silage as the roughage source were found to have a higher incidence of liver abscesses (Mader et al., 1993).

The method and extent to which grains are processed effects the structure of the grain and therefore the availability of starch. Highly processed grains will cause an increase in ruminal fermentation and digestibility (Theurer et al., 1999; Svihus et al., 2005). The risk of acidosis varies among grain type and extent of processing (Offner et al., 2003). Methods of grain processing that use moisture and heat such as steam-flaking increase the rate of ruminal starch fermentation (Galyean and Rivera, 2003). Distillers' grains with solubles (DGS) are a by-product of the fermentation of cereal grains by yeast during the production of fuel ethanol (Klopfenstein et al., 2008). There has been an increasing interest in optimizing the use of dried DGS (DDGS) in the finishing diets of feedlot cattle across North America (Salim et al., 2014). Replacing a portion of cereal grains with DDGS in feedlot cattle diets may reduce the incidence of acidosis, liver abscesses and other digestive disorders as a result of its low starch and high NDF content (Schingoethe et al., 2009). Salim and colleagues (2014) found that as the concentration of modified-wet DGS (MWDGS) or DDGS in dry whole corn-based diets increased, the liver abscess scores decreased compared to a dry whole corn-based control diet. However, Li et al. (2011) replaced barley grain, and partially or completely replaced silage with wheat DDGS in two experiments. The first had little effect on ruminal pH of eight cannulated heifers (Li et al., 2011). Whereas with 200 feedlot steers, the incidence of liver abscesses rose from 16% (10% silage), to 24% (5% silage) and 50% (no silage) with increasing rates of DDGS replacing silage (Beef Cattle Research Council, 2019). An earlier study, with four cannulated heifers also found a decreasing level of ruminal pH with increasing levels of wheat DDGS, consistent with the conditions of acidosis, and therefore these cattle were at greater risk of developing liver

abscesses (Beliveau and McKinnon, 2009). Further research is required to determine why high levels of DDGS in feedlot cattle diets can increase the risk of acidosis and liver abscess.

1.1.3.2 Influence from Cattle Type

Liver abscesses can develop in all types of cattle. The highest incidence is found in Holsteins (25.0%) raised for beef production rather than beef steers (18.2%) and heifers (19.1%) (Herrick et al., 2018b). Amachawadi and colleagues (2017) found no correlation between the bacterial flora, and the higher incidence of liver abscesses in Holsteins an outcome that is thought to be related to their higher feed intake (12% higher) than beef breeds (Hicks et al., 1990). Within beef breeds, steers tend to have 1 to 3% higher incidence of liver abscess than heifers, possibly because steers require a longer feeding period than heifers to reach finished weight (Nagaraja et al., 1996b).

1.1.4 Economics and Prevalence

Liver abscesses are a major economic concern to the Canadian feedlot cattle industry and is the number one liver disorder in cattle, with an average feedlot prevalence of 12-32% (Brink et al., 1990). In the United States, liver abscesses are the primary cause of liver condemnation at slaughter, accounting for 46% of cases and is responsible for 67% of all liver abnormalities (Brown and Lawrence, 2010)

Generally, liver abscesses have been linked to feeding practices, and although abscesses can occur in cattle of all ages and breeds, including dairy cattle, they have the greatest economic importance in grain-fed cattle. It is common practice in the feedlot industry to finish cattle over a period of 120 days and to feed a grain-based diet to promote fat deposition during this period (Beever and Bach, 2016). This is done in the United States, Canada, Europe, South Africa, and

Japan and to a lesser extent in Australia, Brazil and Argentina by feeding high-energy grains such as corn, wheat, barley and sorghum combined with a small amount of forage, a practice associated with an increase in liver abscesses (Nagaraja et al., 1996b).

Beef quality audits collect information from producers and consumers with an ultimate goal of improving carcass value and providing higher quality beef. The information collected can be used to pinpoint quality defects and develop strategies to reduce the incidence and severity of liver abscesses. Livers are discounted based on their suitability for either human consumption, pet food or if they are condemned (Beef Cattle Research Council, 2018). Of the top ten quality concerns, a United States National Beef Quality Audit ranked liver abscesses as second (1995). The Beef Cattle Research Council performed a Beef Quality Audit (Beef Cattle Research Council, 2018) in Canada and the results showed that incidence of condemned abscessed livers was 13.3%, 23.2%, and 22.0% in 1999, 2011, and 2016, respectively. Economic losses from discounted livers amounted to $20.98/head in 2016, adding up to $61.2 million as compared to $29.9 million in 2011 and $8.8 million in 1999 (Beef Cattle Research Council, 2018). Additional costs are also incurred as a result of higher feed expenses as cattle with severe liver abscesses exhibit reduced feed efficiency and require more time to finish.

The liver accounts for 2% (by weight) of the carcass, and although condemned livers already represent a significant economic loss, the associated decline in animal performance and carcass yield accentuates this loss (Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). Cattle with liver abscesses have reduced feed intake, reduced weight gain, decreased feed efficiency, and decreased carcass dressing percentage (Nagaraja and Chengappa, 1998). The effects of liver abscesses on animal performance have been reported to range from no effect (Smith, 1944; Wieser et al., 1966; Harman et al., 1989) to as high as an 11% reduction in average

daily gain and a 9.7% reduction in feed efficiency (Brink et al., 1990). These responses are usually most evident when the incidence of liver abscesses is rated as severe as outlined in the rating system above.

1.1.5 Etiological Agents

In most incidences, liver abscesses are caused by polymicrobial infections. However, in almost all studies, *Fusobacterium necrophorum* (previously named *Sphaerophorus necrophorus*) is identified as the primary causative agent, with the second most frequently recovered species being *Trueperella pyogenes* (previously named *Arcanobacterium pyogenes*) (Nagaraja and Chengappa, 1998; Amachawadi and Nagaraja, 2016). Various other anaerobic and facultative anaerobic bacteria have been isolated from the liver abscesses of cattle including, *Bacteriodes* spp., *Clostridium* spp., *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Mobilincus* spp., *Pasteurella* spp., *Streptococcus* spp., and additional unidentified gram-negative and gram-positive bacteria (Scanlan and Hathcock, 1983; Nagaraja and Chengappa, 1998).

1.1.5.1 *Fusobacterium necrophorum*

Fusobacterium necrophorum is a gram-negative, rod-shaped, non-spore forming, aerotolerant anaerobe that is a natural inhabitant of the respiratory and gastrointestinal tract of humans and animals, including the rumen of cattle (Langworth, 1977; Nagaraja and Lechtenberg, 2007). *F. necrophorum* is the primary bacterial agent identified in the liver abscesses of cattle with an incidence rate of 81 to 100% in cultured abscesses (Nagaraja and Chengappa, 1998). Most recently, Amachawadi and colleagues (2017) confirmed this by isolating *F. necrophorum* from 100% of 383 cultured abscesses. In addition to liver abscesses, this bacterium is the primary pathogen in necrotic laryngitis (calf diphtheria), foot rot, and foot

abscesses in cattle as well as uterine diseases in dairy cows (Emery et al., 1985; Tan et al., 1996; Bicalho et al., 2012).

The population of *F. necrophorum* increases in the rumen when cattle are transitioned from a high roughage (7×10^5/g) to a high-grain ($3\text{-}7 \times 10^6$/g) diet, as lactate, its major substrate increases with increasing levels of grain in the diet (Tan et al., 1994b; Nagaraja and Lechtenberg, 2007). Therefore, in conditions like acidosis or rumenitis, where there is an accumulation of organic acids in the rumen, there should be an increased population of *F. necrophorum*. In addition to the rumen, *F. necrophorum* has also been isolated from the ruminal wall by Kanoe and associates (1978), but was isolated less frequently from cattle with healthy rumens.

F. necrophorum is classified into two subspecies *necrophorum* and *funduliforme*, previously named biotypes A and B, respectively (Shinjo et al., 1991). These two subspecies differ based on cell morphology, growth patterns, biochemical and biological characteristics (Nagaraja et al., 2005). Of all the possible virulence factors, leukotoxin production is the major factor contributing to the virulence of *F. necrophorum* (Narayanan et al., 2002). Several studies have shown that subsp. *necrophorum* produces more leukotoxin than subsp. *funduliforme,* accounting for its greater virulence and more frequent isolation from liver abscesses (Coyle-Dennis and Lauerman, 1979; Emery et al., 1985; Tan et al., 1992). Of the two subspecies, *necrophorum* is isolated more frequently (71 to 95%) from liver abscesses, often as a pure culture (up to 75%), as compared to the recovery of subsp. *funduliforme* (5 to 29%), which is more often associated with mixed cultures (up to 78% and 22%, respectively (Lechtenberg et al., 1988).

1.1.5.2 *Trueperella pyogenes*

Trueperella pyogenes is a gram-positive, rod-shaped, facultative anaerobe that is also part of the natural flora of the respiratory and gastrointestinal tract of animals (Biberstein, 1990). After *F. necrophorum*, it is the second most frequently recovered bacterium from liver abscesses, with a recovery ranging from 2 to 20% (Nagaraja et al., 1996a; Tan et al., 1996). Since *T. pyogenes* is a facultative anaerobe, it is more commonly isolated from the ruminal wall where low concentrations of oxygen diffuse in from surrounding capillaries, rather than from the strictly anaerobic contents of the rumen (Narayanan et al., 1998). Of all the various virulence factors, a hemolysin, called pyolysin is the major virulence factor contributing to the pathogenicity of *T. pyogenes* (Billington et al., 1997). *T. pyogenes* is frequently recovered from liver abscesses along with *F. necrophorum,* suggesting a nutritional or pathogenic synergy between these two species (Tadepalli et al., 2009). Lechtenberg and colleagues (1993) observed that *T. pyogenes* only formed liver abscesses when it was injected intraperitoneally with *F. necrophorum* or its leukotoxin. This suggests that *T. pyogenes* assists, but is not the primary causative agent of liver abscesses (Nagaraja and Lechtenberg, 2007).

1.1.5.3 Other Bacterial Species

Aside from *Fusobacterium necrophorum* and *Trueperella pyogenes* there are a number of other bacterial agents that have been isolated from the liver abscesses of cattle. These additional bacterial species have been identified by standard aerobic and anaerobic culture methods and through whole genome sequencing of isolated bacteria (Nagaraja and Chengappa, 1998; Amachawadi and Nagaraja, 2016). The additional anaerobic and facultative bacteria isolated include *Bacteriodes* spp., *Clostridium* spp., *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Mobilincus* spp., *Pasteurella* spp., *Streptococcus* spp., and various other unidentified gram-

negative and gram-positive bacteria (Scanlan and Hathcock, 1983; Nagaraja and Chengappa, 1998).

Since previous attempts were not able to characterize all microorganisms found in liver abscesses and their reduction remain a concern to the industry, a completed bacteriological understanding may provide insight into strategies to mitigate liver abscesses. The full polymicrobial community of liver abscesses has been reported recently (Weinroth et al., 2017) using metagenomic sequencing to identify all the specific genera present and their ratios from the purulent material (pus inside the capsule) of abscesses. A sample of livers with abscesses from both dairy and beef cattle in the United States were collected and the purulent material was aseptically removed from one abscess per liver (Weinroth et al., 2017). DNA was successfully extracted from 34 liver abscess samples and 16S rRNA sequencing of the V4 region was used to characterize the DNA into 5 phyla, 13 classes, and 17 bacterial orders (Weinroth et al., 2017). A total of 13 bacteria were found in all liver abscess samples (Table 1.1). Of the bacteria identified, 82% were gram negative, whereas current antimicrobials used in liver abscess therapy target gram positive bacteria. This could explain the limited efficacy of current antimicrobials that are used to try and control liver abscesses. A further understanding of the microbial communities in liver abscesses as well as the factors that influence their bacterial makeup is necessary in order to design an efficacious strategy to control and prevent liver abscess development.

Table 1. 1. Genera from purulent material of liver abscesses in cattle characterized using 16S rRNA sequencing

Present in all abscesses[1]	Percentage	Additional bacteria	Percentage
Bacteroides	18%	F: Rhodobacteraceae	4%
Fusobacterium	15%	O: Lactobacillales	2%
Porphyromonas	14%	Peptostreptococcus	2%
Pseudomonas	6%	Filifactor	2%
F: Enterobacteriaceae	4%	Neisseria	2%
Trueperella	3%	Serratia	2%
Sneathia	3%	Halomonas	2%
Parvimonas	3%	F: Aeromonadaceae	2%
Helcococcus	3%	F: Moraxellaceae	2%
Psychrobacter	3%	Balneola	1%
Atopobium	2%	F: Saprospiraceae	1%
Campylobacter	2%	Sphingobacteriales	1%
Haemophilus	2%		

Adapted from (Weinroth et al., 2017).
[1] Letter preceding name indicates higher classification; F= Family, O= Order.

1.1.6 Pathogenesis

Diets with grains that result in high rates of starch fermentation such as wheat, barley, high-moisture corn and steam-flaked corn cause an accumulation of organic acids which promote greater fluctuations in ruminal pH leading to acidosis, rumenitis and finally, liver abscesses (Fulton et al., 1979; Stock et al., 1987; Stock et al., 1990; Nagaraja and Lechtenberg, 2007), an outcome that increases with days on feed (Figure 1.1) . If the protective surface of the ruminal wall is compromised by the acidity of ruminal contents, or aggravated by foreign objects (e.g., nails, sharp metal particles, glass, etc), opportunistic bacteria such as *F. necrophorum* and *T.*

pyogenes may invade and form lesions in the rumen epithelium (Jensen et al., 1954; Nagaraja and Lechtenberg, 2007). From there, bacteria can enter the blood through the portal circulation where they can enter the liver and possibly form abscesses (Narayanan et al., 1997; Nagaraja et al., 1999).

Lesions begin with the formation of a micro-abscess which then progresses to a coagulative necrosis of the surrounding hepatocytes (Nakajima et al., 1986). The abscess itself consists of a thick fibrotic wall that encapsulates a necrotic, pus-filled centre with a surrounding zone of inflammation (Lechtenberg et al., 1988; Nagaraja et al., 2005). The entire process from initial lesion to true abscess takes between 3-10 days based on studies where abscesses have been experimentally induced (Nakajima et al., 1986).

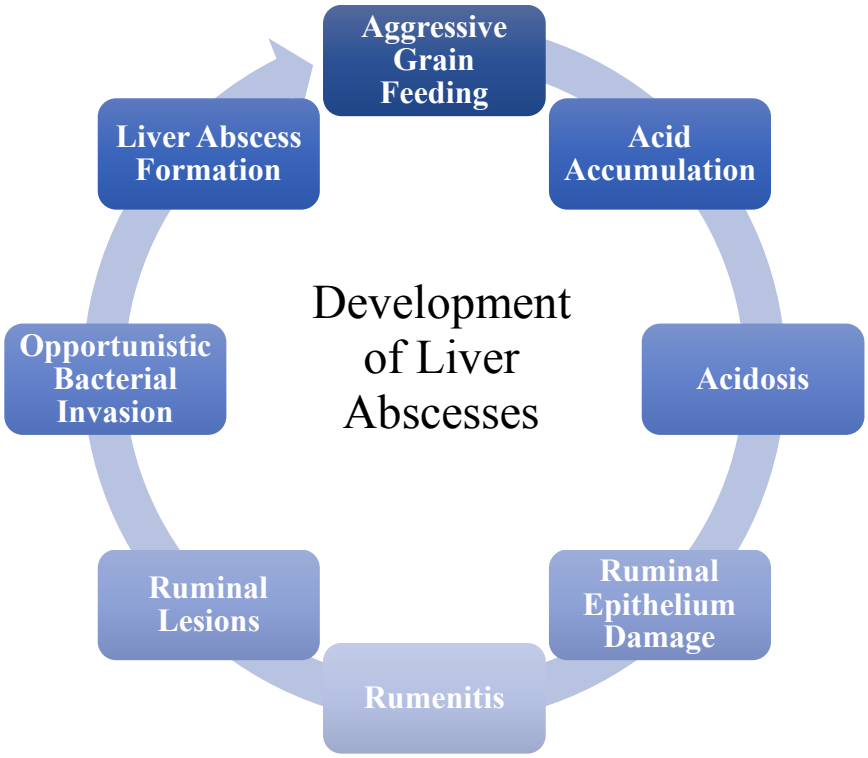

Figure 1. 1. Pathogenesis of liver abscesses in beef cattle fed a high grain diet (Nagaraja and Lechtenberg, 2007).

Smith (1944) first discovered the relationship between ruminal lesions and liver abscesses when he found that 42% of cattle simultaneously had ruminal lesions and liver abscesses, whereas only 9% of cattle with liver abscesses lacked rumen lesions. Although Wieser et al. (1966) found no correlation between the incidence of liver abscess and ruminal lesions, other studies found evidence that supported the observations of Smith (Jensen et al., 1954; Tamate et al., 1973). Consequently, the rumenitis-liver abscess complex has become an accepted term to describe the relationship between ruminal lesions and the development of liver abscesses in feedlot cattle (Smith, 1944). A more recent study observed that 32% of cattle with mild or severe rumenitis had liver abscesses as compared to 19% in cattle with healthy ruminal walls (Rezac et al., 2014). The positive relationship between liver abscesses and ruminal lesions initiated the hypothesis that *F. necrophorum* and *T. pyogenes* isolated from liver abscesses originated from the rumen (Narayanan et al., 1997; Narayanan et al., 1998). Indeed, gel electrophoresis has shown a genetic linkage between both *F. necrophorum* and *T. pyogenes* isolates obtained from ruminal contents, the ruminal wall and liver of an individual host, providing more direct evidence that these bacteria originate from the rumen (Narayanan et al., 1997; Narayanan et al., 1998).

The virulence factors of *F. necrophorum* and *T. pyogenes* play a critical role in the invasion and colonization of the ruminal epithelium, and the establishment of infection. Since the liver is highly oxygenated and protected by phagocytic cells, *F. necrophorum* must proliferate within an oxygen limited environment and circumvent immune responses in order to proliferate and initiate infection (Nagaraja and Lechtenberg, 2007). In addition, the combination of leukotoxin and hemolysin virulence factors produced by *F. necrophorum* and *T. pyogenes*

respectively, appears to be an important factor in inducing the formation of liver abscesses (Nagaraja et al., 1996a).

The size, number, location and pattern of liver abscesses can vary. The typical number of abscesses on a liver is between 2 and 10, although there can be as few as one and over a hundred. The diameter of the abscesses can range from a pinpoint to over 15 cm (Nagaraja and Lechtenberg, 2007). There appears to be no consistent pattern in the distribution of abscesses across lobes of the liver, although smaller abscesses tend to be distributed throughout the liver whereas larger abscess form closer to the point of portal entry (Nagaraja and Lechtenberg, 2007).

1.1.7 Symptoms and Diagnosis

Liver abscesses are undetectable until the time of slaughter as they induce no clinical symptoms in the host. Occasionally, cattle can experience abdominal pain, or if an abscess ruptures, severe peritonitis or systemic infection can occur, possibly resulting in death (Nagaraja and Chengappa, 1998). Liver function tests do not seem to be a good indicator of liver abscesses (Holtenius and Jacobsson, 1966; El Sabban, 1971). Only through experimental inoculation with *F. necrophorum*, have liver abnormalities been shown to be associated with elevated serum protein, bilirubin and certain enzymes concentrations such as γ- glutamyl transferase and sorbitol dehydrogenase (Itabisashi et al., 1987; Lechtenberg and Nagaraja, 1991). Ultrasound has also been used to document the formation of liver abscesses (Lechtenberg and Nagaraja, 1991), however, this method is costly for use in feedlot cattle that naturally develop abscesses (Nagaraja and Lechtenberg, 2007). In addition, visualization of abscesses can be difficult depending on the liver position and ruminal fill, furthermore, observation of the liver can be obscured by the right lung or rumen, or by abdominal fat stores (Nagaraja and Lechtenberg, 2007).

A recent study at West Texas A&M University attempted to develop a mathematical model to predict the severity of liver abscesses based on trends in blood parameters (Herrick et al., 2018a). Results showed that cattle with severe liver abscesses undergo metabolomic changes which may be observed by increased platelets and decreased hemoglobin in whole blood analysis and increased total proteins and decreased aspartate aminotransferase in serum compared to carcasses without abscesses (Herrick et al., 2018a). Blood parameters could represent a promising tool for rapid detection of liver abscess and provide better management strategies for feedlot producers.

1.2 Management Strategies

The prevention and control of liver abscesses has largely been dependent on antimicrobial therapy in combination with sound nutritional management. However, in recent years, due to both public concern and increased regulatory oversite, additional strategies have been explored in order to reduce industry reliance on antimicrobials. The high prevalence of liver abscesses in beef cattle, and the fact that there are no economical detection methods for the disease until slaughter, it is important that the beef industry identify prudent management strategies. There is no silver bullet for liver abscesses and it may require a combination of sound nutritional management, vaccination, antimicrobial therapy and/or antimicrobial alternatives.

1.2.1 Sound Nutritional Management

The development of liver abscesses is largely affected not only by diet, but also by feeding practices. It is the careful combination of the two that create a solid foundation to minimize the occurrence of liver abscesses (Nagaraja and Chengappa, 1998).

It is a common feeding practice in North America to finish cattle on a high-energy grain-based diet in order to achieve high levels of fat deposition. However, it is also known that a high grain diets promote acidosis, rumenitis and, eventually, liver abscesses. Diets that are high in starch include cereal grains (wheat, barley, oats, rye) and corn. When these high-concentrate diets are implemented too quickly, high rates of starch fermentation create excess organic acids which will cause the previously mentioned ruminal problems. Therefore, it is recommended to start with a concentrate: roughage ratio of 1:3 and gradually shift the ratio over 4 weeks (Blood and Henderson, 1963). Frequent and consistent feeding in order to moderate fermentation and avoid ruminal acid build up is recommended and can also help prevent over-, or undereating (Elam, 1976). Adequate bunk space is important so cattle have clean, fresh and available room for feed and water consumption in order to reduce competition between individuals and maintain a consistent feeding pattern. Abnormal feeding due to competitiveness has been hypothesized to increase ruminal acidosis and consequently liver abscesses (Nagaraja and Chengappa, 1998). Just a few quick and easy changes in cattle feeding practices can help minimize inconsistencies in ruminal fermentation and reduce the occurrence of liver abscesses.

1.2.2 Vaccination

Although current antimicrobial strategies are the most common method for reducing the incidence of liver abscesses, they do not eliminate the problem. Vaccination could not only control liver abscesses but also lower the public health concern of inclusion of medically important antimicrobials in feed. Antimicrobials and growth promoting hormones are prohibited in natural beef programs from birth to harvest, whereas vaccines are allowed (Canadian Food Inspection Agency, 2019b). Most beef cattle are routinely vaccinated for a variety of diseases, most commonly against bovine respiratory and clostridial diseases (Checkley et al., 2005).

There have been quite a few attempts to develop a vaccine to prevent liver abscesses. The approach has been to use information on the pathogenicity and virulence of *F. necrophorum* to establish an effective vaccine. Past attempts involve the two most prevalent bacteria in liver abscesses, *Fusobacterium necrophorum* and *Trueperella pyogenes* and their associated virulence factors leukotoxin and pylosin, respectively.

There have previously been two commercially available vaccines targeting liver abscesses, one with a *F. necrophorum* bacterin (Fusogard®) and the other with *F. necrophorum* toxoid and *T. pyogenes* bacterin (Centurion™) which is no longer commercially available (Amachawadi and Nagaraja, 2016). A 2006 natural feedlot study compared the efficacy of two vaccines (i.e., Fusogard or Centurion) against an unvaccinated control group (Fox et al., 2009). There was no difference in the incidence (56%) or severity (39% with A/A+) of liver abscess between vaccinated and control cattle. Others have suggested that vaccines significantly reduce the number of severely abscessed livers when fed a forage based diet, but did not evoke the same response in cattle fed high grain diets (Checkley et al., 2005; Fox et al., 2009). Prior studies also evaluated the efficacy of Fusogard and Centurion (Jones et al., 2004; Checkley et al., 2005), and although protection was limited, overall incidence of liver abscess was lower (<30%) than in the study of Fox et al. (2009), likely due to the greater number of days to reach finish (238 d).

Since previous vaccines have proven to have limited efficacy, a new vaccine is being developed which will hopefully provide livestock producers with a useful alternative. Researchers from the Kansas State University (KSU) have recently created a patent which incorporates another antigen of *F. necrophorum* along with the leukotoxoid vaccine which disables the adhesive properties of the bacteria (Roney, 2016). Adhesion is a critical step in order for bacteria to attached and establish infection. The discovery and characterization of this

adhesive protein have allowed the KSU research team to induce an immune response in cattle against *F. necrophorum* bacteria, which prevents its attachment to the rumen epithelium, possibly preventing it from entering the portal blood stream and causing infection in the rumen (Roney, 2016). The research for this vaccine is still ongoing and is currently being tested in cattle.

1.2.3 Antimicrobial Use

Antimicrobials are frequently used in livestock production for the treatment and prevention of infectious diseases caused by bacteria. There are three commonly described routes of antimicrobial administration: prophylaxis, metaphylaxis and growth promotion. Prophylaxis is the preventative treatment of individual animals that are at risk to developing disease whereas, metaphylaxis provides treatment to an entire herd and includes both treatment of sick, or prophylaxis to unaffected or susceptible animals. Growth promotion typically involves administration of the antimicrobial at a low or sub-therapeutic dose either in feed or water for the majority of the feeding period. This approach attempts to improve weight gain and feed efficiency and can include the benefits of prophylaxis. Prophylaxis and metaphylaxis are referred to as therapeutic levels of treatment and are less controversial than the use of antimicrobials for growth promotion.

The administration of in-feed antimicrobials is the most effective and common method of reducing the incidence of liver abscesses in feedlot cattle. According to the United States Feed Additive Compendium, there are six antimicrobials approved to reduce the incidence of liver abscesses in feedlot cattle including bacitracin methylene disalicylate, chlortetracycline, neomycin sulfate in combination with oxytetracycline, oxytetracycline, tylosin, and virginiamycin (Lundeen, 2013). In Canada, only tylosin and virginiamycin are approved for the

claim of reducing liver abscesses in cattle (Canadian Food Inspection Agency, 2019a). These antimicrobials differ in their ability to inhibit *F. necrophorum* and *T. pyogenes* as well as in their effectiveness in preventing liver abscesses (Lundeen, 2013). Bacitracin is the least effective, although Embry et al. (1964) found bacitracin an effective control, Haskins et al. (1967) found it to not reduce the incidence of liver abscesses, as 72% of livers in their study were condemned. Whereas, tylosin, is the most effective and widely used antimicrobial in North America for reducing liver abscesses (Beukers et al., 2015). Tylosin is primarily effective against gram positive bacteria, but gram negative *F. necrophorum* is also sensitive to this antimicrobial (Tan et al., 1994b; Lechtenberg et al., 1998).

There has been significant evidence demonstrating that the administration of tylosin in-feed reduces the incidence of liver abscesses in cattle between 40-70% (Brown et al., 1975; Heinemann et al., 1978; Pendlum et al., 1978; Brink et al., 1990; Tan et al., 1994a). Although tylosin significantly reduces the incidence of liver abscesses, it does not completely prevent their occurrence (Nagaraja et al., 1999). Some possible reasons for this include: the inclusion of tylosin in the feed may reduce *F. necrophorum* population in the rumen, but induce the growth of other opportunistic pathogens that cause liver abscesses. Feeding tylosin can also select for antimicrobial resistant microflora that can still cause liver abscesses (Nagaraja et al., 1999).

Tylosin is typically administered at sub-therapeutic levels (low dosage; 11 ppm or 11 mg/kg/head/day) in-feed or water throughout the entire feeding period. The argument whether this repeated subtherapeutic exposure to macrolides is contributing more to the proliferation of AMR bacteria than a single therapeutic dose has been explored. Zaheer et al. (2013) compared a single therapeutic subcutaneous injection of the macrolides, tilmicosin and tulathromycin to the sub-therapeutic, continuous administration of tylosin in-feed for 28 days. There was no

significant difference between injectable or in-feed macrolides in the incidence of erythromycin resistant enterococci (Zaheer et al., 2013). Since there is increasing concern regarding the emergence of antimicrobial resistant bacteria, researchers and livestock producers have examined how the duration and the frequency of tylosin administration in-feed effects AMR and the efficacy of liver abscess control. Such attempts include withdrawing tylosin 4 weeks prior to slaughter or intermittently administering the antimicrobial in-feed (Beukers et al., 2015; Müller et al., 2018). Reducing the duration that tylosin is administered to cattle during the feeding period could be an effective approach to reducing antimicrobial use if it does not adversely affect the incidence of liver abscesses or the growth performance and health of feedlot cattle.

1.2.4 Antimicrobial Alternatives

Although tylosin is still used widely in North America, the exploration of alternatives has been of increasing interest. The most popular strategies have included essential oils, probiotics, and direct fed microbials in combination with or compared to tylosin. Although there are many different potential alternatives (McAllister et al., 2011), few have been examined for their ability to reduce the incidence of liver abscesses.

Desirable properties of secondary plant metabolites, such as essential oils, would include their ability to improve animal performance, feed efficiency, ruminal fermentation and overall host health (Cobellis et al., 2016). Like most antimicrobial alternatives, data that supports the aforementioned responses is limited. However, Meyer et al. (2009) evaluated the effects of commercial essential oil mixtures in high concentrate diets of four hundred beef cattle over a 115-day finishing period. Compared to the control, a mixture of essential oils (thymol, eugenol, vanillin, guaiacol, limonene) or an experimental blend containing guaiacol, linalool and α-pinene reduced the incidence of liver abscesses by 39% and 2%, respectively, whereas the

reduction with the mixture of essential oils and tylosin was 68% and 76% with monensin + tylosin, respectively (Meyer et al., 2009). Samii et al., (2016) demonstrated that the essential oil limonene linearly decreased ($P = 0.03$) ruminal concentrations of *F. necrophorum* in seven ruminally canulated heifers, whereas tylosin ($P = 0.019$) had no effect. However, this magnitude of reduction may not be great enough to lower the incidence of liver abscesses. (Samii et al., 2016). Therefore, further research is needed to evaluate the effectiveness of limonene on liver abscesses in feedlot cattle.

Probiotics or direct-fed microbials (DFM) are widely accepted as live microorganisms, naturally occurring bacterial supplements that are used to improve the overall intestinal microbial balance of livestock (Hernández et al., 2014). Some probiotics also contain prebiotics, enzymes, and/or crude extracts in combination with live microbes (Yoon and Stern, 1995). Several different bacterial or yeast species have been explored for their probiotic activity including *Lactobacillus, Enterococcus, Bacillus, Bifidobacterium* and *Saccharomyces.* Many studies have researched the effects of probiotics in calf and cattle diets and have found either lower incidence of diarrhea, decreased fecal load of *Escherichia coli* O157, higher daily gain and/or total feed intake (Agarwal et al., 2002; Brashears et al., 2003; Younts-Dahl et al., 2004; Frizzo et al., 2010). In addition, several studies have found the effects of probiotics to be inconsistent on animal performance (Krehbiel et al., 2003; Peterson et al., 2007). Ran et al. (2018a) found that inclusion of active dried yeast (ADY; *Saccharomyces cerevisiae*) in-feed resulted in fewer severely abscessed livers ($P < 0.05$) compared to tylosin fed cattle, whereas dry matter intake, final body weight, gain-to-feed ratio and average daily gain were unaffected.

Essential oils, probiotics and direct-fed microbials, are just a few of the many natural, antimicrobial alternatives that are continuously explored in livestock diets. A common goal

between all antimicrobial alternatives are to reduce the current reliance on antimicrobials while promoting health and optimizing productivity. However, their unpredictability and inconsistency makes the currently proposed antimicrobial alternatives more of an additional preventative measure rather than a proven control.

1.3 Antimicrobial Resistance

In North America, animal agriculture has become dependent on antimicrobials for animal health, welfare, and productivity. The administration of antimicrobials in livestock feed has been a growing concern for consumers and policy makers because of the risk of this practice promoting the emergence of antimicrobial resistant bacteria (Aarestrup, 1999). Although some resistance occurs naturally, the use of antimicrobials can select for antimicrobial resistant bacteria. The concern with antimicrobial resistant bacteria residing in food animals is two-fold. First, antimicrobial resistant pathogens could enter into the food chain causing foodborne illness in humans and there is a risk that antimicrobial resistant genes in livestock microflora could transfer to human pathogens (Barton, 2000). Second, antimicrobial therapy becomes less efficacious in animals colonized with antimicrobial resistant bacteria (Barton, 2000). The ecological interplay (Figure 1.2) between humans, animals and the environment plays a major role in the transfer of antimicrobial resistance in bacteria and resistance genes (Woolhouse and Ward, 2013). It is estimated that by 2050, the global number of deaths attributed to antimicrobial resistant bacteria will reach 10 million (O'Neill, 2016). Without a united, worldwide change in behavior, a post-antimicrobial era could develop in which minor infections that are easily treated with antimicrobials will again emerge as a major health threat.

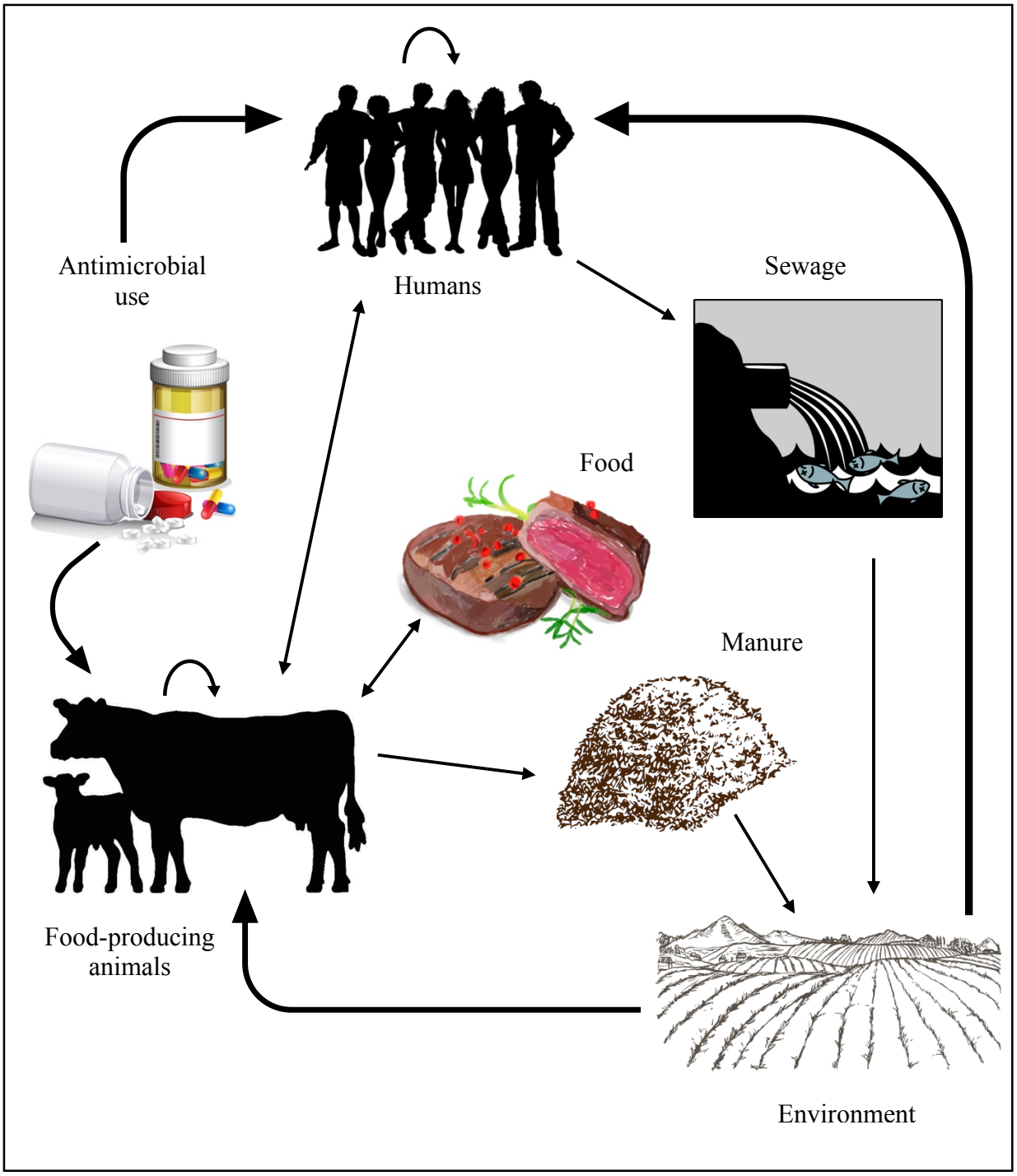

Figure 1. 2. Representation of the routes of antimicrobial resistance transmission between humans, food producing animals and the environment.
Adapted from (Woolhouse and Ward, 2013).

1.3.1 History Between Antimicrobial Use and Resistance

Ever since the dawn of the antimicrobial era, the complex relationship between antimicrobial use (AMU) and development of resistance has been closely followed. Penicillin was the first antimicrobial discovered in 1928 and was not available for clinical use until 1940 (Mahoney et al., 1943; Fleming, 2001). Since then, many new classes of antimicrobials have been identified and have undoubtedly saved numerous lives. However, because of the overuse and abuse of such antimicrobials, many bacteria have become resistant to these drugs. As early as 1942, penicillin resistance in *Staphylococcus aureus* was recognized (Rammelkamp and Maxon, 1942). To solve this dilemma, a derivative form of penicillinase-stable methicillin was developed in 1960, and within a year the first reported case of methicillin-resistant *S. aureus* (MRSA) emerged (Jevons et al., 1961). Since then, the MRSA "superbug" has become the most prevalent hospital acquired infection with resistance to several common antimicrobial families including aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, and tetracyclines (Clinical and Laboratory Standards Institute, 2016).

In addition to MRSA, there are other antimicrobial resistant bacteria of particular concern not only in Canada, but across the globe. Multidrug resistant (MDR) *Clostridum difficile*, carbapenemase-producing Enterobacteriaceae (CPE) which is often MDR, vancomycin-resistant enterococci (VRE), and antimicrobial resistant *Neisseria gonorrhoeae*, just to name a few (Mithani and Tam, 2018).

1.3.2 AMU in Livestock and AMR in Humans and Public Health

There is no doubt that the leading cause of antimicrobial resistant bacteria of relevance to human health is the overuse of antimicrobials in human medicine. Current agriculture practices rely heavily on antimicrobials for the health, welfare and productivity of animals. However, there

is a general acceptance that the administration of antimicrobials in food-producing animals is a major contributor to the development of antimicrobial resistant bacteria. There is potential for resistance genes to transfer from one bacteria to another which could present a major public health concern. In addition, antimicrobial resistant pathogens and their associated genes can transfer to humans through contaminated food, water, soil, crops and meat (Xiong et al., 2015a; Xiong et al., 2015b). Since the transmission pathway is complex, linking the on-farm use of antimicrobials to resistance in humans is difficult.

Direct contact of AMR bacteria from animals is more of a concern for farm workers or veterinarians who closely handle animals. However, there is the additional risk of indirectly transmitting resistant bacteria through person-to-person contact. The first recorded case of this occurred when a worker on a poultry farm transferred a chloramphenicol-resistant *Escherichia coli* to a family member, with this same bacterium being isolated within the poultry barn (Levy et al., 1976). A child in the United States was also infected with a ceftriaxone-resistant strain of *Salmonella enterica* serotype typhimurium that were identical to isolates obtained from nearby cattle herds (Fey et al., 2000). In both cases, the route of transmission was unknown, however there was clear evidence that transmission occurred from animals to humans.

Indirect transmission of AMR bacteria from animals through the food chain is more concerning. Alexander et al. (2009) followed cattle from the farm-to-fork and found approximately 81% of the 165 AMR *Escherichia coli* isolates from the carcass or ground beef were genetically linked to at least one hide or digesta isolate collected during processing. Another 12 year study in the United States tested over 22,000 retail meats, including nearly 6,000 ground beef samples and isolated enterococci from 92%, most notably *E. faecium* and *E. faecalis* (Tyson et al., 2018). The resistance profiles, and the exact source of bacterial

contamination from this study were unknown, but it clearly demonstrated that animal protein could be a source of antimicrobial resistance genes that could be potentially be present in human pathogens within the food chain. Adequate cooking of food can efficiently kill AMR bacteria, but proper cooking times and temperature guidelines need to be followed. There is also a question to the degree that ingested AMR bacteria from foods can survive and colonize humans. A study in Denmark followed individuals before and after ingestion of either glycopeptide- or streptogramin-resistant enterococci isolated from store bought chicken or pork, respectively (Sørensen et al., 2001). In both cases, researchers were able to detect the excreted resistant enterococci strains up to 14 days after ingestion (Sørensen et al., 2001). Although the use of antimicrobials in farm animals is linked to the emergence of antimicrobial resistant bacteria (Schechner et al., 2013), not all antimicrobial resistance in human pathogens originates from food producing animals (Xiong et al., 2018).

1.3.3 Antimicrobial Resistant Enterococci

The genera *Enterococcus* (formerly *Streptococcus*) are a group of gram positive cocci that are ubiquitous in nature and occupy a wide range of habitats including soil, water, sewage, plants, animals and humans (Giraffa, 2002). Enterococci can be a commensal of the gastrointestinal tract of humans and animals, but can also be serious nosocomial pathogens. MDR bacterial infections are a major concern for hospitals because ineffective antimicrobial therapy can lead to prolonged hospital stays, increased cost of treatment and risk of morbidity and mortality. Although not a direct threat to healthy people, some strains are opportunistic and in immunocompromised individuals can lead to bacteraemia, endocarditis, urinary tract and other infections (Morrison et al., 1997). *Enterococcus faecalis* and *Enterococcus faecium* are more commonly associated with infections in humans (Poh et al., 2006; Franz et al., 2011).

Enterococcus hirae is the predominant species found in cattle and is not normally associated with infections in humans (Anderson et al., 2008).

Antimicrobial resistance in livestock is of particular interest in the enteric populations of bacteria including *Escherichia coli, Salmonella* spp., thermophilic campylobacters and enterococci (Barton, 2000). Macrolides are among the most common antimicrobials used in feedlots and are classified as a category 2 antimicrobial by Health Canada (2009) which ranks this family of antimicrobials as highly important for use in human medicine. Whereas, the World Health Organization (2016) ranks macrolides as highest priority critically important antimicrobial. The macrolide, tylosin, is only used in food producing animals, although there is evidence that the use of this macrolide causes resistance to other members of the macrolide family (Portillo et al., 2000). If these resistant enterococci enter the food chain it is possible that they could colonize the intestinal tract and cause AMR infections in humans (Giraffa, 2002; Jensen et al., 2002). If tylosin administered in-feed creates tylosin resistant enterococci in cattle, it could in turn cross-select for enterococci resistant to erythromycin, a macrolide that is important for treating bacterial infections in humans (Roberts, 2008; Desmolaize et al., 2011).

A recent study investigated the withdrawal of in-feed tylosin 28 days prior to slaughter and found a significant reduction of macrolide resistant enterococci (Beukers et al., 2015). However, the effect of the tylosin withdrawal on the incidence and severity of liver abscesses was not determined. Therefore, the investigation into reducing antimicrobial use in livestock, while still promoting economically viable production practices in agriculture, is of growing interest.

1.3.4 Mechanisms of Macrolide Resistance in Enterococcus

Antimicrobial resistance among enterococci has spread rapidly and has become a major public health concern. Enterococci are often referred to as "resistant gene traffickers" because of their genetic promiscuity (Werner et al., 2013). The resistance mechanisms of enterococci for macrolides can be intrinsic or inducible. Inducible or acquired resistance means the resistance genes are not always expressed, but become expressed in the presence of an antimicrobial, whereas intrinsic genes are naturally expressed, regardless of the presence or absence of antimicrobials (Nakajima, 1999).

There are four generations of macrolide antimicrobials made up of naturally occurring or semi-synthetic derivatives, including erythromycin and tylosin (Bryskier et al., 1995). Cross resistance can occur because macrolides are part of the MLS_B superfamily, in which resistance to one member of the group is known to create resistance to all family members (macrolide-lincosamides-streptogramin B), regardless of their structural differences (Portillo et al., 2000). Acquisition of resistance to MLS_B antimicrobials can occur through modification of the drug target site, active efflux pumps, or inactivation of the drug itself.

The first and most common mechanism, in which the presence of an *erm* (erythromycin resistance methylase) gene causes modification of the 23S rRNA of the 50S subunit, which disables binding of MLS_B antimicrobials (Portillo et al., 2000). Most notably, the *erm*(B) gene has been frequently described in enterococci isolates, irrespective of species (Jensen et al., 1999; Beukers et al., 2015). In tylosin fed cattle, fecal enterococci isolates displaying intermediate resistance or resistance to erythromycin were screened for *erm*(B), with 85 percent of the isolates containing this gene (Beukers et al., 2015). In the same study, the *msr*C gene was also detected, but to a lesser extent in only 19 *E. faecium* isolates (Beukers et al., 2015). The *msr*C gene is also

commonly responsible for macrolide resistance in enterococci. This second mechanism, *msr*C, involves a macrolide efflux pump that essentially ejects toxic or foreign substances such as antimicrobials from the inside to outside of the cell (Webber and Piddock, 2003). Thirdly, MLS_B drug modification is a possibility although a relatively minor and uncommon mechanism of resistance. In this case, an enzyme modifies or inactivates one of the MLS_B antimicrobials, therefore preventing the drug from binding to its target (Catry et al., 2003). Additional, and less prevalent macrolide resistance genes in enterococci also operate as part of the *erm* methylase gene family: *erm*(A), *erm*(C), *erm*(F), and *erm*(T) (Chen et al., 2007).

1.3.5 Action Plan

According to the World Health Organization (WHO), without a behavioural change, antimicrobial resistance will remain a major threat to human health (2018). To prevent and control the spread of resistance, a multifaceted approach is required not only from the agriculture sector, but also from individuals, policy makers, health professionals, and the healthcare industry. The WHO has provided a number of suggestions on how different stakeholders can take part in this movement and a few recommendations are summarized below [Figure 1.3; (WHO, 2018)]. The goal on-farm is to practice prudent use of antimicrobials. This includes reducing or eliminating the use of antimicrobials, not using antimicrobials for growth promotion purposes, and finding "natural" alternatives to antimicrobials or developing new vaccines. In addition, improvements of biosafety and biosecurity practices on-farm are necessary through good hygiene and animal welfare so as to prevent the spread of disease.

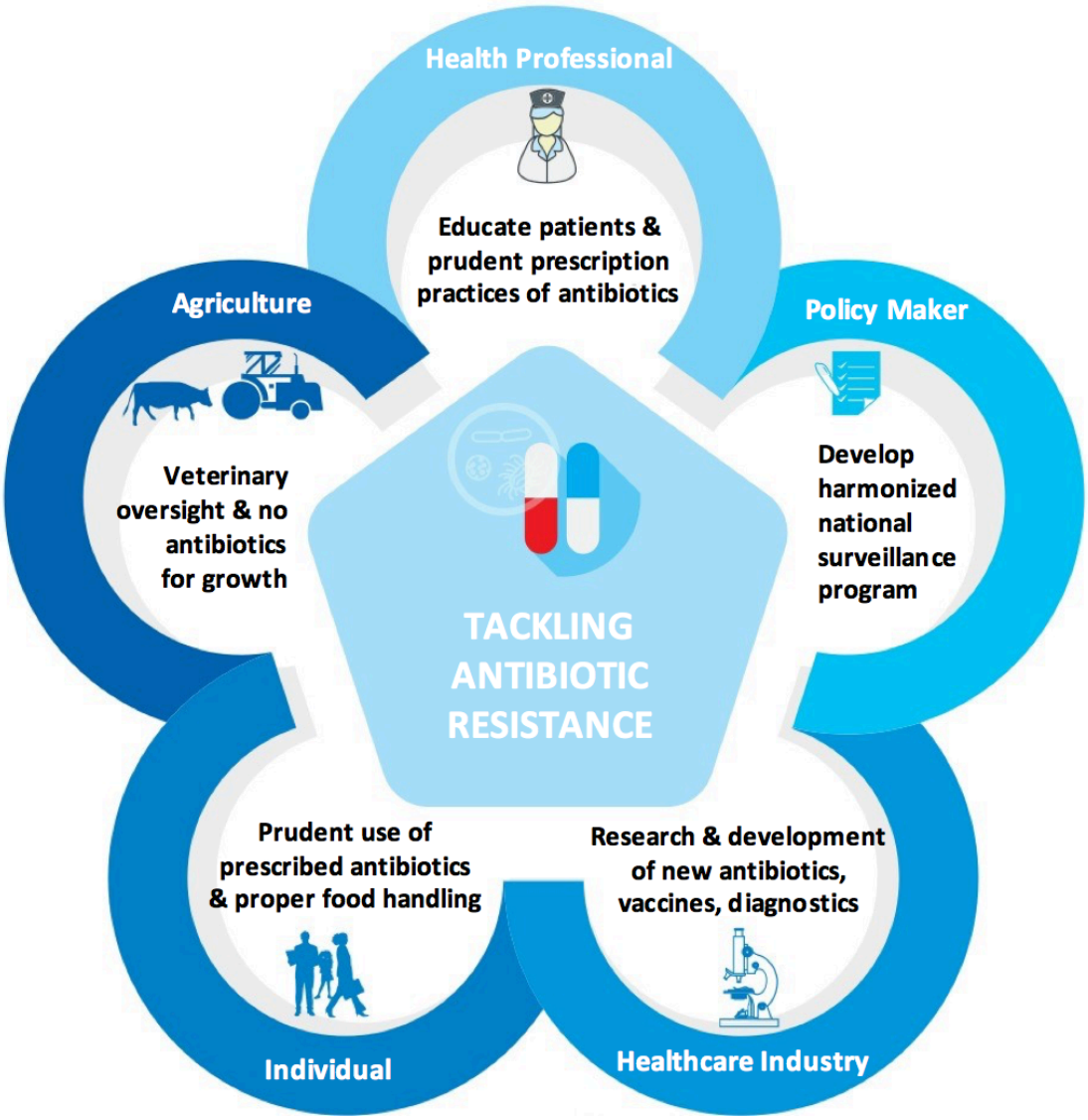

Figure 1. 3. Recommendations for different stakeholders for tackling AMR.
Adapted from (WHO, 2018).

1.4 Conclusions and the Future of Tylosin in Canada

There are few publications about reducing tylosin in-feed for beef cattle, without eliminating the antimicrobial completely. Similar initiatives are underway in the United States with recent studies exploring the intermittent use of tylosin in-feed for a 118 day trial compared

to continuous and no tylosin (Müller et al., 2018). Feeding tylosin on an intermittent basis (1 week on, 2 weeks off), lowers the prevalence of liver abscess to a similar degree as the continuous feeding of tylosin, but did not alter the prevalence of enteric enterococcal populations (Müller et al., 2018). There was no treatment effect on the proportion of erythromycin ($P = 0.63$) or tetracycline ($P = 0.71$) resistant enterococci, but there was a significant period effect ($P < 0.05$). Therefore, with increasing days on feed, equal levels of AMR enterococci from all treatments would have entered the food chain, which is the main reason for wanting to reduce antimicrobials use in the first place. There is no doubt that completely removing antimicrobials from farm animals could negatively impact animal health, welfare, and productivity and result in increased market prices. According to a 2012 report, antimicrobial-free meat sales increased by 25 percent over the previous three years (National Resources Defense Council, 2015). Therefore, antimicrobial-free meat is becoming more popular in the consumer market, but at the expense of an increased cost of production.

Since January 2017 the U.S. Food and Drug Administration implemented the Veterinary Feed Directive (VFD) Program has aimed to reduce the inclusion of all medically important antimicrobials in livestock feed (US Food and Drug Administration, 2015). Antimicrobials can only be used under the supervision of a licensed veterinarian and has been limited to therapeutic uses rather than for promotion of growth and feed efficiency (US Food and Drug Administration, 2015). New restrictions in Canada on antimicrobials have also been phased in since the end of 2017, and by December 1st, 2018 they were fully implemented. All medically important antimicrobials, including tylosin will require a prescription from a veterinarian, and this applies to all food-producing agriculture sectors. Additional changes in policy in Canada include new regulations covering own use importation (OUI), so that producers cannot import medically

important antimicrobials and other animal health products from outside of Canada. Producers will need to come up with a plan and establish a veterinary-client-patient relationship (VCPR) in order to obtain a prescription and treat or prevent diseases in a timely manner. Since the end of 2017, there has already been an 11 percent reduction in antimicrobial use in livestock in Canada, and a 17 percent reduction in the United States (Beef Cattle Research Council, 2018). This demonstrates how industry stewardship and awareness of responsible use and management of antimicrobials can impact change.

Since the Canadian and United States beef industries are closely connected, it is important for Canadian producers to understand the impact of antimicrobial use management on their own barley-based feed production systems. The key objective of this work is to assess the effects of reducing in-feed tylosin on liver abscesses, growth performance, carcass traits, immune response and antimicrobial resistance by evaluating two alternative feeding strategies: 1) *Saccharomyces cerevisiae* fermentation product and 2) shorter duration tylosin. This project is the first in Canada to investigate the simultaneous effects of reducing in-feed tylosin on bacterial antimicrobial resistance profiles, liver abscess incidence and feedlot cattle productivity. Documenting how macrolide usage effects antimicrobial resistance in an indicator species like *Enterococcus* will increase our understanding of the ecology of AMR and help the Canadian beef industry make judicious choices with regard to the use of antimicrobials in beef production.

CHAPTER TWO: Ruminally protected and unprotected *Saccharomyces cerevisiae* fermentation products as alternatives to antibiotics in finishing beef steers

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2.1 Abstract

The objectives of this study were to assess the effects of *Saccharomyces cerevisiae* fermentation products (**SCFP**; NaturSafe®, **SCFPns**; and Original XPC™, **XPC**; Diamond V) on growth performance, carcass traits, immune response and antimicrobial resistance in beef steers fed high-grain diets. Ninety Angus steers (initial body weight [BW], 533 ± 9.8 kg) were assigned to a randomized complete design with 6 treatments (n = 15/treatment): 1) control, 2) low (12 g SCFPns·steer^{-1}·d^{-1}), 3) medium (15 g SCFPns·steer^{-1}·d^{-1}), 4) high SCFP (18 g SCFPns·steer^{-1}·d^{-1}), 5) encapsulated XPC (**eXPC**; 7 g XPC·steer^{-1}·d^{-1} encapsulated with 9 g capsule material), and 6) antibiotics (**ANT**; 330 mg monensin + 110 mg tylosin·steer^{-1}·d^{-1}). Steers were fed *ad libitum* a diet containing 10% barley silage and 90% barley grain concentrate mix (dry matter basis) for 105 d. Increasing SCFPns tended ($P < 0.09$) to linearly increase feed efficiency. Average daily gain (**ADG**) tended ($P < 0.10$) to be greater in steers supplemented with eXPC than control. The SCFPns also tended ($P < 0.10$) to linearly increase marbling score. Proportion of severely abscessed livers tended ($P < 0.10$) to be lower in steers supplemented with medium and high SCFPns, eXPC or ANT. A treatment × days on feed interaction were noticed ($P < 0.01$) for blood glucose, blood urea nitrogen (**BUN**) and acute phase proteins. The concentration of blood glucose responded quadratically ($P < 0.05$) on d 28 and 56, whereas BUN linearly ($P < 0.01$) increased on d 105 with increasing SCFPns dose. The SCFPns linearly increased haptoglobin ($P < 0.03$) and serum amyloid A (**SAA;** $P < 0.05$) concentrations on d 105, and lipopolysaccharide binding protein (**LBP;** $P < 0.01$) on d 56 and 105. The percentage of erythromycin resistant and erythromycin + tetracycline resistant enterococci was greater ($P < 0.05$) with ANT than control, SCFPns and eXPC, while no difference was observed among control, SCFPns and eXPC. No treatment effect was detected on the percentage of tetracycline

resistant enterococci. These results indicate that feeding SCFPns and eXPC were beneficial in improving ADG, feed efficiency and decreasing liver abscesses in a manner comparable to ANT. Unlike antibiotics, SCFPns or eXPC did not increase antimicrobial resistance. Both SCFPns and eXPC are potential alternatives to in-feed antibiotics.

2.2 Introduction

Antibiotics like monensin and tylosin have been widely used in beef feedlot operations to improve feed efficiency and reduce the incidence of liver abscesses (Nagaraja and Chengappa, 1998; Meyer et al., 2009). However, increasing concern over the use of antimicrobials in livestock leading to the emergence of antimicrobial resistant bacteria with adverse impacts on human health has resulted in their ban for growth promotion in many countries. Therefore, there is an increasing need for alternatives to antimicrobials for use in livestock production. *Saccharomyces cerevisiae* fermentation products (**SCFP**) are produced through anaerobic fermentation and have been widely used in livestock feed. The SCFP contains B vitamins, amino acids, organic acids and other nutritional elements that stimulate the growth of fiber-digesting and lactic acid-utilizing bacteria (Wiedmeier et al., 1987; Callaway and Martin, 1997). As with the antibiotic monensin (Tedeschi et al., 2003), SCFP has also been reported to improve feed efficiency in feedlot beef cattle (Wagner et al., 2016).

However, production responses to SCFP in beef cattle have been variable, possibly due to differences in dosage, and diet, particularly high grain diets where the rumen pH is often well below the point that reduces fiber digestion (Swyers et al., 2014; Geng et al., 2016). Studies have reported a reduction (Swyers et al., 2014), and no difference (Geng et al., 2016) in the finishing performance of beef cattle fed diets supplemented with SCFP. Geng et al. (2016) explained that the failure of SCFP to improve the growth performance of finishing cattle was related to the

inclusion of monensin in the diet. Wagner et al. (2016) concluded from a meta-analysis study that the marginal improvement in feed efficiency of finishing cattle fed SCFP reflected increases in nutrient digestibility and the ruminal production of propionate. A previous study showed that feeding SCFP to finishing beef heifers improved ruminal and total tract digestibility of OM and NDF (Shen et al., 2018), with responses in rumen fermentation to SCFP often being dose-dependent (Lascano et al., 2012; Shen et al., 2018).

In addition, until recently, most research studies focused on evaluating the effects of SCFP on rumen fermentation and microbial activity. In studies with swine, SCFP improved gut morphology and immune activity (Van der Peet-Schwering et al., 2007; Shen et al., 2009). The improvement in immune activity suggests that SCFP may confer health benefits to swine, but the possibility of this response has not been explored in beef cattle. Recently, Shen et al. (2018) evaluated the effect of SCFP on intestinal function by directly delivering it into the duodenum, a method of administration that tended to increase fecal IgA as compared to when it was orally fed. Elevated IgA is desired and suggests that SCFP may benefit intestinal mucosal immunity if it escapes ruminal degradation and remains biologically active in the lower digestive tract (Suzuki et al., 2004)

Feeding SCFP modulated ruminal microbial profiles (Mullins et al., 2013) and reduced detectable markers of virulence in *Salmonella* (Feye et al., 2016), which can alter the antimicrobial resistant profile of resident bacteria (Kirkup and Riley, 2004). We hypothesize that feeding SCFP will improve feed efficiency, reduce liver abscesses and antimicrobial resistant enterococci in a dose-dependent manner in finishing beef steers. Thus, this study was conducted to 1) determine the effect of adding ruminally protected and unprotected SCFP at varying dosages on growth performance, feed efficiency, carcass traits, animal health and immune

response including fecal IgA and acute phase proteins in finishing beef steers; and 2) evaluate the effects of adding SCFP on antimicrobial resistance in enterococci.

2.3 Materials and Methods

Experiments with steers were reviewed and approved by the Institutional Animal Care and Use Committee at the Lethbridge Research and Development Centre (#1731; Lethbridge, AB, Canada). Cattle were cared for and managed according to the guidelines of the Canadian Council on Animal Care (2009).

2.3.1 Encapsulation of SCFP

Two different SCFP products (NaturSafe®, **SCFPns**; and Original XPC™, **XPC**; Diamond V, Cedar Rapids, IW) were used. Both products were composed of dried *S. cerevisiae* fermentation products, but were different SCFP preparation. The XPC (**eXPC**) was encapsulated in a stearic acid and palm oil-based capsule material (King Techina Feed Co., Ltd, Hangzhou, China) as reported by Wang et al. (2011). The eXPC consisted of approximately 45% of XPC and 55% of capsule material. The stability of eXPC was assessed by incubating it *in vitro* with rumen inoculum for 6, 12 or 24 h and measuring DM disappearance (**DMD**). Similarly, the release of eXPC in the intestine was estimated by measuring DMD from bags incubated under simulated abomasal and intestinal conditions with the incubation solution containing lecithin, bile salts, bile acids and trypsin (Shen et al., 2017). The DMD after 24 h of *in vitro* ruminal incubations was 82% for XPC and only 25% for eXPC. The sum of DMD in the simulated ruminal and intestinal models was 88% and 71% for XPC and eXPC, respectively. These results suggest that a large portion of eXPC would reach the intestinal tract. Although one cannot definitively determine whether the more soluble fermentation metabolite components within

eXPC were made available in the rumen or in the intestine, a significant part of eXPC escapes rumen fermentation and would be expected to be active in the intestine.

2.3.2 Animals, Design and Treatments

Ninety Angus steers (15 head/treatment) were purchased at local auction market from the same source and housed in individual feedlot pens (4.9 × 1.8 m) in a sheltered barn bedded with sawdust. Steers were gradually adapted to a high-grain finishing diet over 4 weeks before starting the experiment, ear tagged and vaccinated against infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI-3) virus and *Haemophilus somnus* (Resvac 2/Somubac, Pfizer Animal Health, Parsippany-Troy Hills, NJ) and against *Clostridium* spp. (Tasvax 8, Schering-Plough Animal Health, Upper Hutt, NZ). Steers (initial BW, 533 ± 9.8 kg) were blocked by BW and assigned to a randomized complete design to 1 of 6 treatments. The treatments were: 1) control (no antibiotics, no SCFP); 2) low (12 g SCFPns·steer^{-1}·d^{-1}); 3) medium (15 g SCFPns·steer^{-1}·d^{-1}); 4) high (18 g SCFPns·steer^{-1}·d^{-1}); 5) eXPC (16 g eXPC·steer^{-1}·d^{-1}); and 6) antibiotics (**ANT**; 330 mg monensin + 110 mg tylosin·steer^{-1}·d^{-1}). The dose of SCFPns used was based on the manufacture's recommendation. All the treatments were mixed with 50 g ground barley and 8 g molasses, and top dressed onto feed once daily at feeding. Steers in the control group received 50 g ground barley and 8 g molasses.

Table 2. 1. Ingredient and chemical composition of the total mixed diet

Item	
Ingredient, % DM	
Barley silage[1]	10.0
Barley grain,[2] dry-rolled	87.0
Supplement	
Barley, ground	1.64
Canola meal	0.29
Calcium carbonate	0.73
Molasses	0.07
Salt	0.15
Feedlot premix[3]	0.03
Urea	0.06
Vitamin E (500,000 IU/kg)	0.002
Canola oil	0.03
Chemical composition, % DM	
DM	79.8
OM	96.6
NDF	23.1
ADF	7.20
Starch	54.2
CP	13.2

[1]Composition (DM basis): 39.4% DM, 46.6% NDF, 24.7% ADF, 22.1% starch and 12.9% CP based on 4 samples composited by period.
[2]Composition (DM basis): 90.2% DM, 20.8% NDF, 5.3% ADF, 58.7% starch and 13.4% CP based on 4 samples composited by period.
[3]Supplied per kilogram of dietary DM: 15 mg Cu, 65 mg Zn, 28 mg Mn, 0.7 mg I, 0.2 mg Co, 0.3 mg Se, 6,000 IU vitamin A, 600 IU vitamin D, and 47 IU vitamin E.

Steers were fed a total mixed ration (**TMR**) *ad libitum* once daily at 0900 for 105 d. The TMR contained 10% barley silage, 87% dry-rolled barley and 3% vitamin and mineral supplement (DM basis) to meet the nutrient requirements of finishing beef steers at a daily growth rate of 1.8 kg (National Academies of Sciences, 2016). The diets were prepared daily using a Data Ranger feed mixer (American Calan Inc., Northwood, NH). Fresh water was continuously available to all steers throughout the experiment.

The amount of feed offered was recorded daily for each steer and refusals were weighed weekly. Rolled barley, barley silage, TMR and refusals were collected weekly and oven dried at

55°C for 48 h to measure DM content. The daily DMI was calculated as the daily feed offered minus the weekly feed refused divided by 7 (DM basis). The steers were weighed on two consecutive days at the beginning and end of the experiment, and every 28 d in between. The ADG was calculated by subtracting initial BW from BW at the end of the experiment and dividing by the number of days on-feed. Feed efficiency (ratio of gain to feed, **G:F**) was determined as the ratio of ADG to daily DMI.

2.3.3 Blood Sampling and Analysis

Ten steers from each treatment were randomly selected for blood sampling. Blood samples were collected via jugular venipuncture on day 0, 28, 56 and 105. Two 10-mL vacuum tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing Na heparin or without additive, were used to collect plasma and serum, respectively. Plasma samples were obtained by centrifuging at 3,000 × g for 20 min at 4°C, and serum samples were obtained by centrifuging at 2,000 × g for 20 min at 4°C. Both plasma and serum samples were stored at -20°C. A subsample (1 mL) of the plasma was centrifuged at 16,000 × g for 2 min at 4°C to remove fibrinogen, and the supernatants were analyzed for blood urea N (**BUN**), and blood glucose using a dry chemistry analyzer (VetTest analyzer, model 8008, IDEXX Lab, Westbrook, ME). The serum non-esterified fatty acid (**NEFA**) was determined using a commercial enzymatic colorimetric procedure (NEFA-HR 2, Wako Chemicals Inc., Richmond, VA). Concentrations of serum amyloid A (**SAA;** SEA885Bo), plasma haptoglobin (**Hp;** SEA817Bo), and lipopolysaccharide binding protein (**LBP;** SEB406Bo) were determined using bovine ELISA kits (Cloud-Clone Corp., Katy, TX) following the manufacturer's instructions. The minimum detectable dose of SAA, Hp and LBP is typically less than 0.067 ng/mL, 5.900 ng/mL and 0.287 ng/mL, respectively.

2.3.4 Fecal Sampling and Analysis

Fecal samples (approximately 400 g wet) were collected concurrent with blood collection. After sampling, fecal pH was measured immediately using a pH meter (B20PI, SympHony Benchtop Meters; VWR, Edmonton, AB, Canada). Fecal samples were subdivided into 4 portions for the enumeration of generic *Escherichia coli* (***E. coil***), antimicrobial resistant enterococci, estimation of fecal IgA concentration and DM measurement. For enumeration of *E. coli*, 1 g of fresh feces was weighed into a 16 × 125 mm culture tube containing 9 mL of 0.1% peptone dilution water and vortexed vigorously. Serial dilutions of 10^{-4}, 10^{-5} and 10^{-6} were prepared and 5 mL were then spread plated onto *E. coli* Petrifilm media (3M Canada, London, ON, Canada) and incubated at 35°C for 24 h. Colonies that appeared blue and exhibited gas bubbles were counted as *E. coli*. Antimicrobial resistance in enterococci populations was determined as described by Beukers et al., (2015). Briefly, fecal samples were weighed (1.0 g) into sterile 4.0 mL tubes of 1 × phosphate buffered saline and vortexed for 30 sec. Samples were serially diluted 10-fold and 100 µL of the 5^{-3}, 5^{-4} and 5^{-5} were plated in duplicate onto Bile Esculin Azide (**BEA**) agar containing no antibiotics, and 5^{-1}, 5^{-2} and 5^{-3} onto BEA amended with erythromycin (8 µg/mL; $\mathbf{BEA^{E}}$), BEA amended with tetracycline (16 µg/mL; $\mathbf{BEA^{T}}$), and BEA amended with a mixture of erythromycin and tetracycline (8 µg/mL; 16 µg/mL; $\mathbf{BEA^{ET}}$). For greater feasibility, SCFPns at 15 g/d was not included in the AMR portion of this study, justified by including the upper (18 g/d) and lower (12 g/d) concentrations in feed. In addition, only the control, SCFPns at 18 g/d, and ANT treatments were enumerated on BEA^{T} and BEA^{ET}. Antibiotic selective media was chosen based on importance in human medicine and observed resistance in enterococci in recent studies (Beukers et al., 2015). The concentration of antibiotics used in all plates were set at the breakpoint standards for defining resistance as described by the

Clinical and Laboratory Standards Institute guidelines (2016). All plates were incubated for 48 h at 37°C and isolates that grew on these plates were considered resistant to erythromycin (**Ery^R**), tetracycline (**Tet^R**) or both tetracycline and erythromycin (**Ery^RTet^R**). The percentage of enterococci resistant to "X" on the selective plates was calculated according to (Alexander et al., 2009), in which: [(number of colonies on selective BEA^X plates / total colonies on non-selective BEA plates) × 100%]. Fecal IgA was analyzed using ELISA kits (Bovine IgA ELISA Quantitation Set, Bethyl Laboratories, Montgomery, TX).

2.3.5 Carcass Traits

At the end of the experiment, steers were transported to a commercial abattoir for slaughter. Hot carcass weights (**HCW**, with kidneys removed), dressing percentage, 12th-rib back fat thickness (**BFT**), longissimus muscle (**LM**) area, marbling score, quality grade, saleable meat yield, and liver abscess score were recorded for each carcass. The percentage of HCW on final BW was calculated as dressing percentage. Marbling score was estimated according to pictorial standards from 1 (devoid) to 10 (abundant marbling; USDA, 1989). Canada AAA standards were used to estimate the quality grade with Canada grade A being equivalent to USDA Standard; AA to USDA Select; and AAA to USDA Choice. Saleable meat yield was calculated using an equation that considered the length, width, and fat cover over the rib eye muscle between the 11th and 12th rib: Saleable meat (%) = (57.96 – 0.027 HCW + 0.202 LM area – 0.703 BFT)%. Liver abscess scores were generated according to the ranking scale used by the Canadian Beef Grading Agency as described by Brink et al. (1990). A liver was defined as abscessed if it had at least one abscess, and severe liver abscesses were defined as a liver with at least four small abscesses or at least one abscess with diameter larger than 2.5 cm.

2.3.6 Statistical Analysis

Data of DMI, BW, ADG, G:F and carcass traits were analyzed using the Mixed procedure of SAS (Version 16.0.0, SAS Inst. Inc. Cary, NC) as a completely randomized design. The model included treatment as a fixed effect and steers within treatment as a random effect to account for blocking by initial BW. After a log transformation of *E. coli* counts and levels of antimicrobial resistance in enterococci, these indicators were analyzed together with blood metabolites, acute phase proteins, fecal pH and IgA, using the Mixed procedure of SAS as a completely randomized design with treatment, days on-feed and their interaction as fixed effects and steers within treatment as a random effect. The mixed model also included d 0 as covariate for analysis of blood metabolites and acute phase proteins. For repeated measures, various covariance structures were tested and AR(1) was selected based on the lowest value for Akaike's information criteria. Orthogonal contrasts were conducted to determine linear and quadratic SCFPns dose responses. The GLIMMX procedure of SAS (version 16.0.0, SAS Inst. Inc., Cary, NC) was used to analyze meat quality and liver abscess data. Least square means were compared using the Tukey correction for multiple comparisons, and treatment effects were declared significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

2.4 Results

2.4.1 Growth Performance and Carcass Traits

Across treatments, DMI averaged 12.0 kg/d and did not differ among treatments (Table 2.2). Final BW and ADG were not affected by either SCFP supplementation or monensin and tylosin, whereas the ADG tended ($P < 0.10$; P value not shown in table) to be greater with eXPC than control. Feed efficiency tended ($P < 0.09$) to be greater with eXPC than control, and linearly improved with increasing SCFPns in the diet.

Treatments had no effect on carcass traits, except that marbling score tended ($P < 0.10$) to linearly increase with increasing SCFPns (Table 2.2). Although the proportion of total abscessed livers did not differ, abscesses in steers supplemented with SCFPns at 15 and 18 g/d, eXPC or ANT tended ($P < 0.10$) to be less severe than in control steers.

Table 2. 2. Growth and carcass traits of finishing steers fed a diet supplemented with, *Saccharomyces cerevisiae* fermentation product (SCFPns), encapsulated Original XPC (eXPC) or antibiotics (ANT)

	SCFPns (g/d)							$P <$[2]		
Item	0	12	15	18	eXPC	ANT[1]	SEM	Treat	L	Q
No of steers	15	15	15	15	15	15				
Growth										
Initial BW, kg	533	533	533	533	533	533	9.8	1.00	0.99	0.96
Final BW, kg	703	719	721	709	722	716	13.4	0.36	0.59	0.45
DMI, kg/d	11.8	12.2	11.9	11.6	12.5	11.9	0.36	0.84	0.95	0.23
ADG, kg/d	1.62	1.77	1.79	1.68	1.80	1.75	0.08	0.49	0.26	0.15
G:F, g/kg	137	145	150	146	144	147	4.9	0.54	0.09	0.42
Carcass traits										
HCW, kg	414	423	420	417	421	421	8.4	0.97	0.64	0.49
Dressing, %	58.9	58.8	58.2	58.8	58.5	59.0	0.37	0.73	0.56	0.66
Back fat, mm	20.4	22.0	20.8	20.1	19.3	20.8	1.71	0.92	0.97	0.40
LM area, cm^2	80.3	80.3	81.1	78.5	80.2	77.1	2.64	0.75	0.74	0.53
Marbling score[3]	5.51	6.33	5.96	6.00	5.96	5.75	0.22	0.29	0.10	0.15
Saleable meat[4], %	48.7	47.3	48.3	48.4	49.2	47.5	1.46	0.93	0.84	0.53
Quality grade[5], %	80	100	80	87	93	87	--	0.97		
Liver score, %										
Abscessed[6]	66.7	60.0	53.3	60.0	53.3	60.0	--	0.97		
Severely[7]	53.3	33.3	20.0	20.0	20.0	6.7	--	0.10		

[1] ANT = 330 mg monensin + 110 mg tylosin/d; eXPC = 16 g eXPC/d.
[2] Treat = treatment; L, Q = linear and quadratic effects of SCFPns (0, 12, 15, 18 g/d).
[3] According to pictorial standards (from 1 = devoid to 10 = abundant marbling; USDA, 1989).
[4] Estimated lean yield = 57.96 - 0.027 HCW + 0.202 LM area - 0.703 Back fat
[5] Canada grade AAA = equivalent to USDA choice.
[6] The percentage of liver with at least 1 abscess.
[7] The percentage of liver with at least 4 small abscesses or at least 1 abscess with a diameter greater than 2.5 cm.

2.4.2 Blood Metabolites and Acute Phase Proteins

Concentration of blood glucose did not differ among treatments on d 0 and 105, but responded quadratically on d 28 ($P < 0.01$) and 56 ($P < 0.05$) to increased SCFPns (Table 2.3). Blood glucose was also higher ($P < 0.03$) with the low dose of SCFPns than ANT. On d 28, steers receiving eXPC tended ($P < 0.07$) to have lower blood glucose concentration than control, without differing from ANT. Serum NEFA concentration did not differ among treatments throughout the feeding period, except for a quadratic ($P < 0.08$) change in NEFA concentration on d 28 with increasing SCFPns. No treatment effect on BUN concentration was observed on d 0, 28 or 56, whereas concentration of BUN linearly ($P < 0.01$) increased with increasing SCFPns on d 105. Steers supplemented with eXPC or ANT also had greater ($P < 0.04$) BUN concentrations than control steers on d 105. The concentration of BUN did not differ between eXPC and ANT steers over the entire feeding period.

Table 2. 3. Blood metabolites of finishing steers fed a diet supplemented with, *Saccharomyces cerevisiae* fermentation product (SCFPns), encapsulated Original XPC (eXPC) or antibiotics (ANT)

	SCFPns (g/d)							$P <$[2]		
Item	0	12	15	18	eXPC	ANT[1]	SEM	Treat	L	Q
No of steers	10	10	10	10	10	10				
Glucose, mg/dL										
Day 0	94.3	97.3	91.1	94.4	98.1	90.4	4.99	0.85	0.89	0.77
Day 28	101.6[ab]	109.9[a]	98.2[ab]	87.0[b]	87.3[b]	92.0[b]	5.43	0.03	0.16	0.01
Day 56	106.2	90.0	98.7	105.2	100.7	94.7	6.04	0.40	0.58	0.05
Day 105	108.6	92.3	96.5	105.2	95.0	100.0	7.46	0.62	0.43	0.12
NEFA, μ*M*										
Day 0	146	125	164	201	209	204	40.0	0.58	0.43	0.26
Day 28	105	120	105	79	113	142	15.4	0.12	0.44	0.08
Day 56	85	86	78	97	77	90	19.9	0.98	0.83	0.68
Day 105	125	139	152	132	137	136	16.8	0.93	0.50	0.53
BUN[3], mg/dL										
Day 0	15.0	14.3	14.2	13.5	15.6	13.2	1.51	0.87	0.52	0.86
Day 28	20.6	20.1	16.7	23.6	22.5	19.0	1.83	0.13	0.82	0.11
Day 56	19.6	19.5	19.3	21.0	24.4	21.9	1.57	0.18	0.71	0.51
Day 105	22.5[b]	27.4[ab]	26.7[ab]	30.8[a]	30.2[a]	30.8[a]	1.56	0.01	0.01	0.53

[a,b] Means within a row with different superscripts differ ($P < 0.05$).
[1]ANT = 330 mg monensin + 110 mg tylosin/d; eXPC = 16 g eXPC/d.
[2]Treat = treatment; L, Q = linear and quadratic effects of SCFPns (0, 12, 15, 18 g/d); treatment × day was significant ($P < 0.01$) for glucose and BUN concentration.
[3]BUN = Blood urea N.

There was a treatment × days on feed interaction ($P < 0.01$) for plasma Hp, SAA and LBP concentration (Table 2.4). Blood concentrations of Hp, LBP and SAA differed among treatments at d 0, thus these values were used as covariate to analyze the data for d 28, 56 and 105. There were no differences in plasma Hp concentration among treatments on d 28 and 56, but it quadratically ($P < 0.03$) increased with increasing SCFPns on d 105. Steers fed eXPC on d 105 had less ($P < 0.01$) Hp than steers receiving the medium or high dose of SCFPns, and tended to have lower ($P < 0.10$) Hp concentration than control and ANT. SCFPns linearly increased plasma LBP concentration on d 56 ($P < 0.05$) and 105 ($P < 0.09$). Plasma SAA concentration responded quadratically ($P < 0.01$) on d 28 and 56 or linearly ($P < 0.01$) on d 105 to increasing

SCFPns in the diet. The SAA concentration tended ($P < 0.10$) to be greater with eXPC than control on d 28 and 105, and also greater ($P < 0.01$) than ANT on d 28.

Table 2. 4. Plasma acute phase protein concentration in steers fed a diet supplemented with, *Saccharomyces cerevisiae* fermentation product (SCFPns), encapsulated Original XPC (eXPC) or antibiotics (ANT)

	SCFPns (g/d)							$P <$[2]		
Item[3]	0	12	15	18	eXPC	ANT[1]	SEM	Treat	L	Q
No of steers	10	10	10	10	10	10				
Hp, µg/mL										
Day 0	1535^{b}	1094^{c}	1243^{c}	1173^{c}	1176^{c}	1898^{a}	123.5	0.01	0.03	0.28
Day 28	140	141	128	132	136	128	6.3	0.50	0.27	0.52
Day 56	146	133	130	146	124	124	9.3	0.31	0.59	0.19
Day 105	124^{bc}	126^{bc}	128^{b}	146^{a}	113^{c}	126^{bc}	5.5	0.01	0.04	0.03
LBP, µg/mL										
Day 0	743^{a}	516^{b}	413^{bc}	336^{c}	335^{c}	753^{a}	67.6	0.01	0.01	0.01
Day 28	135	145	161	109	104	97	35.3	0.72	0.89	0.41
Day 56	57	101	106	84	80	95	13.7	0.14	0.05	0.09
Day 105	79	83	103	112	87	103	12.6	0.35	0.09	0.30
SAA, µg/mL										
Day 0	84.7	74.0	46.1	63.5	62.0	76.6	11.81	0.28	0.07	0.89
Day 28	7.2^{bc}	18.7^{a}	9.0^{bc}	10.2^{b}	11.8^{b}	3.0^{c}	2.27	0.01	0.26	0.01
Day 56	9.5^{c}	23.9^{ab}	31.9^{a}	10.6^{c}	9.2^{c}	13.5^{bc}	4.71	0.01	0.13	0.01
Day 105	13.2	19.4	28.6	29.5	22.2	22.4	4.46	0.14	0.01	0.50

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).
[1]ANT = 330 mg monensin + 110 mg tylosin/d; eXPC = 16 g eXPC/d.
[2]Treat = treatment; L, Q = linear and quadratic effects of SCFPns (0, 12, 15, 18 g/d); treatment × day was significant ($P < 0.01$).
[3]Hp = haptoglobin; SAA = serum amyloid A; LBP = lipopolysaccharide binding protein.

2.4.3 Fecal pH, IgA and Total *E. coli*

The fecal pH, IgA concentration and total *E. coli* counts did not differ among treatments. A treatment × days on feed interaction was significant ($P < 0.01$) for all fecal variables measured (Table 2.5). Fecal pH linearly ($P < 0.04$) increased with increasing SCFPns on d 28. The eXPC tended ($P < 0.09$) to increase fecal pH compared with control on day 28 and 105. No treatment effect was observed on fecal IgA on d 28 and 56, whereas steers receiving eXPC had lower fecal IgA than control ($P < 0.08$) and other treatments ($P < 0.05$). Fecal *E. coli* counts linearly

decreased with increasing SCFPns on d 56 ($P < 0.07$) and d 105 ($P < 0.02$). Steers supplemented with eXPC had lower ($P < 0.05$) *E. coli* compared with control and ANT on d 105.

Table 2. 5. Fecal pH, IgA and *E. coli* counts in finishing steers fed a diet supplemented with *Saccharomyces cerevisiae* fermentation product (SCFPns), encapsulated Original XPC (eXPC) or antibiotics (ANT)

	SCFPns (g/d)							$P <$[2]		
Item	0	12	15	18	eXPC	ANT[1]	SEM	Treat	L	Q
No of steers	10	10	10	10	10	10				
Fecal pH										
Day 0	6.19	6.40	6.49	6.25	6.28	6.23	0.12	0.42	0.28	0.15
Day 28	6.22^{b}	6.37^{b}	6.22^{b}	6.63^{a}	6.49^{ab}	6.74^{a}	0.10	0.01	0.04	0.13
Day 56	6.23	6.17	6.38	6.30	6.54	6.63	0.14	0.18	0.61	0.71
Day 105	6.37	6.38	6.60	6.52	6.60	6.66	0.10	0.17	0.16	0.65
IgA, µg/g										
Day 0	2.41	2.34	2.29	1.28	1.07	1.78	0.50	0.25	0.24	0.20
Day 28	1.39	2.11	1.93	1.92	1.17	1.18	0.44	0.49	0.31	0.54
Day 56	5.29	5.67	7.61	6.18	5.26	5.09	1.18	0.66	0.36	0.90
Day 105	3.78^{ab}	4.42^{a}	3.28^{ab}	4.56^{a}	1.82^{b}	5.12^{a}	0.78	0.05	0.71	0.23
E. coli counts[3]										
Day 0	7.15	7.21	7.45	7.32	7.39	7.43	0.14	0.58	0.24	0.97
Day 28	7.28^{bc}	7.09^{c}	7.61^{a}	7.24^{bc}	7.44^{ab}	7.51^{ab}	0.13	0.05	0.62	0.72
Day 56	7.42^{a}	6.93^{b}	7.30^{ab}	7.14^{b}	7.25^{b}	7.43^{a}	0.11	0.02	0.07	0.08
Day 105	7.50	7.14	7.15	7.17	7.18	7.31	0.12	0.23	0.02	0.35

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).
[1]ANT = 330 mg monensin + 110 mg tylosin/d; eXPC = 16 g eXPC/d.
[2]Treat = treatment; L, Q = linear and quadratic effects of SCFPns (0, 12, 15, 18 g/d); treatment × day was significant ($P < 0.01$).
[3]*E. coli* counts showed on $\log_{10}$ basis.

2.4.4 Antimicrobial Resistance of Enterococci

The percentage of Ery^{R} enterococci did not differ among treatments on d 0 (Figure 2.1). However, on d 28 and 56, the percentage of Ery^{R} enterococci was greater ($P < 0.02$) in steers receiving antibiotics compared to other treatments. On d 105, although no significant treatment effects were observed, the percentage of Ery^{R} enterococci in steers receiving antibiotics tended ($P < 0.06$) to be greater than other treatments. The percentage of Tet^{R} enterococci was greater ($P < 0.01$) for steers supplemented with SCFPns and antibiotics than control on d 0, whereas no

difference among treatments was observed from d 28 to 105. The percentage of $Ery^{R}Tet^{R}$ enterococci was greater ($P < 0.02$) for steers receiving SCFPns compared with control and antibiotics on d 0, with no difference on d 28. From d 56 to 105, there was an increase of $Ery^{R}Tet^{R}$ ($P < 0.05$) in steers administered antibiotics as compared to control and SCFPns steers.

Table 2. 6. Antimicrobial resistance of enterococci in finishing steers fed a diet supplemented with, *Saccharomyces cerevisiae* fermentation product (SCFPns), encapsulated Original XPC (eXPC) or antibiotics (ANT)

	SCFPns, g/d						$P <$[2]		
Item[3,4]	0	12	18	eXPC	ANT[1]	SEM	Treat	L	Q
BEA, cfu/g feces									
Day 0	3.59	4.21	3.68	4.54	4.67	0.61	0.63	0.80	0.46
Day 28	4.59[bc]	4.31[c]	5.36[b]	6.73[a]	4.15[c]	0.31	0.01	0.17	0.04
Day 56	3.97	4.15	4.36	4.17	3.09	0.33	0.08	0.43	0.85
Day 105	3.20	2.48	3.11	3.02	2.17	0.61	0.70	0.79	0.38
BEA^{E}, cfu/g feces									
Day 0	0.79	2.33	2.52	0.97	2.09	0.60	0.15	0.03	0.60
Day 28	2.99	2.11	3.79	3.86	2.56	0.56	0.14	0.53	0.05
Day 56	1.58	1.23	2.08	1.63	1.84	0.54	0.85	0.65	0.32
Day 105	0.88	0.64	2.06	0.92	1.36	0.45	0.21	0.13	0.08
BEA^{T}, cfu/g feces									
Day 0	3.17	N/A[4]	4.49	N/A	4.40	0.73	0.15		
Day 28	4.49[b]	N/A	5.24[a]	N/A	4.01[b]	0.32	0.04		
Day 56	3.99[a]	N/A	4.32[a]	N/A	2.64[b]	0.40	0.02		
Day 105	2.17	N/A	2.35	N/A	1.63	0.57	0.66		
BEA^{ET}, cfu/g feces									
Day 0	0.47	N/A	2.51	N/A	1.08	0.58	0.06		
Day 28	3.01	N/A	3.71	N/A	2.89	0.51	0.49		
Day 56	1.96	N/A	2.10	N/A	1.96	0.61	0.98		
Day 105	1.11	N/A	1.54	N/A	1.71	0.50	0.69		

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).
[1]ANT = 330 mg monensin + 110 mg tylosin/d; eXPC = 16 g eXPC/d.
[2]Treat = treatment; L, Q = linear and quadratic effects of SCFPns (0, 12 and 18 g); treatment × day was significant ($P < 0.01$).
[3]Media types: BEA = Bile Esculin Azide (**BEA**) agar (total enterococci); BEA^{E} = BEA amended with erythromycin (8 µg/mL); BEA^{T} = BEA amended with tetracycline (16 µg/mL); BEA^{ET} = BEA amended with a mixture of erythromycin and tetracycline (8 µg/mL; 16 µg/mL).
[4]N/A: not applicable, treatment 12 g SCFPns and eXPC were not plated on tetracycline or erythromycin + tetracycline. For greater feasibility, SCFPns at 15 g/d was not included in the AMR portion of this study, justified by including the upper (18 g/d) and lower (12 g/d) concentrations in feed. In addition, only the control, SCFPns at 18 g/d, and ANT treatments were enumerated on BEA^{T} and BEA^{ET}. No treatment × day effects occurred.

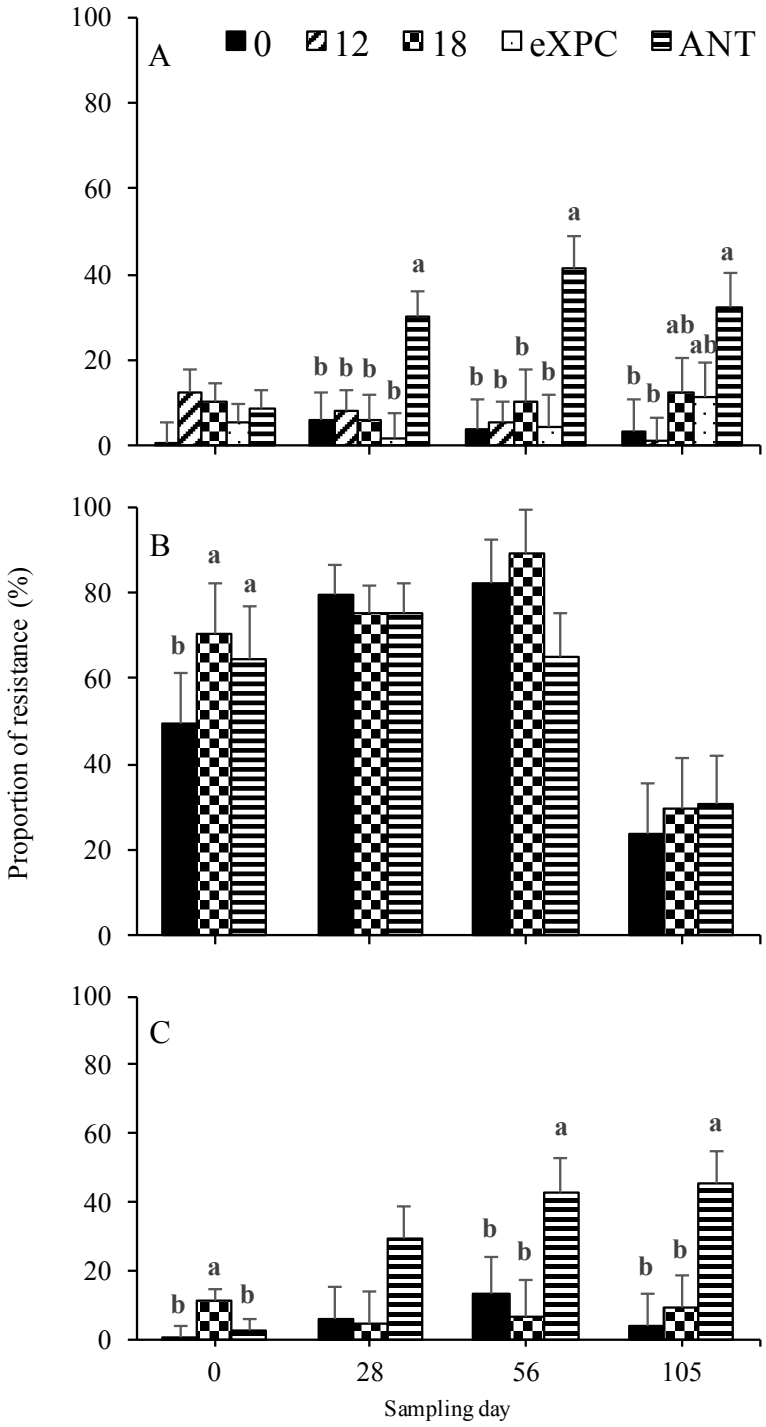

Figure 2. 1. Proportion of resistance to (A) erythromycin, (B) tetracycline, or (C) erythromycin and tetracycline in fecal enterococci isolates collected across sampling days. [a,b] Means within a day with different superscripts differ ($P < 0.05$). ANT = 330 mg monensin + 110 mg tylosin/d; eXPC = 7 g XPC·steer^{-1}·d^{-1} encapsulated with 9 g capsule material.

2.5 Discussion

2.5.1 Effects of SCFPns and eXPC on Growth Performance and Carcass Traits

The absence of an effect of SCFPns on DMI confirms previous findings that DMI of beef heifers fed a high barley grain diet with SCFPns did not differ from control heifers (Shen et al., 2018). Other studies also found no effect of SCFP on DMI in either beef cattle (Swyers et al., 2014) or dairy cows (Acharya, 2018). Wagner et al. (2016) conducted a meta-analysis by compiling 18 experiments evaluating the effects of SCFP (Diamond V, Cedar Rapids, IW) on feedlot performance and found that although cattle receiving SCFP had greater DMI, the difference in DMI between treatments was subtle (control vs. SCFP; 7.69 vs. 7.77 kg/d; $P < 0.05$). Allen (2000) proposed that ruminal propionate production played an important role in decreasing DMI. Ruminal propionate is absorbed across the rumen wall into liver where it is oxidized and yields sufficient ATP to evoke satiety by the vagus nerve, thus reducing DMI. Total VFA concentration and molar proportion of propionate was not affected by adding SCFPns to the high-barley grain diet in a previous study (Shen et al., 2018). This could partly explain the lack of SCFPns effect on DMI in the present study. However, compared to control cattle, there was a slight but consistently greater DMI with either duodenally delivered SCFPns (+0.8 kg DMI·d^{-1}) (Shen et al., 2018) and the rumen protected eXPC in the present study (+0.7 kg DMI·d^{-1}).

The trend of improved feed efficiency with SCFPns is consistent with the improvement in ruminal and total tract digestibility of OM, NDF and CP observed in a previous study using beef heifers fed a high grain diet and supplemented with the same SCFPns product (Shen et al., 2018). Swyers et al. (2014) indicated that the most consistent response to SCFP was a stimulation of rumen cellulolytic bacteria. Although the dietary fiber content of the high-grain diet in our study

was relatively low (23.1% NDF), the improved feed efficiency is likely associated with the increased fiber and protein digestibility, as well as an increase in rumen pH (Shen et al., 2018). However, the trend of greater ADG with eXPC than control, appeared to be primarily due to numerically greater DMI in steers fed eXPC (12.5 vs. 11.8 kg/d). Although the XPC was encapsulated to protect it from rumen degradation, approximately 25% of eXPC (including both capsule and XPC) was released in *in vitro* rumen fermentation as we described in the preparation of eXPC. Neither SCFPns nor eXPC had an effect on growth performance or G:F as compared to ANT, which is consistent with Scott et al. (2017). These authors reported that the growth performance did not differ between beef heifers fed a combination of monensin and tylosin and heifers fed 18 g/d of SCFPns. The lack of difference in growth performance of steers fed SCFPns and ANT compared with control in the current study appears to be inconsistent with previous findings in beef heifers provided SCFPns (Shen et al., 2018). In that study, ruminal and total tract digestibility of OM and NDF improved with supplementation of SCFPns (18 g/d) compared with ANT and control. Nevertheless, the energy provided by the present high-grain diet exceeded energy requirements (National Academies of Sciences, 2016), and therefore any improvements in the total tract OM digestibility with SCFP vs. control (80.7 vs. 77.2%) (Shen et al., 2018) may have limited the impact on growth performance.

The lack of an overall treatment effect on growth performance likely accounts for the similar carcass traits among treatments (Swyers et al., 2014; Geng et al., 2016). The greater marbling score with addition of SCFPns is in agreement with other studies that reported beneficial effects of feeding SCFP in beef steers. In a recent meta-analysis, supplementation of SCFP including different SCFP-based products developed by Diamond V such as YC, XP, XPC, compared to beef cattle fed conventional diets, resulted in an improvement in quality grade

(more USDA Choice and less USDA Select carcasses; Wagner et al., (2016). The improved marbling quality with feeding SCFP could be a result of increased ruminal propionate production, which is converted to glucose in the liver. Shen et al. (2018) showed that there tended to be a greater amount ($P = 0.10$) of OM (0.7 kg/d) fermented in the rumen with SCFP than control without a difference in the molar proportion of propionate, indicating that more propionate was produced with SCFP. Glucose is the preferred carbon source for fatty acid synthesis by intramuscular adipocytes (Smith and Crouse, 1984). The trend of reduced severely abscessed livers with SCFPns may be explained due to alleviation of rumen acidosis by feeding SCFPns as observed previously (Shen et al., 2018). Rapid starch digestion in the rumen of steers fed high-grain diets can lower ruminal pH and cause digestive disturbances such as acidosis and rumenitis, increasing the incidence of liver abscesses (McAllister et al., 1990; Nagaraja and Lechtenberg, 2007). Similarly, rapid starch digestion can also occur in the hindgut of steers fed high-grain diets, increasing the release of endotoxin and the translocation of pathogens (Li et al., 2012). The decrease in severely abscessed livers with eXPC addition suggests that feeding eXPC might have some potential benefits in decreasing the release of endotoxin and protect the cattle from translocating pathogens in the hindgut. In addition, although there was a trend ($P < 0.09$) for a linear effect of increasing SCFPns dosage on G:F and marbling score, differences in final BW, ADG and carcass traits among low, medium and high doses were minimal.

The lack of effect of ANT on carcass characteristics observed is consistent with previous studies that feeding monensin had no effect on carcass traits (Swyers et al., 2014; Ran et al., 2018b). However, the reduction in severely abscessed livers in steers fed ANT was expected as a result of the inclusion of tylosin in the diet. Antibiotics, particularly tylosin, are commonly used to control liver abscesses in feedlot cattle by reducing acidosis and subsequent rumenitis

(Nagaraja and Lechtenberg, 2007; Amachawadi and Nagaraja, 2016). The fact that tylosin reduced the severity, but not the total number of abscessed livers, is consistent with a recent report of finishing cattle fed barley-based diet (Ran et al., 2018b).

2.5.2 Effects of SCFPns and eXPC on Blood Metabolites and Immune Response

Blood glucose and NEFA concentrations are two important indicators for evaluating energy balance in cattle. Glucose can be synthesized from propionate in the liver, or directly absorbed from the small intestine, whereas NEFA can be absorbed from feed, or mobilized from adipose tissue. Since lipid levels were low in the diet and no difference was observed in DMI, the exogenous NEFA should be similar among treatments. Blood glucose can be used to assess energy intake, while blood NEFA reflect the mobilization of body fat (Herdt, 2000). Usually a higher concentration of blood glucose is indicative of greater energy intake, reduced fat mobilization, and as a result, lower blood NEFA. In the present study, the energy provided by the finishing diet exceeded energy requirements for finishing beef cattle (National Academies of Sciences, 2016), thus fat mobilization would be minimal. Hence, the absence of differences in blood NEFA concentration among treatments is not surprising. However, the biological impetus for quadratic changes of blood glucose concentration with increasing SCFPns on d 28 and 56 is difficult to explain. The trend of greater blood NEFA concentration with ANT on d 28 was consistent with previous reports that the NEFA concentration increased from 0.13 to 0.20 m*M* in beef steers supplemented with 330 mg monensin and 110 mg tylosin·steer^{-1}·d^{-1} (Ran et al., 2018b). We suggest that the greater serum NEFA concentration in steers supplemented with ANT was caused by lower DMI due to monensin.

The BUN is a useful indicator to evaluate protein balance in cattle, with the value being driven by protein digestibility and requirement (Kohn et al., 2005). The protein requirement depends on age and growth rate as more protein is required when cattle grow faster. In the present study, the obvious greater BUN on d 105 compared to d 28 and 56 is likely a reflection of steers depositing more fat and less muscle with increasing days of finishing. The linear increase in BUN with increasing SCFPns on d 105 suggests increased intestinal absorption of AA. In fact, feeding SCFPns to heifers fed a high-grain diet increased protein digestibility in the total digestive tract without changing ruminal NH_3-N concentration or ruminal protein degradability (Shen et al., 2018). In addition, the greater BUN in cattle fed ANT than cattle fed the control diet may be indicative of reduced degradation of ruminal protein as a result of monensin, and an increase in feed protein digestion in the small intestine (Tedeschi et al., 2003). However, greater BUN with eXPC than control could result from either decreased or increased rumen protein degradability as we did not measure rumen NH_3–N concentration with eXPC.

In ruminants, both external and internal stimuli including inflammatory response, tissue injury, and infection can trigger systemic reaction and release acute phase proteins like SAA, Hp and LBP (Ceciliani et al., 2012; Tothova et al., 2014). Lipopolysaccharide (**LPS**) release increases from Gram-negative bacteria in cattle fed highly fermentable grain, and LPS is a strong pro-inflammatory agent that stimulates the acute phase response (Plaizier et al., 2012). Both LBP and SAA can bind LPS and play an important role in LPS clearance (Ceciliani et al., 2012). The linear or quadratic response in the concentration of LBP and SAA with increasing SCFPns suggests an improved immune response. Cattle fed high grain diets are also at greater risk of hindgut acidosis, where LPS is easier to translocate into blood circulation than through the rumen wall (Plaizier et al., 2012). The Hp is the principal scavenger of free hemoglobin, and

prevents oxidative damage (Ceciliani et al., 2012). However, greater blood Hp of steers administered the highest dose of SCFPns was not clear as blood hemoglobin concentration was unaffected (data not shown). Feeding eXPC did not result in differences in acute phase proteins compared with control or ANT, suggesting that eXPC did not elicit the same improvement in immunity as SCFPns. Blood acute phase proteins are also associated with elevated stress (Tothova et al., 2014). The greater concentration of SAA, Hp and LBP on day 0 in the present study demonstrated that the steers were likely under additional immunological stress at the start of the study.

2.5.3 Effects of SCFPns and eXPC on Fecal Microflora and Immune Status

Fecal pH could be affected by the amount of feed digested in the hindgut. The linear increase in fecal pH with increasing SCFPns on d 28 with no difference in fecal pH on d 56 and 105 is consistent with previous findings with short period feeding (28 d) (Shen et al., 2018). We found that feeding SCFPns to beef heifers resulted in greater ruminal and lower intestinal digestion of OM (Shen et al., 2018). The absence of an effect of SCFPns on fecal IgA is consistent with a previous study (Shen et al., 2018) where a trend for greater fecal IgA concentration was found when SCFPns was added directly to the duodenum but not when it was included in the feed. In contrast, lower fecal IgA concentration was observed when eXPC was fed. The IgA secreted by the gut plays a crucial role in mucosal defense, and fecal IgA concentrations can be used as an indicator of mucosal immunity (Suzuki et al., 2004). The decreased fecal IgA concentration with eXPC suggests a reduction in the immune response or immune suppression. It appeared that adding either SCFPns or eXPC potentially decreased fecal total *E. coli* counts on d 56 and 105, suggesting possible beneficial effects of SCFPns and eXPC

on the intestinal ecosystem. Although the reduction of *E. coil* counts was less than one log, it may still be biologically relevant.

2.5.4 Effects of SCFPns and eXPC on Antimicrobial Resistance

Enterococci are ubiquitous in nature and occupy a wide range of environments including soil, water and plants, as well as acting as commensals in animals and humans (Giraffa, 2002). Enterococci are widely used as 'indicator' bacteria to determine antimicrobial resistance (**AMR**) in food-producing animals. The fact that they are easy to culture, can be readily isolated from healthy animals, and exhibit resistance to most clinical antibiotics makes them useful as an indicator of antimicrobial resistance. Tetracycline is the most widely used antibiotic in both humans and animals. Although tylosin is only administered in food producing animals, it is a member of the macrolides, the same family as erythromycin which is widely used for clinical treatment of humans (Zaheer et al., 2013). Antimicrobial resistance to both erythromycin and tetracycline has been reported to be increasing worldwide (Inglis et al., 2006). In the present study, although the percentage of Ery^R and Ery^RTet^R enterococci were considerably lower (< 12%) on day 0, the Tet^R enterococci was quite high (50 - 70%), likely reflecting the widespread bacterial resistance to tetracycline in a number of environments. In the present study, the percentage of Tet^R enterococci appeared to unexpectedly decrease during the experimental period, across all treatments. The percentage of Ery^R and Ery^RTet^R enterococci was not affected by SCFPns or eXPC, whereas it was increased by adding ANT. In a previous study in Alberta (Beukers et al., 2015), the proportion of Ery^R enterococci (<10%) did not differ between the control or tylosin fed cattle upon arrival. However, with increasing days on feed the proportion of Ery^R enterococci significantly increased in steers administered tylosin in feed. Tylosin promotes erythromycin resistance as both antibiotics are macrolides and members of the MLS_B

(macrolide-lincosamides-streptogramin B) superfamily, and as a result resistance to one antibiotic in this group can confer resistance to all members (Portillo et al., 2000). The proportion of Ery^R, Tet^R and Ery^RTet^R enterococci in beef cattle feces has not been previously documented, although the identification of resistant gene determinants in related cattle studies has been extensively researched (Chen et al., 2008; Amachawadi et al., 2015; Beukers et al., 2015). Enterococci are known for their genetic 'promiscuity' due to their ability to acquire and transfer resistance genes (Werner et al., 2013). The macrolide and tetracycline resistance genes *erm*(B) and *tet*(M) are often linked, and evidence of these genes within individual fecal enterococci isolates has been described (Beukers et al., 2015). Although the cattle in our study were not administered tetracycline, co-selection for tetracycline resistance may have occurred as a linkage between *tet* and *erm* genes in enterococci isolated from ca the feces of tylosin-fed cattle has been identified (Chen et al., 2008). Therefore, administration of tylosin in-feed is not only problematic for selection for macrolide resistance, but also possibly for the co-selection of tetracycline resistance.

In conclusion, inclusion of SCFPns in a high grain diet tended to linearly improve feed efficiency. As with the inclusion of tylosin in the diet, it also tended to reduce the number of severely abscessed livers, possibly as a result of a reduction in ruminal acidosis. The impact of SCFPns or eXPC supplementation on blood glucose and NEFA appeared minor and inconclusive, whereas the increase in BUN with SCFPns or eXPC suggests increased intestinal absorption of amino acids. The increased blood acute phase protein concentrations in steers supplemented with SCFPns or eXPC suggests that adding SCFPns or eXPC in high-grain diets may have improved immune responses. The effect of SCFPns addition on fecal IgA was not apparent, whereas, the decreased fecal IgA concentration with eXPC suggests a reduction in the

need for an immune response. Supplementing monensin and tylosin potentially increased the proportion of enterococci resistance to both erythromycin and tetracycline. Whereas, feeding SCFPns (12 or 18 g/d), or eXPC did not alter the level of antimicrobial resistant enterococci in the feces of finishing cattle. The present results suggest that feeding SCFPns or eXPC to feedlot steers may exert benefits in feed efficiency and health comparable to antibiotics, without increasing the level of antimicrobial resistance within fecal microbial populations. Thus, SCFPns may be used as an alternative to in-feed antibiotics in beef production systems.

CHAPTER THREE: Investigation of the reduction in tylosin on liver abscesses and antimicrobial resistance in enterococci in feedlot cattle

This chapter has been submitted: Davedow, T., Narvaez-Bravo, C., Zaheer, R., Sanderson, H., Rodas-Gonzalez, A., Klima, C., Booker, C.W., Hannon, S.J., Bras, A.L., Gow, S., and McAllister, T. 2019. Investigation of the reduction in tylosin on liver abscesses and antimicrobial resistance in enterococci in feedlot cattle. Frontiers in Veterinary Science.

3.1 Abstract

Recent concerns over linkages between antimicrobial resistance in human pathogens and antimicrobial use in livestock have prompted to investigate the management strategies that can reduce the current reliance on in-feed antimicrobials such as tylosin that is frequently administered to reduce liver abscesses in feedlot cattle. A total of 7576 crossbred yearlings were allocated to the study (~ 253 animals/pen, 10 replicate pens per treatment) and individually randomized to one of three treatments. Tylosin phosphate (11 ppm) was included in-feed 1) for the first 125 days on feed (DOF) (FIRST-78%), 2) for DOF 41 to 161 (LAST-75%), or 3) for the entire feeding period (CON; day 0 to 161). Fecal composites were collected from the pen floor on days 0, 81, and 160 of the finishing period. Appropriate serial dilutions were spread plated for enumeration of enterococci on Bile Esculin Azide (BEA) agar and BEA amended with 8 μg/ml erythromycin. Results indicated that although the proportion of Ery^R enterococci increased with DOF ($P < 0.01$), neither treatment ($P = 0.34$) or treatment x DOF ($P = 0.37$) affected antimicrobial resistance. Of the 538 isolates, 97% were confirmed as enterococci, with an increase in *Enterococcus hirae* from 82 to 100% over the study period. Isolates were most frequently resistant to tylosin (86%), erythromycin (84%) and doxycycline (31%). Macrolide and tetracycline resistant isolates harbored *erm*(B), *msr*C and *tet*(L), *tet*(M), *tet*(O) genes, respectively. Overall, the proportion of Ery^R enterococci increased in all three treatments over the feeding period. Cattle administered tylosin for a shorter duration had more severe liver abscesses compared to the controls; but there was no difference in total liver abscesses, growth performance, carcass traits, morbidity or mortality. These results support the potential to reduce the duration and therefore quantity of tylosin administration in feedlot cattle without impacting animal productivity.

3.2 Introduction

Liver abscesses have a major economic impact on the North American beef cattle industry, with an average prevalence in feedlot cattle ranging from 12-32% (Brink et al., 1990) but incidences as high as 95% have been reported (Nagaraja and Lechtenberg, 2007). Cattle with severely abscessed livers can exhibit compromised growth performance as a result of reduced feed intake and carcass weight (Brown et al., 1975; Booker et al., 2015). In Canada, economic losses as a result of condemned and discounted livers are estimated at $60 million annually (Beef Cattle Research Council, 2018).

Antimicrobials are the primary tool used to prevent liver abscesses in cattle fed high-grain finishing diets. The macrolide, tylosin phosphate, is the most common antimicrobial included in feed to control liver abscesses in beef cattle in North America (Canadian Food Inspection Agency, 2019a), and is typically subtherapeutically administered in-feed to lower the prevalence of liver abscesses caused by *Fusobacterium necrophorum* and *Trueperella pyogenes* (Amachawadi and Nagaraja, 2016). However, despite tylosin use, the prevalence of liver abscesses in slaughter cattle often exceeds 15% (Beef Cattle Research Council, 2018).

The use of antimicrobials in-feed has come under scrutiny by both the public and regulators over concerns that their use selects for antimicrobial resistant bacteria that pose a risk to public health (Hoelzer et al., 2017). Tylosin belongs to the MLS_B superfamily (macrolide-lincosamide-streptogramin B) which are classified as a category II antimicrobial in terms of their importance for use in human medicine (Health Canada, 2009). Although tylosin is not used in human medicine, it cross-selects for resistance to other antimicrobials within this superfamily, including erythromycin, a macrolide widely used in humans (Roberts, 2008).

It is essential to evaluate new strategies to reduce liver abscesses in feedlot cattle while reducing industry reliance on medically important antimicrobials in livestock production. According to recently implemented restriction in the United States (US Food and Drug Administration, 2015) and Canada (Health Canada, 2018), all medically important antimicrobials require a veterinary prescription and cannot be used for growth promotion.

Enterococci are commensal bacteria of humans and animals that can cause serious hospital acquired infections (Giraffa, 2002). The most prevalent species associated with infections in humans are *E. faecium* and *E. faecalis* (Poh et al., 2006), whereas *E. hirae* is the predominant species in cattle (Anderson et al., 2008). Few studies have investigated the link between tylosin administration and antimicrobial resistance in enterococci. The most recent study in Canada, withdrew tylosin 28 days prior to slaughter in a small-scale (100 steers) trial and found a reduction in macrolide resistance in enterococci (Beukers et al., 2015). Another feedlot study in the United States investigated the impact of intermittent (1 week on, 2 weeks off) and continuous administration vs. no tylosin on erythromycin resistance (Ery^R) in enterococci and found no difference in the occurrence of liver abscesses between the intermittent and continuously fed tylosin treatments but a significantly higher ($P < 0.01$) occurrence of liver abscesses in the tylosin-free cattle. (Müller et al., 2018). As such, it is important to continue to investigate ways to optimize tylosin use while promoting antimicrobial stewardship, supporting productivity, and working to minimize use of antimicrobials in livestock that are of importance in human medicine.

The present study investigated and compared the effect of tylosin administration in the first 78 or last 75 percent of the feeding period on antimicrobial resistance, liver abscess score, animal health, feedlot performance, and carcass traits of feedlot cattle.

3.3 Materials and Methods

All procedures involving cattle were reviewed and approved by the Feedlot Health Management Services Ltd (Okotoks, Alberta) and Lethbridge Research Centre Animal Care Committees in accordance with guidelines of the Canadian Council on Animal Care (2009). Informed consent for use of the cattle was received from the owners of the cattle.

3.3.1 Experimental Design

This study was conducted at a large commercial feedlot in southern Alberta over an average 161-day finishing period. Cattle (n = 7576) for this study were crossbred beef yearling steers and heifers (394 ± 5.49 kg body weight) that arrived between June 11, 2018 and July 7, 2018. Upon arrival, cattle were randomly assigned to one of three treatments; **FIRST-78%**, **LAST-75%**, or **CON**. The experimental unit was the pen, with 10 pens (6 steer, 4 heifer) allocated to each treatment. Average pen capacity was 253 head/pen. Upon arrival, feedlot cattle were randomly assigned to one of the three treatments and placed into a corresponding pen. Once a pen was full then newly arriving cattle assigned to that treatment were allocated to a new pen for a second replicate of that treatment with this process continuing until all 10 pens per treatment were full.

Cattle were fed tylosin phosphate (Tylosin 40, Bio Agri Mix LP, Mitchell, Ontario) at an inclusion level of 11 ppm (100% dry matter basis [DM]) for: 1) the first 125 days of the 161-day feeding period (**FIRST-78%**), 2) the last 120 days of the feeding period (**LAST-75%)**, starting at an average of 41 days on feed (DOF) and continuing to slaughter at an average of 161 DOF or 3) continuously throughout the 161-day feeding period (**CON**). Tylosin was administered in the diet at 11 ppm, the concentration approved for the prevention of liver abscesses in beef cattle (Canadian Food Inspection Agency, 2019a).

Upon arrival, individual animals were managed as per standardized commercial Canadian feedlot practices, receiving an ear tag for identification, a growth promoter implant (Revalor®-XS, Merck Animal Health, Intervet Canada Corp, Kirkland, Québec), a subcutaneous modified live virus vaccine (Pyramid 2 + Type II BVD®, Boehringer Ingelheim Ltd, Burlington, Ontario), a multivalent clostridial bacterin-toxoid (Ultrachoice®8, Zoetis Canada, Kirkland, Québec), and a pour-on endectocide (Noromectin® Pour-On, Norbrook Laboratories Ltd, Newry, Co. Down, Northern Ireland). Heifers received an intramuscular cloprostenol injection (Estrumate®; Merck Animal Health) and intramuscular dexamethasone injection (Dexamethasone 5; Vetoquinol N.-A. Inc, Lavaltrie, Québec) for pregnancy termination. No antimicrobials were administered to the cattle upon arrival.

All diets were fed twice daily, and cattle were offered *ad libitum* access to feed and water. Cattle were gradually transitioned to a high-concentrate finishing diet (dry matter basis) consisting of 85.8% concentrate, 11.5% roughage and 2.8% supplement. The concentrate portion consisted of 70% corn with the remainder being temper rolled barley or wheat. Monensin sodium was also included in diets at 33 ppm over the feeding period (Monensin Premix; Bio-Agri Mix LP, Mitchell, Ontario) according to the medicating ingredient brochure (Canadian Food Inspection Agency, 2019a).

3.3.2 Sample Collection and Processing

Composite, fresh, pen-floor fecal samples from 20 different pats were collected from each pen using a standardized pen sampling plan. Samples were collected at allocation (0 DOF) before any tylosin was administered, in the middle of the feeding period (avg. 81 DOF), and just prior to shipment for slaughter (avg. 160 DOF). Samples were collected in sterile Whirl Pak bags and stored at 4°C for an average of one day prior to transport to the Agriculture and Agri-Food

Canada Lethbridge Research Center, Lethbridge, Alberta for microbial analysis. Samples were processed within one day of arrival at Lethbridge.

At the lab, each fecal sample was thoroughly mixed, weighed (1.0 g) and diluted 1:5 into 4.0 mL of sterile phosphate buffered saline and vortexed for 30 sec. Samples were then 10-fold serially diluted and 100 µL of the appropriate dilution were plated in duplicate onto Bile Esculin Azide (BEA) agar containing no antimicrobials and BEA amended with erythromycin (8 µL/mL; BEA^E). The concentration of erythromycin added into the BEA plates was set at the breakpoint standards for defining resistance as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines (2016). After incubation for 48 h at 37°C, colonies that exhibited esculin hydrolysis (black precipitate) and morphology typical of enterococci were enumerated. Isolates that grew on BEA^E were considered resistant to erythromycin. The percentage of enterococci resistant to erythromycin was calculated according to (Alexander et al., 2009), in which: [(number of colonies on selective BEA^E plates / total colonies on nonselective BEA plates) x 100%].

For each sample, three enterococci colonies from each BEA and BEA^E plates (6 colonies in total) were subcultured onto their respective media and incubated for 48 h at 37°C, for purification and further characterization. To prepare template DNA for PCR one colony from each plate was suspended in 100 µL of TE (10 mM Tris, 1 mM EDTA, pH 8.0) and heat lysed for 5 min at 98°C with shaking at 1000 RPM in an Eppendorf thermomixer (VWR, Missisauga, ON, Canada). Heat lysed cell suspensions were stored at -80°C for later use. Growth from subcultures was suspended in brain heart infusion (BHI) broth containing 15% glycerol and archived at -80 °C for subsequent use.

3.3.3 Characterization of *Enterococcus* species

A total of 538 presumptive enterococci isolates representing approximately six isolates from each pen on each sampling day were saved in TE as mentioned above. Tubes containing heat lysed cells were thawed and centrifuged at 10,000 x g for 5 min. The supernatant was used as template DNA in a multiplex PCR to identify *Enterococcus* species using *groES-EL* (Ent-ES-211-233-F, Ent-EL-74-95-R) (Zaheer et al., 2012) and *mur*-2 (muramidase) primers to distinguish *Enterococcus hirae* (Arias et al., 2006) from other *Enterococcus* spp. (Zaheer et al., 2013; Beukers et al., 2015). A commercially available Multiplex Master Mix Kit (25 µL, Qiagen Canada, Inc., Mississauga, ON, Canada) was used for the PCR reaction with 2 µL of template DNA and thermocycler conditions of: 5 min at 95ºC, followed by 45 cycles of 30 s at 94ºC, 30 s at 49ºC, 30 s at 72 °C and a final extension for 10 min at 72ºC. The PCR products were resolved on a 1.8% agarose gel. Isolates that were *groES-EL* positive, but *mur*-2 negative were sent for sequencing of the *groES-EL* intergenic region PCR product to identify enterococci species.

3.3.4 Antimicrobial Susceptibility Testing

A subset of 176 characterized isolates were randomly chosen to represent one isolate from each media type and from all samples, with the exception of four discarded samples from the BEA plates that were characterized as non-*Enterococcus* species. Antimicrobial susceptibility testing for enterococci was performed against 12 antibiotics using disc diffusion methodology according to the CLSI guidelines, documents M02-A12, M100-S26, and VET-01S (Clinical and Laboratory Standards Institute, 2015b, a, 2016). The antimicrobial panel, supplier, disk content and zone diameter for determining break points are listed in Table 3.1. *Staphylococcus aureus* ATCC® 25923 and *Enterococcus faecalis* ATCC® 29212 were used as standards and were included in each panel. Zone diameters were read using the BioMic V3

imaging system (Giles Scientific, Inc., Santa Barbara, CA, USA), and each enterococci isolate was classified as either susceptible, intermediate or resistant according to CLSI guidelines for 10 antimicrobials, or EUCAST for tigecycline (The European Committee on Antimicrobial Susceptibility Testing, 2019). Tylosin does not have an established interpretive criteria for *Enterococcus* spp., by CLSI or EUCAST, there is an acceptable quality control range for 30 µg tylosin discs for *S. aureus* ATCC® 25923 set at 18-26 mm (Clinical and Laboratory Standards Institute, 2015a). For tylosin, previously published minimum inhibitory concentration (MIC) established at our lab (Beukers et al., 2015) were used as breakpoints in the current study. Isolates that were resistant to three or more antimicrobials were defined as being multidrug resistant.

Table 3. 1. Antimicrobials and zone diameters used for disk susceptibility testing.

Antimicrobial	Supplier	Disk content (µg)	Zone diameter (mm) breakpoints[e]		
			S	***I***	***R***
Ampicillin[a]	BD	10	≥17	n/a	≤16
Doxycycline[a]	BD	30	≥16	13–15	≤12
Erythromycin[a]	BD	15	≥23	14–22	≤13
Gentamicin[a]	BD	120	≥10	7–9	6
Levofloxacin[a]	BD	5	≥17	14–16	≤13
Linezolid[a]	BD	30	≥23	21–22	≤20
Nitrofurantoin[a]	BD	300	≥17	15–16	≤14
Quinupristin-dalfopristin[a]	BD	4.5/10.5	≥19	16–18	≤15
Streptomycin[a]	BD	300	≥10	7–9	6
Tigecycline[a]	Oxoid	15	≥18	n/a	<18
Tylosin[c]	Mast Group	30	≥18	n/a	≤12
Vancomycin[b,d]	BD	30	≥17	15–16	≤14

[a]M100-S26: Performance standards for antimicrobial susceptibility testing (CLSI, 2016).
[b]Breakpoint tables for interpretation of MICs and zone diameters (EUCAST, 2019).
[c](Beukers et al., 2015).
[d]Vancomycin required 24h of incubation, while the remaining antimicrobials were assessed after 18h.
[e]Zone diameters (mm) are interpreted to indicate: S = susceptible, I = intermediate, R = resistant, n/a = not available

3.3.5 Resistant Gene Determinants

The isolates displaying intermediate resistance or resistance to erythromycin or tylosin were screened by PCR for macrolide resistance genes *erm*(B), and *msr*C (Portillo et al., 2000), using the primers of Chen et al. (2007) and Beukers et al. (2015), respectively. Reactions were processed as a multiplex PCR with an initial denaturation for 5 min at 95ºC, followed by 35 cycles of denaturation for 30 s at 94ºC, annealing for 30 s at 58ºC and a final extension for 10 min at 72ºC. Isolates displaying intermediate resistance or resistance to doxycycline were also screened by PCR for *tet*(L), *tet*(M), and *tet*(O) as previously described (Ng et al., 2001). All PCRs were prepared as a 20 µL reaction with 2 µL DNA template and resolved on a 1.5%

agarose gel. Conventional PCR was performed using HotStarTaq Master Mix Kit, and multiplex reactions using the Mulitplex Master Mix Kit (Qiagen Canada, Inc., Mississauga, ON, Canada).

3.3.6 Animal Performance, Liver Abscesses and Carcass Traits

Upon allocation, initial body weight (BW) and hip height were measured as baseline variables for each individual animal to assess homogeneity in each treatment. Animal performance variables (final BW; daily dry matter intake, DDMI; average daily gain, ADG; feed-to-gain ratio, F:G) were calculated for each pen to describe feedlot performance. Final BW represents the average net (with account for gut fill) live weight of cattle sold for slaughter. The DDMI was calculated by the total quantity of feed consumed divided by the number days in the trial and animals within a pen. The ADG was determined by the total net slaughter weight plus total weight of cattle shipped for salvage slaughter plus total weight of animals that died minus total allocation weight; divided by the number of days in the trial. Feed efficiency, (F:G) was determined as DDMI divided by ADG on a live weight basis. Cattle were monitored twice daily by animal health personnel for evidence of disease. Individual cattle that were deemed "sick" were separated out of the pen and moved to a hospital facility for diagnosis and treatment. The overall mortality was defined as the number of mortalities divided by the number of animals allocated.

All animals from this study were slaughtered at a single processing plant. All cattle from assigned pens were shipped for slaughter based on reaching finish as assessed by standard feedlot production practices. At slaughter, all livers were scored for severity and prevalence of liver abscesses by trained personnel, using a modified Elanco Liver Check System (Elanco, Greenfield, IN, USA). Livers that had no abscesses (normal healthy liver) were assigned a liver score of 0. Livers with one or two small active abscesses/scars or up to four well organized

abscesses with a diameter of less than 2.5 cm were assigned a liver score of A. Livers with one or more large abscesses (diameter > 2.5 cm) or more than four small/old abscesses of a diameter < 2.5 cm were assigned a liver score of A+ (severe).

Canadian quality grade (QG), yield grade (YG), and weight of each carcass were collected using the data capture system in place at a single processing plant. The average carcass weight was determined by the total carcass weight at slaughter divided by the number of cattle sold for slaughter. The dressing percentage was calculated by the total carcass weight at slaughter divided by the total weight at slaughter expressed as a percentage.

3.3.7 Statistical Analysis

Data were analyzed using SAS® for Windows, Release 9.4 (SAS Institute Inc, Cary, North Carolina). Prior to analysis, microbial enumeration data were normalized through a log10 transformation and analyzed using the MIXED procedure of SAS with a completely randomized design with factorial arrangements and repeated measures. The treatments (**FIRST-78%**, **LAST-75%**, **CON**) and sampling days (0, 81, 160) and their interaction were analysed as fixed effects with replicates as the random effect.

Risk ratios were used to determine the risk of the exposed group (**FIRST-78%**, **LAST-75%**) to having severely abscessed livers compared to the group that was continuously administered tylosin (**CON**).

The baseline (initial BW and hip height), feedlot performance, and carcass trait variables were analyzed using GLIMMIX in SAS. Baseline variables were tested as covariates of the feedlot performance variables and included in the model if statistically significant. Sex (steers or heifers) was included as a fixed effect in the model for feedlot performance. Morbidity and

mortality data were analyzed using the GENMOD procedure in SAS with Poisson regression in a log linear model for treatment effects and adjusted for clustering of disease (pen nested within replicate) with generalized estimating equations. For all tests, level of significance was set at $P < 0.05$.

3.4 Results

3.4.1 CFU Counts of Enterococci and Proportion of Erythromycin Resistance

Enterococci were isolated from fecal composite samples from all 30 pens on all sampling days with the exception of four pens on day 81, where selected colonies were not enterococci. No difference ($P > 0.05$) was observed between **FIRST-78%**, **LAST-75%**, and **CON** cattle with regard to total enterococci, Ery^R enterococci or Ery^R enterococci within the total enterococci population (Table 3.2). However, there was a decrease ($P < 0.01$) in total enterococci with increasing days on feed. The proportion of Ery^R was highest on day 81 ($P < 0.01$) for all treatments (Figure 3.1). Compared to arrival, Ery^R enterococci isolated just prior to slaughter increased by 52, 187, and 89% ($P < 0.01$) in the **FIRST-78%**, **LAST-75%**, and **CON**, respectively.

Table 3. 2. Enterococci counts of the total population and Ery^R enterococci isolated from feedlot cattle feces from cattle fed tylosin for **FIRST-78%**, **LAST-75%**, or continuously (**CON**) during the feeding period.

Item[1]	Treatments[2]			SEM	*P* – value[3]		
	FIRST-78	LAST-75	CON		T	D	T x D
No. of Enterococci (log_{10} CFU/g feces)							
Day 0	6.0	6.5	6.2				
Day 81	5.2	5.7	5.9	0.24	0.14	< 0.01	0.12
Day 160	5.3	5.3	5.3				
No. of Ery^R Enterococci (log_{10} CFU/g feces)							
Day 0	4.4	4.5	4.9				
Day 81	5.0	5.4	5.4	0.41	0.18	0.02	0.98
Day 160	4.5	4.7	4.9				

[1] Cattle were sampled upon arrival and after 81 and 160 d on feed. Total enterococci were enumerated on BEA = bile esculin azide agar; and erythromycin resistant (Ery^R) enterococci were enumerated on BEA^E amended with erythromycin (8 μg/ml).
[2] Tylosin inclusion at 11 ppm; FIRST-78% = tylosin in-feed from d 0 to d 125; LAST-75% = tylosin in-feed from d 41 to d 161; CON = control, continuous feeding of tylosin (d 0 to d 161).
[3] Treatment, T; Days on feed, D; Treatment X Days on feed, T x D.

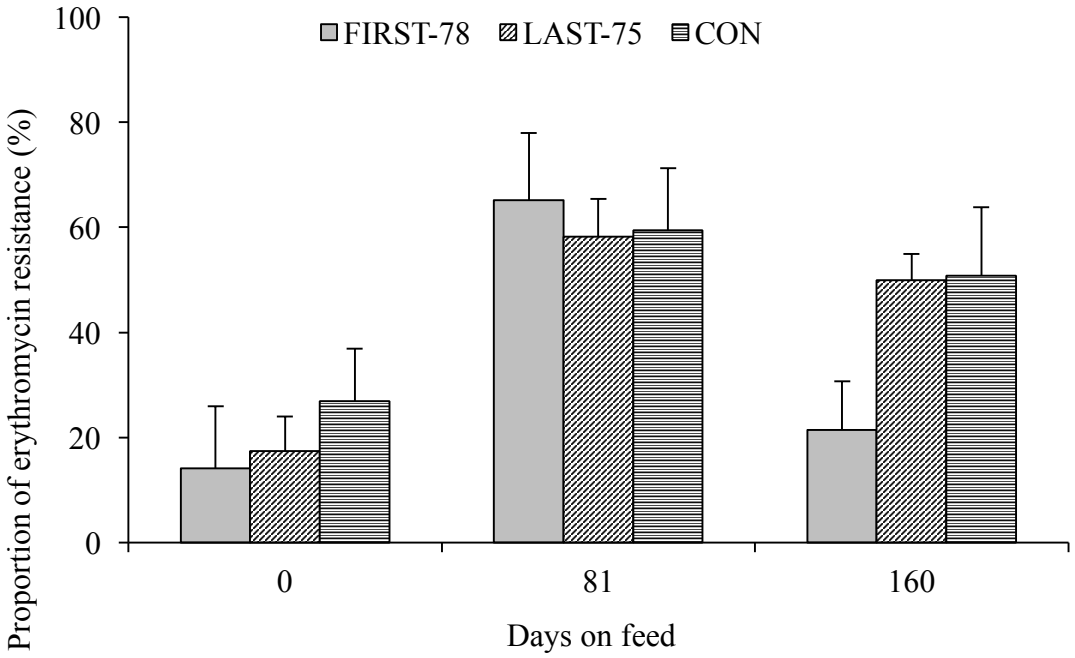

Figure 3. 1. Proportion of erythromycin resistant fecal enterococci isolates from the feces (sampled 0, 81, 160 d) of feedlot cattle administered tylosin in the **FIRST-78%**, **LAST-75%**, or continuously **(CON)** during a 161 d feeding period. Treatment, $P = 0.34$; Treatment X Days on feed, $P = 0.37$; Days on feed, $P < 0.01$.

3.4.2 Characterization of Enterococci

Of the 538 isolates collected throughout the trial, 97% were confirmed as enterococci by PCR. Speciation of 522 enterococci isolates revealed that 93.9% were *E. hirae* (n = 490), 3.3% were *E. villorum* (n = 17), 2.5% were *E. faecium* (n = 13), and 0.4% were *E. durans* (n = 2) (Figure 2). Out of the 32 non-*hirae* enterococci isolated, 41% (n = 13) were collected from non-selective BEA, whereas 59% (n = 19) were isolated from selective BEA^{E}. The diversity of enterococci tended to be greater at arrival than at later sampling times during the feeding period.

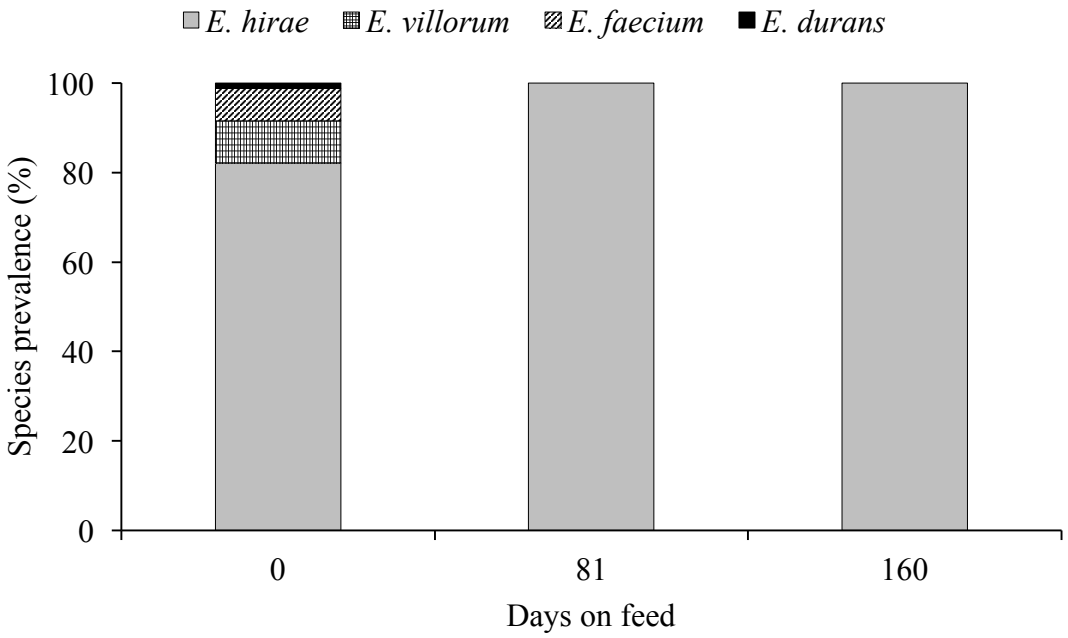

Figure 3. 2. *Enterococcus* species distribution of characterized isolates from feedlot cattle feces upon arrival (d 0), in the middle (d 81), and at the end (d 160) of feeding period. Isolates are pooled across all pens, treatments and media type.

3.4.3 Antimicrobial Susceptibility Testing

A total of 86% (n = 151), 84% (n = 147), and 31% (n = 54) of isolates displayed intermediate resistance or resistance to tylosin, erythromycin and doxycycline, respectively (Table 3.3). Of the 153 isolates that were not susceptible to erythromycin and/or tylosin, 95% (n

= 145) displayed either intermediate resistance or resistance to both erythromycin and tylosin. In total, 16 antibiogram phenotypes were observed, ranging from no resistance (A1) to resistance to six antimicrobials (A16) (Table 3.3). No isolates displayed intermediate resistance or resistance to ampicillin, gentamicin, levofloxacin, or vancomycin; but at least one isolate was resistant to all of the other antimicrobials tested. The three most common antimicrobial resistance phenotypes across all treatments and days were A1 (No resistance), A5 (ERY-TYL), and A7(dox-ERY-TYL), representing 82% of all observed susceptibility patterns. Multidrug resistance (≥ 3 antimicrobials) occurred in 9.7% (n = 17) of isolates, and did not appear to be influenced by treatment.

Table 3. 3. Antibiograms of enterococci (n=176) isolated from feedlot cattle feces from cattle fed tylosin for the **FIRST-78%**, **LAST-75%**, or continuously (**CON**) during the feeing period.

Profile	Phenotype[3]	No. isolates (%) within treatments and days[1,2]									Total
		FIRST-78			LAST-75			CON			
		d 0 (n=20)	d 81 (n=20)	d 160 (n=20)	d 0 (n=20)	d 81 (n=18)	d 160 (n=20)	d 0 (n=20)	d 81 (n=18)	d 160 (n=20)	
A1	No Resistance	6 (30.0)		2 (10.0)	6 (30.0)	1 (5.6)		5 (25.0)	1 (5.6)		21
A2	NIT			1 (5.0)							1
A3	tyl						1 (5.0)		1 (5.6)	1 (5.0)	3
A4	ery-nit	1 (5.0)									1
A5	ERY-TYL	7 (35.0)	16 (80.0)	4 (20.0)	7 (35.0)	7 (38.9)	10 (50.0)	7 (35.0)	12 (66.7)	11 (55.0)	81
A6	nit-tyl						1 (5.0)	1 (5.0)			2
A7	dox-ERY-TYL	2 (10.0)	3 (15.0)	9 (45.0)	1 (5.0)	10 (55.6)	5 (25.0)	3 (15.0)	4 (22.2)	5 (25.0)	42
A8	ery-lin-NIT				1 (5.0)						1
A9	ERY-nit-TYL		1 (5.0)	1 (5.0)	1 (5.0)		1 (5.0)	1 (5.0)			5
A10	ERY-q-d-TYL	1 (5.0)		2 (10.0)			1 (5.0)				4
A11	ERY-str-TYL									1 (5.0)	1
A12	lin-NIT-TYL	1 (5.0)									1
A13	DOX-ERY-NIT-TYL	2 (10.0)		1 (5.0)	2 (10.0)		1 (5.0)	2 (10.0)		2 (10.0)	10
A14	ery-NIT-TIG-tyl							1 (5.0)			1
A15	dox-ERY-NIT-q-d-TYL				1 (5.0)						1
A16	dox-ery-lin-NIT-TIG-TYL				1 (5.0)						1

[1] Enterococci were isolated from BEA and BEA[E] media.

[2] Tylosin inclusion at 11 ppm; FIRST-78% = tylosin in-feed from d 0 to d 125; LAST-75% = tylosin in-feed from d 41 to d 161; CON = control, continuous feeding of tylosin (d 0 to d 161). Fecal samples were collected on d 0, d 81 and d 160.
[3] Doxycycline, DOX; Erythromycin, ERY; Linezolid, LIN; Nitrofurantoin, NIT; Quinupristin-dalfopristin, Q-D; Streptomycin, STR; Tigecycline, TIG; Tylosin, TYL. Upper case denotes complete resistance and lower case denotes intermediate resistance.

Table 3. 4. Distribution of enterococci isolates from feedlot cattle feces grouped according to macrolide (n = 153) and tetracycline (n= 54) resistance genes and by cattle fed tylosin for the **FIRST-78%**, **LAST-75%**, or continuously (**CON**) during the feeding period.

Treatment[1]	No. Positive (%)[2]								
	Macrolide				Tetracycline				
	n	*erm*(B)	*msr*C	Negative	n	*tet*(L)	*tet*(M)	*tet*(O)	Negative
FIRST-78	51	49 (96.1)	1 (2.0)	2 (3.9)	17	13 (61.9)	13 (61.9)	4 (19.0)	0 (0)
LAST-75	51	48 (94.1)	3 (5.9)	3 (5.9)	21	18 (85.7)	19 (90.5)	2 (9.5)	0 (0)
CON	51	47 (92.2)	2 (3.9)	4 (7.8)	16	10 (62.5)	10 (62.5)	5 (31.3)	1 (6.3)
Total	153	144 (94.1)	6 (3.9)	9 (5.9)	54	41 (75.9)	42 (77.8)	11 (20.4)	1 (6.3)

[1] Tylosin inclusion at 11 ppm; FIRST-78% = tylosin in-feed from d 0 to d 125; LAST-75% = tylosin in-feed from d 41 to d 161; CON = control, continuous feeding of tylosin (d 0 to d 161).
[2] Isolates pooled across all media types and sampling days.

3.4.4 Identification of Resistant Gene Determinants

Of the 153 enterococci isolates displaying intermediate resistance (n_{Ery} = 8; n_{Tyl} =7) or resistance (n_{Ery} = 139; n_{Tyl} = 144) to erythromycin or tylosin, the *erm*(B) gene was detected in 144 (Table 3.4) with representatives of *E. hirae*, *E. faecium*, and *E. villorum*. Within these isolates, six [*E. hirae* (n =1), and *E. faecium* (n = 5)] collected on day 0 were also positive for *msr*C. Nine isolates from BEA displayed intermediate resistance to either erythromycin or tylosin, but were negative for both macrolide resistance genes.

Within the 153 isolates screened for macrolide resistance genes, 39 displayed intermediate resistance and 15 were resistant to doxycycline. These isolates were further screened for tetracycline resistance genes, with 41positive for both *tet*(M) and *tet*(L), and one positive for *tet*(M). Eleven isolates were positive for *tet*(O), with only one intermediate doxycycline resistant isolate being negative for all *tet* genes.

3.4.5 Liver Abscesses, Animal Performance and Carcass Traits

Although the risk of having severe liver abscesses (A+) if fed tylosin for the **FIRST-78%** or **LAST-75%** was greater than **CON** (Table 3.5). The risk of having liver abscesses overall (A and A+) was similar among treatments.

Table 3. 5. Liver abscesses of feedlot cattle from cattle fed tylosin for the **FIRST-78%**, **LAST-75%**, or continuously (**CON**) during the feeding period.

	Liver Abscesses (%)[2]				Risk Ratio[3]	
Treatment[1]	Abscessed	Severe	None	Total	Abscessed	Severe
FIRST-78	61.00	23.47	39.00	100.00	0.99	1.18
LAST-75	64.15	22.95	35.85	100.00	1.04	1.16
CON	61.88	19.83	38.12	100.00	1.00	1.00

[1] Tylosin inclusion at 11 ppm; FIRST-78% = tylosin in-feed from d 0 to d 125; LAST-75% = tylosin in-feed from d 41 to d 161; CON = control, continuous feeding of tylosin (d 0 to d 161).
[2] Severely abscessed (A+), total liver abscesses (A and A+), and no liver abscesses (none = 0).
[3] Reference group = CON; Comparison groups = FIRST-78 and LAST-75

There were no significant differences detected between the **FIRST-78%** or **LAST-75%** and the **CON** for any of the morbidity or mortality outcomes (Table 3.6). The incidence of morbidity was less than 3% and the overall mortality rate ranged from 0.9 to 1.4 % for all treatments.

Table 3. 6. Morbidity and mortality outcomes of feedlot cattle from cattle fed tylosin for the **FIRST-78%**, **LAST-75%**, or continuously (**CON**) during the feeding period.

Item[1]	Treatments[2]			*P* – values	
	FIRST-78	LAST-75	CON	FIRST-78% vs CON	LAST-75% vs CON
Morbidity					
Initial BRD Treatment (%)	2.7	2.5	2.2	0.33	0.44
Initial Bloat Treatment (%)	0.3	0.2	0.2	0.71	0.74
Initial Lameness Treatment (%)	1.1	1.0	1.6	0.57	0.44
Initial Other Treatment (%)	0.6	0.7	1.1	0.16	0.29
Chronicity (%)	0.4	0.2	0.3	0.79	0.25
Wastage (%)	0.2	0.1	0.2	0.74	0.28
Mortality					
Overall Mortality (%)	1.4	0.9	1.3	0.66	0.19
BRD Mortality (%)	0.2	0.1	0.1	0.34	1.00
Lameness Mortality (%)	0.1	0.0	0.0	n/a	n/a[3]
Metabolic Mortality (%)	0.6	0.4	0.7	0.57	0.20
Other Mortality (%)	0.6	0.4	0.5	0.67	0.53

[1] BRD = bovine respiratory disease. Lameness treatment includes arthritis, foot rot, papillomatous digital dermatitis, and lameness. Other treatment includes rider, musculoskeletal injury, rectal or vaginal prolapses, diphtheria. Other mortality includes mortality of animals with lesions consistent with *Histophilus somni* infection (n=1), and mortality due to causes other.
[2] Tylosin inclusion at 11 ppm; FIRST-78% = tylosin in-feed from d 0 to d 125; LAST-75% = tylosin in-feed from d 41 to d 161; CON = control, continuous feeding of tylosin (d 0 to d 161).
[3] n/a = not available - model would not properly converge because of the small number of events.

The treatments were homogenous ($P \geq 0.05$) at allocation with respect to average initial weight (kg) and average hip height (m) (Table 3.7). Growth performance of feedlot cattle did not differ ($P > 0.05$) between the **FIRST-78%** and **CON** or **LAST-75%** and **CON** for ADG or F:G (Table 3.7). Carcass weight was greater (absolute difference of 3.3 kg; $P = 0.04$) for cattle in the **FIRST-78%** compared to **CON** (Table 3.7). There was no difference detected between the **FIRST-78%** or **LAST-75%** and **CON** for dressing percentage (Table 3.7). With respect to yield grade or quality grade outcomes, there were no differences among treatments (Table 3.7).

Table 3. 7. Growth performance and carcass traits of feedlot cattle for cattle fed tylosin for the **FIRST-78%**, **LAST-75%**, or continuously (**CON**) during the feeding period.

Item	Treatments[1]				*P* – values	
	FIRST-78	LAST-75	CON	SEM	FIRST-78 vs CON	LAST-75 vs CON
No. of Cattle	2525	2526	2525			
Growth[2]						
Initial Hip Height (m)	1.2	1.2	1.2	0.01	0.51	0.39
Initial BW (kg)	393.5	395.2	393.6	5.49	0.99	0.22
Final BW (kg)	681.0	680.0	677.5	9.25	0.25	0.40
DMI (kg/d)	11.9	11.9	11.8	0.14	0.80	0.22
ADG (kg/d)	1.8	1.8	1.7	0.03	0.25	0.69
F:G	6.7	6.8	6.8	0.07	0.23	0.70
Carcass Traits						
Carcass Weight (kg)	410.2	408.1	406.9	5.72	0.04	0.45
Dress Percentage (%)	60.2	60.0	60.1	0.1	0.20	0.61
Yield Grade						
Canada 1 (%)	21.9	21.6	20.9	3.91	0.74	0.82
Canada 2 (%)	35.9	39.2	39.0	2.11	0.11	0.92
Canada 3 (%)	42.2	39.3	40.2	5.35	0.55	0.80
Quality Grades						
Canada Prime (%)	1.0	0.8	1.2	0.25	0.62	0.25
Canada AAA (%)	69.2	64.4	66.7	2.69	0.31	0.35
Canada AA (%)	25.8	30.4	27.3	2.72	0.48	0.16
Canada A (%)	0.6	0.8	1.0	0.24	0.09	0.43
B4 (%)	3.3	3.4	3.6	1.33	0.75	0.80
Other (%)[3]	0.1	0.2	0.2	0.11	0.59	0.62

[1] Tylosin inclusion at 11 ppm; FIRST-78% = tylosin in-feed from d 0 to d 125; LAST-75% = tylosin in-feed from d 41 to d 161; CON = control, continuous feeding of tylosin (d 0 to d 161).
[2] DMI = dry matter intake; ADG = average daily gain; F:G = feed-to-gain ratio, calculated as DMI divided by ADG (live weight basis).
[3] Canada quality grades B2, B3, D2, D3, and E were combined into "Other" off grades category.

3.5 Discussion

For the purpose of this study, enterococci were chosen as the fecal indicator bacteria for assessing macrolide resistance as the most commonly employed indicator bacterium, *Escherichia coli,* is intrinsically resistant to this antimicrobial family (Mao and Putterman, 1968). Enterococci, notably *E. faecalis* and *E. faecium* are seen with increasing prevalence in clinical infections in humans (Poh et al., 2006). In the present study, *E. faecalis* was not detected, and *E. faecium* was only isolated from cattle upon arrival. Consistent with previous reports (Zaheer et al., 2013; Beukers et al., 2015; Tymensen et al., 2017), there was a decrease in the diversity of enterococci over the feeding period, with *E. hirae* being the predominant species isolated from beef cattle feces, a species not frequently associated with infections in humans (Chan et al., 2011). Beukers et al. (2015) proposed that this shift in fecal enterococci species may arise from the transition of cattle from a forage-based to a grain-based finishing diet during the finishing period. Others have proposed that it may also be influenced by age of the host (Devriese et al., 1992; Shanks et al., 2011). In the present study, cattle were transitioned from a high (40%) to low (11.5%) forage diet over the first 20 days of the feeding period. Therefore, cattle pens sampled upon allocation had less concentrate in their diets compared to those sampled on days 81 and 160 when the high concentrate diet was being fed.

Tylosin was administered to cattle at the concentration approved for the prevention of liver abscesses (Canadian Food Inspection Agency, 2019a). Since this study revolved around the feeding regime of tylosin, the main focus was on Ery^R enterococci isolated from beef cattle feces. Antimicrobial susceptibility testing of enterococci indicated that all isolates initially collected from the selective BEA^E were resistant to erythromycin.

Due to the large number of animals enrolled in this study, and the importance of tylosin in liver abscess prevention (Nagaraja and Chengappa, 1998; Meyer et al., 2009) it was not possible for our industry partner to bare the possible financial burden of having a negative control group of cattle that did not receive tylosin. Several studies have shown that in-feed tylosin increases Ery^R enterococci in cattle as compared to those that do not receive this antimicrobial (Jacob et al., 2008; Zaheer et al., 2013; Beukers et al., 2015), an observation that aligned with our findings.

The amount of Ery^R enterococci did not differ among treatments at any of the three sampling days. However, between the time of allocation and mid-sampling the proportion of Ery^R enterococci increased, and then decreased by the end of the feeding period, an observation that coincides with Beukers et al. (2015). In a smaller scale study, Beukers et al. (2015) compared macrolide resistance in fecal enterococci in cattle fed tylosin for the first 197 days and after withdrawal 28 days prior to slaughter. They observed a reduction in macrolide resistance, just prior to and after the withdrawal of tylosin. Müller et al. (2018) explored the intermittent use (1 week on, 2 weeks off) of tylosin compared to continuous or no tylosin and found no difference in Ery^R enterococci between tylosin treatment at each time point, but did record a significantly higher percentage of Ery^R enterococci with increasing days on feed between day 20 and day 118. The beneficial effect of reducing tylosin in-feed is difficult to predict because antimicrobial resistant bacteria are present in nearly all environments (Vikram et al., 2017). However, shortening the duration of tylosin administration could help reduce the selection pressure that exacerbates the occurrence of antimicrobial resistance (Beukers et al., 2015). In relation to the present study, to realize the impact of the removal of tylosin on the reduction in macrolide resistance, a much longer than 25% of the tylosin-free feeding period may be required.

Cattle feces are a natural vector for the transmission of bacteria and their antimicrobial resistant genes into the environment (Durso et al., 2011). Enterococci are known as antimicrobial resistance gene traffickers because they can readily transfer and acquire antimicrobial resistance genes (Werner et al., 2013). Enterococci have emerged as a major public health concern, especially vancomycin resistant *E. faecalis* and *E. faecium* which are more difficult to treat (Public Health Agency of Canada, 2018). Of the 176 isolates screened for antimicrobial resistance, all were susceptible to vancomycin, consistent with previous studies indicating that cattle feces do not represent a major source of vancomycin-resistant enterococci (Beukers et al., 2015; Ngbede et al., 2017). In the present study, resistance to tylosin, erythromycin and doxycycline was most prevalent among isolated enterococci. It has been proposed that the administration of tylosin may co-select for enterococci with resistance to tetracycline, even in the absence of tetracycline use (Amachawadi et al., 2015). Müller et al. (2018) reported increased proportion of Tet^R enterococci in cattle feces with increasing days on feed, but found no relationship between Tet^R occurrence and the administration of tylosin in feed. Although tetracycline was absent in the diet, Müller et al. (2018) observed an initially high proportion of Tet^R enterococci in cattle feces at approximately 10% on day 0, with increases between day 20 (~ 20%) and day 118 (~ 40%). These results coincide with the present study, where initially high number of enterococci isolates with intermediate or resistant phenotypes to doxycycline (23%) was detected, with this level only increasing slightly between days 81 (34%) and 160 (31%).

Resistance of enterococci to erythromycin and tetracycline are commonly encoded by *erm*(B), *msr*C, and *tet* (L), *tet*(M), *tet*(O) resistance genes, respectively (Beukers et al., 2015; Ngbede et al., 2017). The resistance gene *msr*C, is universally present in all *E. faecium* (Portillo et al., 2000), and was detected in all isolates of this species, as well as in one *E. hirae* isolate.

Other Ery^R genes in enterococci include *erm*(A) and *erm*(C) (Portillo et al., 2000), but as these genes are infrequently observed in enterococci isolated from beef cattle (Chen et al., 2008; Zaheer et al., 2013; Beukers et al., 2015), we elected to not screen for them. Nine isolates were negative for both macrolide resistance genes and it is possible that these isolates contained unknown or other known macrolide resistance genes not screened for (Chen et al., 2008; Vikram et al., 2017).

The occurrence of multiple resistance genes within a single isolate suggests the presence of mobile genetic elements (MGE). Both *tet*(M) and *erm*B are known to be frequently associated with the *Tn*916 family of MGE that are common in enterococci (Jurado-Rabadan et al., 2014). Therefore, feeding tylosin, may create selective pressure for not only macrolide resistance, but also tetracycline resistance (Amachawadi et al., 2015). Although erythromycin and tetracycline are seldom used to treat enterococcal infections, they are used to treat other bacterial infections in humans (Arias et al., 2010). If resistant enterococci serve as a reservoir of these MGE-associated antimicrobial resistance genes, they could present a public health risk (Ngbede et al., 2017).

Previous studies noted that liver abscesses, especially livers scored as severe (A+) result in reduced feed intake, and a lower final body weight (Brink et al., 1990). Tylosin is frequently administered in-feed throughout the entire feeding period and in the past was found to lower the prevalence of liver abscesses 40-70% (Nagaraja and Chengappa, 1998). However, the incidence of liver abscesses in feedlot cattle has increased over time, even with the inclusion of tylosin in the diet (Beef Cattle Research Council, 2018). The reasons why tylosin does not completely control liver abscesses are unknown, but there are speculations that it may promote the growth of opportunistic pathogens, select for resistance strains, or that its concentration in the rumen is too

low to be affective against causative bacteria (Nagaraja et al., 1999). Although there is little evidence of tylosin selecting for macrolide resistant *F. necrophorum* or *T. pyogenes* (Nagaraja et al., 1999; Jost et al., 2003; Amachawadi et al., 2017).

There was approximately a 25% decrease in the amount of tylosin consumed by the two reduced tylosin feeding programs (**FIRST-78%**, **LAST-75%**) as compared to continuous administration treatment (**CON**). In the current study, the risk of having severely abscessed (A+) livers was greater in the **FIRST-78%** (1.18 x) and **LAST-75%** (1.16 x) compared to the **CON**. However, the risk of total liver abscesses was not effected when tylosin was administered for shorter durations during the feeding period. Despite the greater risk of severe liver abscesses with shorter duration tylosin programs, there was no difference ($P < 0.05$) between the **FIRST-78%** or **LAST-75%** and the **CON** for any of the morbidity or mortality outcomes. Overall, the mortality rate for the present study was < 2 % which is within the typical range (0 to 15%) of feedlot cattle in North America (Kelly and Janzen, 1986). Causes of mortality included bovine respiratory disease, lameness, metabolic disorder and *Histophilus somni* infection, all of which are not prevented or treated with tylosin.

Walter et al. (2018) evaluated liver abscess prevalence in cattle (n = 3360) fed tylosin during the first 42, first 84, last 84, and first 126 out of 162 days on feed compared continuous administration and no tylosin administration. They observed a linear total decrease in abscessed and A+ livers as days of tylosin feeding increased. Cattle that were fed tylosin in the first 84 d had fewer A+ livers than cattle fed tylosin for the last 84 d suggesting that the greatest risk of liver abscess development and subsequent greatest efficacy of including tylosin in feed was early in the feeding period (Walter et al., 2018). However, in the present study, the lack of difference of A+ liver score between treatments suggests that some risk of severe liver abscess formation is

still possible later in the feeding period. Also similar to our study, a decrease in overall edible livers (score 0) was observed with reduced tylosin administration. Using feedlot performance as a secondary indicator of animal health and welfare, no difference in mortality, ADG, G:F, hot carcass weight, marbling score or other carcass traits were observed.

The cost of administering tylosin phosphate at the manufacturer's recommended concentration (11 ppm) was about $0.02/head/day, representing an average cost of $3.20/head over the160-day feeding period. For the control group that received tylosin throughout the duration of the study the cost of the drug application was ~$8,080 for 2525 cattle. By reducing the amount of tylosin at either the beginning or end of the feeding period, the resulting cost of drug application was reduced to $2.50/head for the **FIRST-78%** and $2.40/head for the **LAST-75%.** The occurrence of condemnable liver abscesses in the **CON**, **FIRST-78%** and the **LAST-75%** was 61.9%, 61%, and 64.2% respectively. With the average estimated economic loss due to condemned or discounted livers in Canada at a rate of $20.98/head (Beef Cattle Research Council, 2018), and the average number of animals per treatment set at 2525, the cost due to the loss of livers associated with the treatment was -$476.8 for the **FIRST-78%** and $1218.4 for the **LAST-75%**. Therefore, a reduced duration of tylosin in-feed at the beginning of the feeding period could result in a savings of approximately $0.70/head in tylosin costs, while lessening the economic burden associated with liver abscesses by $0.19/head.

3.6 Conclusion

Few studies have investigated the effect of a reduced tylosin feeding in feedlot cattle. Based on the results of the current study, shortening the duration of tylosin feeding is likely to result in slightly more severe liver abscess scores, but the overall impacts on morbidity and mortality, animal performance and carcass traits were negligible. This study demonstrated that

reduced feeding of tylosin either at the beginning or end of the feeding period is unlikely to change the proportion of resistant enterococci in the feces at the time of slaughter. The measured levels of Ery^R and antimicrobial susceptibility patterns in enterococci reflect the selection pressure in the bacterial population as a whole, which may have resulted from administering tylosin to feedlot cattle. Additionally, *E. hirae*, was the predominant species of enterococci associated with feedlot cattle fed a high grain finishing diet, a species that is not commonly associated with infections in humans. Findings of this study support the potential for producers to reduce antimicrobial inputs and reduce cattle exposures to tylosin, a medically important class of antimicrobials related to public health. However, such practices are unlikely to reduce the amount of macrolide resistant enterococci excreted in beef cattle feces.

CHAPTER FOUR: Conclusions and Prospects

Although liver abscesses have been studied for over half a century, they are a continuing problem in the Canadian beef cattle industry. High-grain diets are the most common and efficient strategy for reaching a desired finishing weight for market cattle. Consequently, these starchy and highly fermentable diets lead to metabolic disorders such as ruminal acidosis, a sequela of liver abscesses (Nagaraja and Lechtenberg, 2007). The annual economic loss associated with liver abscesses in Canada has doubled between 2011 to 2015 from $29.9 to $61.2 million, and is expected to continue to rise (Beef Cattle Research Council, 2018).

Current strategies in controlling liver abscesses rely on nutritional management and antimicrobial therapy. Tylosin phosphate is frequently administered to cattle in North America at subtherapeutic levels in-feed throughout the finishing period (Brown et al., 1975). Although tylosin does not completely prevent liver abscesses, it has previously been found to reduce the incidence from 40-70% (Nagaraja and Chengappa, 1998). Additionally, increasing concerns over the use of antimicrobials in food-producing animals and their role in the dissemination of antimicrobial resistant bacteria remains an important public health concern (Hoelzer et al., 2017). Administering macrolides to cattle promotes resistance regardless if they are administered through a subcutaneous injection (therapeutic) or continuously in-feed (subtherapeutic) (Zaheer et al., 2013). New antimicrobial regulations in the United States (US Food and Drug Administration, 2015) and Canada (Health Canada, 2018) have recently been implemented. Currently the new regulations require veterinary oversight for medically important antimicrobials such as tylosin and a prescription for its use to control liver abscesses. The ability to continue to include tylosin in the feed of beef cattle to control liver abscesses is uncertain. For this reason, several antimicrobial alternatives have been explored for their effectiveness at preventing illness

while maintaining animal productivity. The probiotic, *Saccharomyces cerevisiae* fermentation product (SCFP), has the potential to improve ruminal fermentation and the composition of the microbiota within the bovine digestive tract (Callaway and Martin, 1997), and improve ruminal pH status in feedlot cattle fed a high grain diet (Shen et al., 2018). Responses of SCFP towards growth performance measurements have been variable (Swyers et al., 2014; Geng et al., 2016). Additional reports have found SCFP to lower antimicrobial resistance and fecal shedding of *Salmonella* and *Escherichia coli* O157:H7 in feedlot heifers (Feye et al., 2016). Reducing the use of antimicrobials in food-producing animals and developing an alternative strategy to control liver abscesses in beef cattle is an important area of research.

It was the primary objective of this work to assess the effects of two alternative feeding strategies on liver abscesses, growth performance, carcass traits, and immune responses in beef cattle as well as to characterize antimicrobial resistance in enterococci isolated from cattle feces. An overview of the major impacts from this work are displayed in Figure 4.1.

This work involved two beef cattle studies in Alberta, Canada. In the first study, the cattle (n = 90) were housed in individual pens at a research feedlot at the Lethbridge Research and Development Centre for 105 days to compare the administration of a probiotic, SCFP, to the antimicrobials, tylosin and monensin. In the second study, cattle (n = 7576) were housed in pens at a commercial feedlot in Brooks, AB for 160 DOF, and fed tylosin during the initial or later half of the feeding period as compared to continuous administration. In the first study, feedlot cattle were fed a barley-based finishing diet, while cattle in the second study were fed a corn-based finishing diet.

Fecal samples were collected from the beginning, middle and end in both studies and plated on antimicrobial selective and non-selective media to determine the proportion of antimicrobial resistant enterococci overtime. Both studies demonstrated that administering tylosin to beef cattle increased the percentage of antimicrobial resistance in enterococci throughout the feeding period, a response that aligns with previous studies (Zaheer et al., 2013; Beukers et al., 2015). Shortening the duration of tylosin administration did not reduce erythromycin resistance in enterococci, whereas completely substituting tylosin for SCFP at different concentrations did result in a reduction in both erythromycin and erythromycin + tetracycline resistance. In the first study, there was an initially high proportion of tetracycline resistance in isolates from cattle across all treatments at the beginning and at mid sampling, but this declined near the end, possibly due to an overall decline in enterococci.

In the second study, disc susceptibility testing was conducted to determine the antimicrobial resistant profiles of enterococci. Enterococci exhibited high levels of resistance to tylosin, erythromycin and doxycycline. Levels of macrolide resistance remained consistent throughout the feeding period, whereas tetracycline resistance increased slightly, and did not differ between continuous vs a reduced duration of tylosin administration.

The high level of tetracycline resistance in enterococci in the absence of tetracycline in the diet is a reflection of the widespread presence of tetracycline resistance genes and their prevalence in beef cattle intestinal microflora (Alexander et al., 2008). The increase in tetracycline resistance over the duration of the feeding period highlights the selection pressure applied by administering tylosin. This resistance is likely through the linkage of the *erm*(B) gene conferring resistance to macrolides and its location on the same mobile genetic element as the tetracycline resistance gene, *tet*(M) (Jurado-Rabadan et al., 2014; Amachawadi et al., 2015).

Müller et al. (2018) also found a high proportion of tetracycline resistant enterococci from the feces of cattle that were administered in-feed tylosin and not tetracycline. Previous reports have demonstrated that it is possible to lower the occurrence of antimicrobial resistant enterococci in animals by removing the selection pressure of antimicrobials (Aarestrup et al., 2001; Beukers et al., 2015). However, the present study demonstrated that resistance continued to persist after tylosin removal, likely due to co-selection (Aarestrup et al., 2001). This continued resistance represents a significant challenge in the global effort to reduce antimicrobial resistance. However, it is in the best interest of the public for researchers, veterinarians and producers to continue to investigate strategies to reduce tylosin use in feedlot cattle so that at the very minimum, dependence on antimicrobials for disease prevention is not increasing.

At slaughter, the liver of every cattle in both studies was scored as "0" if normal/healthy, and "A" for minor abscesses or "A+" if it was severely abscessed. As expected, the cattle administered tylosin had a lower incidence of severe liver abscesses than any of the alternative treatments in both studies. In the first study, although there was no difference in total liver abscesses, cattle administered no tylosin or antimicrobials had the highest incidence of severe liver abscesses, and the incidence tended to decrease with increasing concentrations of SCFP. This may be explained by the ability of SCFP to alleviate ruminal acidosis as outlined by (Shen et al., 2018). In the second study, shortening the duration of tylosin administration resulted in a greater incidence of severe liver abscesses; however, the difference between continuous administration was only 3%.

One of the main reasons producers rely on antimicrobials in livestock is because of the negative implications that their restriction may have on animal performance and profitability. Brown and Lawrence (2010) demonstrated that the greatest impact of liver abscesses on animal

performance are those scored as severe (A+). However, based on the current studies, even though there was a greater incidence of severe liver abscesses in the antimicrobial alternative treatments than the antimicrobial fed cattle, the effects on average daily gain (ADG), dry matter intake (DMI), final body weight (BW), and feed efficiency were negligible. In the first study, SCFP improved ADG and feed efficiency compared to tylosin and monensin fed cattle, whereas DMI and the final BW were unaffected. In the second study, there was no difference in ADG, feed efficiency or final BW of cattle administered tylosin for a shorter duration as compared to continuous administration. It is important to note however that the experimental unit for the growth performance measurements was different among the two studies. In the first study, individual cattle were assessed, whereas, in the second study, the experimental unit was the pen. Therefore, the severity of livers may have had minimal overall impact on overall performance, possibly due to a large pen dilution effect.

An important consideration of this work is the economic impact on production practices. The current studies demonstrate that the economic loss associated with lower cattle performance due to liver abscesses would not present a major issue. When tylosin was administered for an approximately 25% shorter duration, producers would save 25% of the cost of the antimicrobial. Additionally, the difference in economic loss associated with condemned or discounted livers between the cattle fed tylosin continuously or for a shorter duration was minimal. In the present study, this would amount to approximately $0.70 and $0.80/head in feed savings, while the cost due to liver discounts is -$0.19 and $0.48/head when withdrawing tylosin either at the end or beginning of the feeding period, as compared to continuous administration. Therefore, withdrawing tylosin at the end of the feeding period resulted in a higher total economic savings ($0.89.3/head) than withdrawing tylosin in the beginning ($0.32/head).

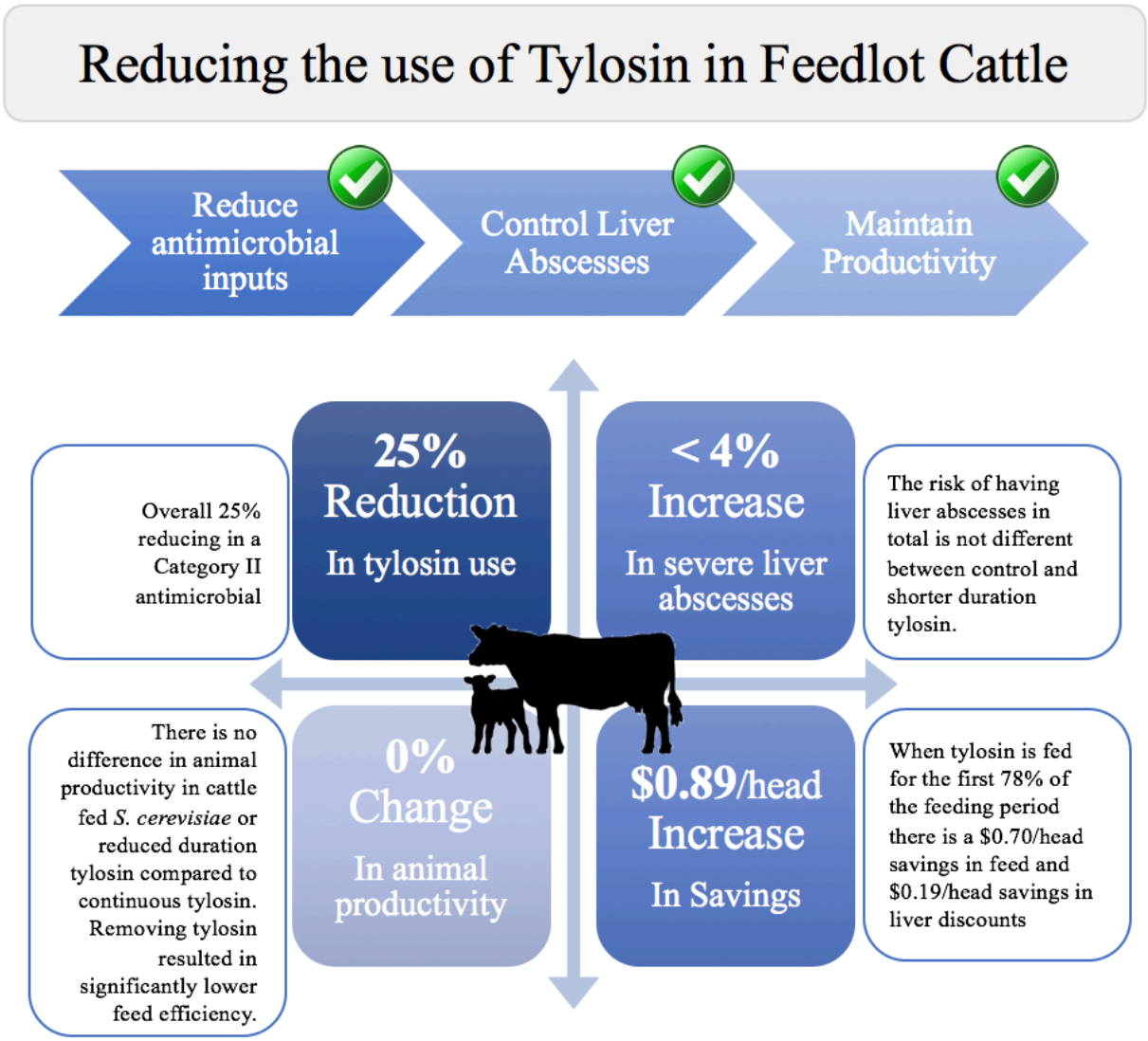

Figure 4. 1. Overall implications of reducing the use of tylosin in feedlot cattle

Whether or not administering antimicrobials in food-producing animals poses a risk for antimicrobial resistance in humans is a concept that is difficult to prove or disprove. Nevertheless, Canada along with the United States are implementing stricter regulations on antimicrobials in an attempt to avoid the harm of antimicrobial resistance to public health even

though this link is not fully understood (Kriebel et al., 2001). Health Canada has developed a pan-Canadian approach to address antimicrobial resistance and antimicrobial use, and it is their vision to protect the health of humans, animals and the environment through conserving the effectiveness of antimicrobials (Public Health Agency of Canada, 2017).

Currently, livestock producers require a veterinary prescription to access any medically important antimicrobials. Health Canada indicates that it is crucial for producers to have a valid veterinary-client-patient relationship in order to develop appropriate protocols for the use of various antimicrobials (Health Canada, 2018). Production practices across Canada and the United States are changing and it is our job as researchers to provide producers and veterinarians with the most up to date scientific information so they can make evidence-based decisions about the use of antimicrobials. According to the Medicating Ingredient Brochure for beef cattle, to maintain effectiveness of tylosin, and reduce the development of resistance, this antimicrobial should be administered for the lowest duration required to achieve the desired outcome (Canadian Food Inspection Agency, 2019a). Therefore, it is the opinion of the author that tylosin could be administered for a shorter duration while still maintaining production standards of the cattle.

There is growing interest from consumers for “clean labels” which includes meat from animals “raised without the use of antimicrobials” (RWA) (Centner, 2016). Meat products with the aforementioned claim on the label can be found in supermarkets across North America and many consumers are buying into the false reality of what the label offers. Conventional beef cattle production practices which administer tylosin or tetracycline in-feed are known to have higher antimicrobial resistance bacteria isolated from their feces compared to cattle RWA (Vikram et al., 2017). However, the levels of antimicrobial resistance in the final meat product

may not be different between conventional and RWA beef due to sanitation interventions in the processing plant (Alexander et al., 2009).

Denmark is an excellent example of the successes of removing antimicrobials in livestock, and switching focus from antimicrobial use to better management systems (Parsonage et al., 2017). Between 1995 and 1999, Denmark banned all nontherapeutic use of antimicrobials in pigs which has resulted in large reductions in antimicrobial resistance without a loss in swine productivity (Aarestrup et al., 2001).

Removing antimicrobials from cattle requires a data-driven and ethical-based approach. At the present time, completely removing antimicrobials in-feed in Canada could have a dramatically negative impact on growth performance, morbidity and mortality rates and the incidence of liver abscesses in beef cattle (Wileman et al., 2009), with no impact on antimicrobial resistant bacteria entering the food chain. Currently, there is no withdrawal period for tylosin before cattle are sent to slaughter (Canadian Food Inspection Agency, 2019a). However, based off of the present research a withdrawal period could be implemented at the end of the feeding period. This will allow Canada to work towards slowly reducing the amount of medically important antimicrobials in-feed while continuing to improve sanitation and management strategies so that in the long term the occurrence of antimicrobial resistance could be reduced.

In conclusion, feeding *Saccharomyces cerevisiae* fermentation product as an alternative to antimicrobials or reducing the duration that tylosin is administered in the feed during finishing, is likely to result in more severe liver abscesses without altering the antimicrobial resistance profiles of enterococci isolated from beef cattle. However, feedlot performance and

carcass characteristics will be maintained to the same extent as when tylosin is fed throughout the entire feeding period. The findings of this work support the potential for producers to reduce tylosin in beef cattle while lowering the inputs of this medically important class of antimicrobials related to public health.

Based on the conclusions of this work, there may be an opportunity to combine the findings from both studies and investigate replacing tylosin with SCFP during an antimicrobial withdrawal period before cattle are sent to slaughter. Additionally, there is ongoing work involving the sequencing of the microbiome associated with liver abscesses in the second study. Identifying difference in the bacterial populations and antimicrobial resistant coding genes among continuous tylosin and reduced duration tylosin treatments will help shed light on future strategies for the prevention of liver abscesses and antimicrobial use in beef cattle.

In both studies, the overall prevalence of liver abscesses was > 50% across all treatments. It appears that the main benefit of adding tylosin or SCFP is to lower the prevalence of severely abscessed livers. Future work that focusses on lowering the prevalence of total liver abscesses is necessary to lower the overall burden of disease. The diets formulated in the current studies included ~86% grain and ~10% roughage which may be contributing to the high overall prevalence of liver abscesses. Reformulation of the finishing diet to include greater amounts of roughage (15-20%) should be explored in combination with a reduced duration of tylosin, SCFP, or a combination of the two in order to evaluate the response in liver abscess prevalence and severity as well as animal performance, carcass traits and antimicrobial resistance as compared to the present study.

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