Probiotics in feedlot cattle: Implications for reducing liver abscess pathogens

by

Maggie Catherine Taylor Moe

B.S., Sam Houston State University, 2018

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Approved by:

Major Professor
T. G. Nagaraja

Copyright

Abstract

Liver abscesses are a significant health and economic issue in feedlot cattle, predominantly caused by *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica*. These abscesses commonly result from ruminal acidosis or rumenitis associated with high-grain diets. While liver abscesses have traditionally been controlled with in-feed antimicrobials like tylosin and chlortetracycline, increasing concern over antimicrobial resistance drives the need for alternative therapies. This study aimed to investigate the potential of lactic acid bacteria (LAB) isolated from liver-abscess-free cattle as natural inhibitors of liver abscess causing pathogens. Between 2 beef processing plants, ruminal content samples from 56 cattle were collected originating from 28 feedlots. From the samples collected, 560 LAB isolates were obtained, and broth cultures were separated into two cell-free aliquots: one with neutralized pH and the other remaining un-altered. Isolates were then screened for antimicrobial activity against the target liver abscess pathogens using agar-well diffusion assays.

Results indicated that non-pH-adjusted LAB supernatants exhibited zones of inhibition for all three pathogens, with F. necrophorum showing the greatest inhibition and highest isolate sensitivity. However, pH-adjusted LAB supernatants displayed no antimicrobial activity, suggesting pH plays a significant role in the inhibitory effects observed. In total, 14 LAB isolates exhibited broad-spectrum inhibition across all pathogens, although repeated trials showed variability between experiments, highlighting the potential influence of LAB supernatant composition and culture conditions.

Our findings suggest that ruminal LAB may exhibit innate antimicrobial effects against liver abscess pathogens, with *Lactobacillus* species showing potential for use in probiotics products for cattle on high-grain diets. Further research is needed to characterize these LAB

isolates, assess their consistency and efficacy, and determine suitability as direct-fed microbial species for livestock production. This study highlights the potential of probiotics as viable substitutes to traditional antimicrobials for managing liver abscesses in feedlot cattle.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to express my deepest gratitude to my committee members, Drs. T.G Nagaraja, Raghu Amachawadi, and Mike Sanderson. Their unwavering support, encouragement, and understanding on this long academic journey have been instrumental in completing my degree. I am especially grateful for Dr. Nagaraja and Dr. Blaine Johnson whose invaluable guidance, expertise, and patience has been essential in getting me through graduate school and now veterinary school. Together, their insight and commitment to excellence has truly inspired me to stay focused and accomplish my professional goals.

A heartfelt thank you goes to all my lab-mates for their endless encouragement and assistance, especially MaRyka Smith and Giovanna Baca. From late-night study sessions to spur-of-the-moment adventures between projects, their support has been invaluable. A special thank you to Leigh Ann Feuerbacher, Neil Wallace, and Tahgreed Mahmood for all their technical assistance and training in microbiology. And finally, a big thanks to Dr. Daniel Thomson who made my packing plant samples possible despite COVID-19 restrictions, and who continues to encourage me in my veterinary career. To everyone who has been a part of this journey, thank you for believing in me and for helping me achieve this milestone.

Chapter 1 - Lactic acid bacterial probiotics as antimicrobial alternatives in cattle: a review

Introduction

The ruminal microbial community, consisting of bacteria, archaea, protozoa, fungi, and viruses, has a major impact on nutrient transformations of carbohydrates, protein and lipids contained in the feeds. The symbiotic relationship between the microbes and the animal host is essential for the health of both the animal and the microbial ecosystem. The animal provides nutrients to the microbes, and in return the microbes provide energy, protein, and other micronutrients to the animal via nutrient fermentation processes. As ingested feed travels through the rumen, it becomes colonized with the microorganisms, gets degraded resulting in production of two major products, volatile fatty acids and gases, carbon dioxide and methene. (end-products of microbial fermentation). The VFA is absorbed from the rumen through the mucosal surface and gases are eructated (Chaucheyras-Durand and Durand, 2010).

Diet composition, feeding regimens, and animal welfare and management have been shown to strongly affect the composition and functionality of the microbial environment in ruminants (Celi et al., 2017 and Wallace et al., 2019). There have been various studies attempting to characterize the rumen microbiota to further understand each microbe's role. The challenge with this is largely due to limitations in microbial culture, identification, and enumeration techniques (Cammack et al., 2018; Foschi et al., 2017; Matthews et al., 2019; Weimer, 2015; Senz et al., 2015). It has been well established that there is a set structure of species called the "core microbiome" present in nearly every animal that begins early in ruminal development. Communities become individually unique based on the animal's environmental setting, regimen, and heritability (Weimer, 2015; Henderson et al., 2015; Wallace et al. 2019).

However, disruptions in the microbiome can cause ruminal dysfunction leading to clinical ruminal or systemic acidosis and bloat. Sub-clinically, producers can see decreased feed efficiency, and decreased milk fat production. Because of these disturbances, there has been growing interest in the addition of beneficial microbial species to enhance or restore ruminal fermentation. In addition, there is a growing interest in utilizing these beneficial microbes as possible antibiotic alternatives due to competitive exclusion and production of antimicrobial compounds (such as bacteriocins and organic acids) (Ayala et al., 2019).

It is worth noting that the terms “probiotic” and “direct fed microbials” are often used interchangeably but are technically not synonymous. Probiotics are loosely defined by the World Health Organization (WHO) as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001). In contrast, Direct-Fed Microbials (DFM) are defined by the United States Food & Drug Administration as “products that are purported to contain live (viable) micro-organisms (bacteria and/or fungi)” and can include their non-viable constituents such as metabolites and end-products (US-FDA, 2015). In other words, DFMs are feed additives containing live microbial species and are considered non-pathogenic, safe, and beneficial to the gastrointestinal tract and the host animal. Numerous beneficial responses have been reported with the use of probiotics or DFM in ruminant diets, such as increased milk yield, improved feed efficiency, reduction of ruminal acidosis, and improved immune function (Adjei-Fremah et al., 2018; Desnoyers et al., 2009; Nalla et al., 2022). In cattle production, probiotics are targeted towards the rumen microbial population to positively affect the fermentation of feedstuffs, particularly digestibility and degradability. Additional benefits include stabilizing ruminal pH and reducing lactate levels.

Although most probiotics are generally recognized as safe, the safety of each microbial species used for animal production entering the human food chain should still be considered. One of the primary concerns regarding probiotics is the carriage of antimicrobial resistance (AMR) genes and the potential for transfer of AMR genes. For example, some *Bacillus* spp. have been included in probiotic products despite potential for transferring AMR genes. Zhu et al., 2016, found several strains of *B. cereus* harboring the *tet*(45) gene for tetracycline resistance. Additionally, Artistimuno Ficoseco et al., 2018 investigated antibiotic susceptibility of lactic acid bacteria species isolated from feedlot cattle and identified 7 of 15 resistance genes (not correlated with phenotypic resistance) in their lactobacilli. The majority of research available discusses the efficacy of probiotics rather than the safety, likely due to their GRAS (Generally Regarded As Safe by the FDA) status. Moreover, oftentimes products are either mislabeled or lack clear labeling of the bacterial strains in the product as well as the number of viable cells (colony-forming units). Still, probiotics are considered safe for implementing in animal feed with the main risks being the potential of transmitting antibiotic resistance genes to pathogenic microorganisms, and the production of enterotoxins. These have the potential to cause negative impacts in the indigenous microbial population or infection of the host itself. Although many experiments have shown positive responses using DFM in dairy and beef cattle, there is still uncertainty regarding the inhibitory properties of lactic acid bacteria, particularly towards common bovine pathogens (Brashears et al., 2003; Pukrop, 2017; Molloy et al., 2011). Identifying autochthonous ruminal lactobacilli with pathogenic inhibition could decrease morbidity and improve our ability to supplement appropriate DFM in cattle diets while also establishing effective antibiotic alternatives. This review summarizes the literature pertaining to lactic acid bacteria and their abilities to inhibit pathogenic bacteria in cattle.

Characteristics of lactic acid bacteria

Lactic acid bacteria (LAB) are described as Gram-positive, cocci, coccobacilli, or rods, and are catalase negative (Leska et al., 2022 and Ismail et al., 2018). Additionally, LAB are acid-tolerant facultative anaerobes that produce organic acids, primarily lactic and acetic acid. Due to the rumen's microaerophilic environment, these traits make the rumen an ideal habitat for facultative anaerobes. In cattle, LAB are not regarded as major contributors to rumen fermentation, however they can be readily isolated from the rumen with species such as *Lactobacillus ruminis*, *L. plantarum*, *L. lactis*, and *Streptococcus bovis* in addition to native species of *Enterococcus*, *Bifidobacteria*, and *Streptococcus* (Uyeno et al., 2015; Brashears et al., 2003; Hernandez et al., 2008).

Various species of LAB can be categorized by their respective bacterial end products. Depending on the species, LAB can produce various proportions of D-lactate and L-lactate in addition to VFAs, such as acetate (Hernandez et al., 2008). These are bacterial end products from the fermentation and breakdown of highly fermentable sugars and starch content in the diet. D-lactate is metabolized by enzymes D-α-hydroxy acid dehydrogenase and D-lactate dehydrogenase in the rumen to produce pyruvate, which is later synthesized to glucose via glucogenesis. However, when dysbiosis occurs, over-production of D-lactate has been attributed to acute ruminal acidosis, a metabolic disorder defined by a decrease in blood pH and bicarbonate in cattle (Hernandez et al., 2014). Ruminal species like *S. bovis* can further decrease environmental pH with the production of L-lactate, a potent acid. As the pH remains critically low, the microbial community begins to favor LAB acidophiles and decreases microbial diversity, particularly the population of lactate-utilizers. As lactate-utilizers dwindle, lactic acid overwhelms the ruminal flora's ability to metabolize efficiently, leading to increased mucosal absorption and consequently systemic acidosis (Khafipour et al., 2016; Hernandez et al., 2016).

On the flip side of this phenomenon, favoring the population of *Lactobacillus* and *Bifidobacterium* species in the rumen can inhibit growth of various Gram-negative pathogenic bacteria from the production of organic acids, bacteriocins, and/or by competitive exclusion (FAO, 2016; Ayala et al., 2019; Rezaee et al., 2019, and Molloy et al., 2011).

Lactobacillus is a principle bacterial genus that is found early in rumen development but gradually decreases in numbers as the animal ages and the rumen microflora develops and diversifies (Uyeno et al., 2015). However, when diets contain higher amounts of rapidly fermentable carbohydrates, *Lactobacillus* species can proliferate rapidly and increase in population size (Matthews et al., 2019). *Lactobacillus* spp. prefer growth conditions between 25-45 C, but because of its acid-tolerant nature, many species can continue to grow as the rumen fermentation environment and subsequent pH change. A review by Gao et al., found that in addition to lactate production, *L. acidophilus* can reduce host cholesterol serum levels via inhibition of intestinal absorption. The researchers also reported improvements of lactose metabolism in milk-fed animals, as well as prevention of colonization by enteric pathogens.

During rumen development, *Bifidobacterium* is beneficial for young pre-ruminant calves (Chaucheyras-Durand and Durand, 2010) and in the hindgut of pre-weaned calves (Uyeno et al., 2015) to stabilize the naive gastrointestinal tract and reduce the risk of pathogen invasion and colonization. It is similar to *Lactobacillus* in the manner of gastrointestinal development of young animals across species and is often used in conjunction with *Lactobacillus*-based probiotic products (Hoffman et al., 2018). Likewise, as the animal ages, numbers of *Bifidobacterium* decrease as well (Uyeno et al., 2015). Other research has described the importance of bifidobacteria for the digestion and fermentation of milk constituents like oligosaccharides and glycoconjugates in pre-ruminant dairy calves. For example, Kelly et al., 2016 found that dietary carbohydrates and host-glycans were the primary fermentation sources for several *Bifidobacterium* species. Furthermore, they described differences in carbohydrate preferences between species (plant vs milk carbohydrates). In summary, *Lactobacillus* and *Bifidobacterium* spp. have been primarily studied regarding pre-ruminant or hind-gut environments. This is due to the innate physiologic changes that take place as they transition from milk-based diets to forage and/or grain-based diets, in addition to the animal industry's push to improve animal health.

Lactic acid bacteria-based probiotics in cattle

Probiotics are primarily formulated into the animal's diet as feed additives or silage inoculants where performance gains can be attributed to improvement of nutrient digestibility with the inclusion of the probiotics (Hernandez et al., 2008; Cammack et al., 2018). Many bacteria do not survive in acidic conditions making LAB ideal candidates for probiotic agents. Common genera used in commercial products are *Lactobacillus, Bifidobacterium*, *Enterococcus*, and yeasts such as *Saccharomyces*. *Lactobacillus* and *Bifidobacterium* are the most common probiotic cultures used to modulate gastrointestinal microflora in many animal species (FOA, 2016, Ghazanfar et al., 2017). These microorganisms can be used as single or multi-strain products, depending on the intended use and conferred benefits. Reports studying the implications and benefits of *L. acidophilus* can be dated back to the early 20th century (Stern and Storrs, 1975; Krehbiel et al., 2001). Despite their extensive history, there is still much to discover regarding efficacy, safety, and mode of action. Commonly studied species include *L. acidophilus*, *L. casei, L. plantarum, B. thermophilum, B. choerinum, E. faecium*, and yeast like S. *cerevisiae* just to name a few (Uyeno et al., 2015; Krehbiel et al., 2001; Beauchemin et al., 2003; and Kelly et al., 2016).

Many commercial products use multi-species and -strains to utilize a broader spectrum or synergistic effects. Some may also include pre- or postbiotics with most marketed products containing live yeast, specifically *Saccharomyces cerevisiae* (Chaucheyras-Durand and Durand, 2010). Yeast cultures are well-liked because of their heat tolerance and innate tolerance to antibiotic use. As antimicrobial use and resistance grows in publicity, interest grows in using probiotics to help decrease antibiotic usage in the production phase (Adjei-Fremah et al., 2018). Many available products are implemented because of their potential to improve animal health and carcass growth in the production animal setting (Muzaffar et al., 2021, Huck et al., 2000). Formulations can be seen chute-side as oral boluses, gels, pastes, as drench or powder suspensions, and as top dressings over feed, or as silage-inoculants. According to the FDA, companies are required to label products with "Contains a source of live (viable), naturally occurring microorganisms" followed by the name of the organism and guaranteed colony-forming units per gram (CFU/g) of product. Products that are labeled for use in animal feeds are regarded as DFM, with the exception of products labeled as strictly silage additives (FAO, 2016, Sanders and Levy, 2011).

Implications of lactic acid bacterial probiotics in cattle

Probiotics can be used in cattle to improve or maintain rumen health throughout feeding changes and periods of stress. There has been growing interest in using targeted probiotic products to support rumen ecology and fermentation efficiency. The goal of implementing these products is to improve growth, mitigate digestive upset, reduce gut pathogen prevalence and fecal shedding, increase milk yield, and more recently, decrease greenhouse gas emissions (Varhidi et al., 2022; Doyle et al., 2019; Markowiak & Śliżewska, 2018).

In feeder cattle, studies show mixed results to increase feed efficiency and enhance weight gain using supplemental probiotics. Additionally, studies are limited regarding benefits from probiotics in fed beef cattle compared to dairy cattle. Many reports exhibiting improved performance utilized yeast species or yeast in combination with the bacteria. Ghazanfar et al., 2015 supplemented a dietary yeast-based commercial product containing *S. cerevisiae* to growing dairy heifers and reported a 7% increase in average final body weight with no change in dry matter intake. In a more recent study using a mixed probiotic product with 3 strains of *Enterococcus faecium* and one strain of *S. cerevisiae* reported improved average daily gain and feed efficiency in *Bos indicus* bulls on feed for 120 days (Dias et al., 2022). Another study by Moreira et al., 2019 showed 12% improvement of average daily gain and feed efficiency in steers fed a combination product (LAB and yeast species). Interestingly, Mackey et al., 2023 found in-feed supplementation of a yeast-derived prebiotic with *Bacillus subtilis* to Angus-type steers improved gain to feed ratio, carcass marbling and grade, and decreased incidence of liver abscesses in comparison to steers supplemented with tylosin and monensin. Other studies have reported benefits without yeast but appear more limited. Recently, Mansilla et al., 2023 reported increased daily gain in cattle supplemented with two Lactobacilli species as well as a 43% reduction in *E. coli* O157:H7 fecal shedding between 40-104 days on feed. But historically, Elam et al., 2003 and Greenquist et al., 2004 found no difference between control groups and animals fed DFM containing *Lactobacillus* spp. on feedlot performance or carcass characteristics. In addition to conflicting outcomes, there are limited studies evaluating *Lactobacillus*-based DFM supplementation for growth performance purposes. Comparatively, silage inoculants commonly use lactobacilli species and may offer probiotic benefits to dairy and feedlot cattle, but survivability is largely dependent on silage pH and temperature (Weinberg et al., 2004).

Apart from growth performance, investigators have made attempts to characterize probiotic species for prevention or mitigation of ruminal acidosis and to support gastrointestinal function in cattle consuming concentrated diets. The concept of including probiotics is to increase the beneficial microflora population and enhance ruminal fermentation. There are two schools of thought to achieve this – the first is to postulate favorable lactic acid producers to stimulate lactate utilizers like, *Megasphaera elsdenii* and *Selenomonas ruminantium* and to competitively exclude *Streptococcus bovis*. The second is to administer lactate utilizers directly to decrease or prevent lactic acid accumulation (Hernandez et al., 2014; Nocek and Kautz, 2006); Thieszen et al., 2015). Beauchemin et al., 2003 and Ghorbani et al., 2002 found diet supplementation of *E. faecium* EF212 (with or without *S. cerevisiae* or *Propionibacterium* P15) had limited effects on ruminal acidosis reduction in animals fed high-grain diets. Investigators concluded that cattle adapted to concentrate diets could maintain intake and digestion despite being in a state of subclinical ruminal acidosis. While *E. faecium* and yeast may not have decreased acidosis together in feedlot cattle, Nocek et al., 2000 reported the addition of *Lactobacillus* with *Enterococcus* and yeast reduced the risk of acidosis in dairy cattle. Similarly, Huffman et al., 1992 and Van Koevering et al., 1994 reported a decrease in the amount of time the rumen pH was below 6.0 and found lower concentrations of D-lactate and total lactate in steers fed *L. acidophilus* alone. These outcomes suggest the inclusion of lactobacilli may provide benefits to manage ruminal acidosis. Meanwhile, results with yeast have been more consistent among studies showing benefits to ruminal health, acidosis mitigation, and animal performance both in growing and lactating cattle (Uyeno et al., 2017). Several studies have demonstrated an increase in ruminal pH among dairy cattle fed live yeast as well as decreased ruminal lactate concentrations. (Dias et al., 2018; Bach et al., 2007; Thrune et al., 2009). In the dairy industry, live yeast have showed enhancement of lactation performance, particularly milk yield (Chaucheyras-Durand & Durand, 2010; Desnoyers et al., 2009; Markowiak & Śliżewska, 2018). The mode of action of yeast is still to be defined but is generally considered to improve fermentation rates and stabilizing pH levels (Uyeno et al., 2017). Similar to bacterial DFM, yeast can stimulate lactate utilizers and ciliate protozoa to support the rumen environment and provide competition against other lactate producers, like *S. bovis* (Amin and Mao, 2021; Tripathi and Karim, 2011).

In pre-ruminant animals, probiotics have been shown to aid in intestinal microbial colonization and limit the risk of enteric pathogen colonization (Chaucheyras-Durand & Durand, 2010; Krehbiel et al., 2003; McAllister et al., 2011). Unlike in dairy and feedlot cattle where probiotics are focused on ruminal modification, in neonates, probiotics have been used to aid establishment and maintenance of the total gastrointestinal microflora. Specifically, it is important for dairy calves to adapt to solid feed quickly, emphasizing the importance of forming a baseline alimentary microflora and minimizing establishment of scour-causing pathogens (Ban and Guan, 2021). Additionally, the microbial population at this stage is extremely sensitive to stressors like pen movements, diet changes, and weaning. Several studies have found that feeding calves viable sources of LAB, can significantly decrease coliform shedding in stool, thereby decreasing the incidence of scours (Lesmeister et al., 2004; Liu et al., 2022; Wang et al., 2023). Timmerman et al., 2003 reported in addition to improved average daily gain, probiotic treatment lessened the incidence of diarrhea and fecal coliform counts. But most intriguingly, they saw a reduction in the number of treatments for digestive and respiratory diseases in veal calves. This suggests that probiotics could also influence respiratory health in calf production.

If we apply these concepts to food safety, we can see that the inclusion of DFMs may offer implications for reducing food borne pathogens. As food demands grow worldwide, more interest has been highlighted towards the development of DFM products that show inhibition or reduction of zoonotic pathogens. *In vitro,* Brashears et al., 2003 cultured 15 LAB species from cattle fecal samples exhibiting significant reduction of *E. coli* counts in rumen fluid. Another study by, Ayala et al., 2019 isolated 28 LAB species from cattle feces within the genus *Enterococcus*, *Pediococcus*, and *Lactobacillus* demonstrating inhibitory properties against *Listeria monocytogenes, Salmonella,* and *E. coli* O157. *In vivo*, Elam et al., 2003 found decreased *E. coli* O157 fecal shedding in beef steers fed DFM containing *L. acidophilus* and *P. freudenreichii* despite no performance and carcass characteristic changes. More recently, Mansilla et al., 2023 found improved growth performance in addition to decreased fecal shedding of *E. coli* O157 in feedlot cattle by as much as 43 percent. In a review by McAllister et al., 2011, they concluded that DFM have the potential to reduce traditional antimicrobial use in production animal settings, but positive responses must be more consistent. Additionally, the relationship between the DFM and exclusion of pathogens should be more well defined to make selection decisions and target DFM efficacy.

Mode of action

The outcome variability within the literature suggests a greater need to understand the underlying mechanisms of direct fed microbials. There is likely more than one mode of action influencing the positive and/or inconclusive results between studies and largely dependent on the bacterial vs yeast species and strain as well as the dose. Currently, benefits are thought to be predominantly attributed to competitive exclusion and bacteriocin production by the bacterial probiotic organisms. Bacteriocins are bioactive peptides that inhibit growth (by bactericidal or bacteriostatic effects) of pathogenic bacteria or enzyme production to hydrolyze toxins (Touza-Otero et al., 2024, Hernández-González et al, 2021). They are generally considered to act on the cell membrane forming pores in the phospholipid bilayer, ultimately causing cell death. They differ from antibiotics in their synthesis, spectrum, and toxicity – these distinctions are important for their use food systems (Molloy and Hill, 2011). Bacteriocins have been studied for use in veterinary medicine and food safety practices. For example, nisin is a bacteriocin produced from *L. lactis* and is an approved food preservative in many countries including the U.S as well as a disinfectant for preventing mastitis in dairy cattle (Touza-Otero et al., 2024, Molloy and Hull, 2011). These compounds are of interest for their use as antibacterial alternatives, and it has been suggested that almost all bacteria produce at least one bacteriocin (McAllister et al., 2011). Naturally, some bacteria have innate and acquired resistance to bacteriocins. Additionally, some studies have shown that exposure to bacteriocins could render cells more resistant to antibiotics. For bacteriocin use to grow, there needs to be more characterization of bacteriocins, their toxicity status, and intended use either in food safety or medicinal applications.

Competitive exclusion in the gastrointestinal tract is the act of beneficial species adhering to the intestinal epithelial cells, preventing colonization of other microorganisms – particularly pathogenic ones. It has been well established with species of *Lactobacillus* to reduce colonization of *E. coli* and *Salmonella*, but few studies have elaborated on the exact mechanisms (Nami et al., 2019, Rodriguez-Palacios et al., 2009, Chen et al., 2020). Some researchers propose that competitive exclusion is possible due to modifications of tight-junction proteins at the epithelial surface (McAllister et al., 2011). Others have attributed success to stimulation of immune response, enhanced gastrointestinal health through restoration of mucosal integrity, and sheer outcompeting for nutrients to limit pathogen growth (O'Tool and Cooney, 2008; Uyreno et al., 2015). Generally, competitive exclusion helps to maintain homeostasis in the microbial population, ultimately improving rumen function (Buntyn et al., 2016).

The use of direct fed microbials in livestock production has also been associated with enhanced host immunity. In a study by Elam et al. 2003, they found steers fed DFM had decreased thickness of lamina propria in the small intestine compared to steers not fed DFM. This suggests that DFM can decrease inflammation within the lower gut of ruminants, thereby improving intestinal absorption. Raabis et al., 2019 further described certain species mediating intestinal inflammatory response through disruption of interleukin-17 production, a pro-inflammatory cytokine. This has also been summarized by Krehbiel et al. 2003, concluding that DFMs can affect cytokine production and T and B cell responses, but are largely dependent on the strains, dose, feeding strategies and host species. While many studies have shown immunologic responses with probiotic supplementation in monogastrics, further research is needed in ruminants to observe and characterize immunological changes in cattle, particularly with established rumen ecologies.

Conclusion

Probiotic species such as *Lactobacillus* and *Bifidobacterium* are studied for their ability to enhance ruminal health and support feed efficiency in cattle, primarily by influencing fermentation processes within the rumen. Lactic acid bacteria improve rumen stability by producing organic acids and promoting lactate-utilizing bacteria, which together can mitigate ruminal upset. Additionally, competitive exclusion and bacteriocin production are mechanisms by which LAB inhibit pathogens like *E. coli* and *Salmonella* – important pathogens in pre-harvest food safety. There is a long history of attempts to alter rumen fermentation with DFM use. However, characterizing the rumen microbial population to change fermentation can prove difficult with the rumen’s vast microbial taxonomy, especially between animals and their respective environments and dietary history.

Despite the Generally Regarded As Safe (GRAS) status of many probiotics, potential concerns include the transfer of antimicrobial resistance genes and the challenge of consistent efficacy due to factors like strain specificity, animal health history, and environmental conditions. Studies on the effects of probiotics in cattle show varying degrees of success in weight gain, feed efficiency, and reduction of acidosis and pathogen colonization. Young, pre-ruminant calves benefit from probiotics in preventing diarrhea and potentially respiratory diseases, helping to establish a resilient gut microflora. It has been demonstrated that neonatal gastrointestinal tracts are more easily altered with DFM supplementation due to their naivety, but once the microbial population has been established, it becomes harder to manipulate. In adult cattle, findings are likely inconsistent due to differences in host microbiomes and external factors like species used in the product, dosing and dosage, animal environment and health history, and much more. Nevertheless, supplementation with DFM have shown to provide at least short-term impacts, increasing the population of LAB and lactate utilizers, creating a more resilient and digestibly efficient rumen. The idea is that lactate production will stimulate lactate-utilizers in the rumen thereby increasing the amount of propionate to be absorbed by the animal and further stimulating beneficial microorganisms. This phenomenon is important for cattle on high-grain diets or in the event of a sudden increase in lactic acid concentrations in the rumen.

The use of LAB probiotics in cattle presents a viable strategy to enhance gut health, improve feed efficiency, and potentially reduce antibiotics use. Conversely, inconsistencies in research outcomes highlight a need for further exploration of the modes of action, particularly regarding the specifics of competitive exclusion, bacteriocin production, and immune modulation. The diverse microbial ecology of the rumen makes probiotic impact highly variable and complex. Going forward, standardized methods and detailed studies on strain specificity, host-microbe interactions, and immunological effects will be essential to realize the full potential of probiotics as sustainable alternatives in cattle production systems.

Literature cited

Adjei-Fremah, S., Ekwemalor, K., Worku, M., & Ibrahim, S. (2018). Probiotics and Ruminant Health. In S. Enany (Ed.), *Probiotics—Current Knowledge and Future Prospects*. InTech. https://doi.org/10.5772/intechopen.72846

Aristimuño Ficoseco, C., Mansilla, F. I., Maldonado, N. C., Miranda, H., Fátima Nader-Macias, M. E., & Vignolo, G. M. (2018). Safety and Growth Optimization of Lactic Acid Bacteria Isolated From Feedlot Cattle for Probiotic Formula Design. *Frontiers in Microbiology*, *9*, 2220. https://doi.org/10.3389/fmicb.2018.02220

Ayala, D. I., Cook, P. W., Franco, J. G., Bugarel, M., Kottapalli, K. R., Loneragan, G. H., Brashears, M. M., & Nightingale, K. K. (2019). A Systematic Approach to Identify and Characterize the Effectiveness and Safety of Novel Probiotic Strains to Control Foodborne Pathogens. *Frontiers in Microbiology*, *10*, 1108. https://doi.org/10.3389/fmicb.2019.01108

Bach, A., Iglesias, C., & Devant, M. (2007). Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. *Animal Feed Science and Technology*, *136*(1–2), 146–153. https://doi.org/10.1016/j.anifeedsci.2006.09.011

Ban, Y., & Guan, L. L. (2021). Implication and challenges of direct-fed microbial supplementation to improve ruminant production and health. *Journal of Animal Science and Biotechnology*, *12*(1), 109. https://doi.org/10.1186/s40104-021-00630-x

Beauchemin, K. A., Yang, W. Z., Morgavi, D. P., Ghorbani, G. R., Kautz, W., & Leedle, J. A. Z. (2003). Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle1,2. *Journal of Animal Science*, *81*(6), 1628–1640. https://doi.org/10.2527/2003.8161628x

Brashears, M. M., Jaroni, D., & Trimble, J. (2003a). Isolation, Selection, and Characterization of Lactic Acid Bacteria for a Competitive Exclusion Product To Reduce Shedding of Escherichia coli O157:H7 in Cattle. *Journal of Food Protection*, *66*(3), 355–363. https://doi.org/10.4315/0362-028X-66.3.355

Buntyn, J. O., Schmidt, T. B., Nisbet, D. J., & Callaway, T. R. (2016). The Role of Direct-Fed Microbials in Conventional Livestock Production. *Annual Review of Animal Biosciences*, *4*(1), 335–355. https://doi.org/10.1146/annurev-animal-022114-111123

Cammack, K. M., Austin, K. J., Lamberson, W. R., Conant, G. C., & Cunningham, H. C. (2018). Tiny but mighty: The role of the rumen microbes in livestock production. *Journal of Animal Science*. https://doi.org/10.1093/jas/skx053

Celi, P., Cowieson, A. J., Fru-Nji, F., Steinert, R. E., Kluenter, A.-M., & Verlhac, V. (2017). Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. *Animal Feed Science and Technology*, *234*, 88–100. https://doi.org/10.1016/j.anifeedsci.2017.09.012

Chaucheyras-Durand, F., & Durand, H. (2010). Probiotics in animal nutrition and health. *Beneficial Microbes*, *1*(1), 3–9. https://doi.org/10.3920/BM2008.1002

Chen, C.-C., Lai, C.-C., Huang, H.-L., Su, Y.-T., Chiu, Y.-H., Toh, H.-S., Chiang, S.-R., Chuang, Y.-C., Lu, Y.-C., & Tang, H.-J. (2020). Antimicrobial ability and mechanism analysis of Lactobacillus species against carbapenemase-producing Enterobacteriaceae. *Journal of Microbiology, Immunology and Infection*, S1684118220300062. https://doi.org/10.1016/j.jmii.2020.01.005

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C., & Sauvant, D. (2009). Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. *Journal of Dairy Science*, *92*(4), 1620–1632. https://doi.org/10.3168/jds.2008-1414

Dias, A. L. G., Freitas, J. A., Micai, B., Azevedo, R. A., Greco, L. F., & Santos, J. E. P. (2018). Effect of supplemental yeast culture and dietary starch content on rumen fermentation and digestion in dairy cows. *Journal of Dairy Science*, *101*(1), 201–221. https://doi.org/10.3168/jds.2017-13241

Dias, B. G. C., Santos, F. A. P., Meschiatti, M., Brixner, B. M., Almeida, A. A., Queiroz, O., & Cappellozza, B. I. (2022). Effects of feeding different probiotic types on metabolic, performance, and carcass responses of *Bos indicus* feedlot cattle offered a high-concentrate diet. *Journal of Animal Science*, *100*(10), skac289. https://doi.org/10.1093/jas/skac289

Doyle, N., Mbandlwa, P., Kelly, W. J., Attwood, G., Li, Y., Ross, R. P., Stanton, C., & Leahy, S. (2019). Use of Lactic Acid Bacteria to Reduce Methane Production in Ruminants, a Critical Review. *Frontiers in Microbiology*, *10*, 2207. https://doi.org/10.3389/fmicb.2019.02207

Elam, N. A., Gleghorn, J. F., Rivera, J. D., Galyean, M. L., Defoor, P. J., Brashears, M. M., & Younts-Dahl, S. M. (2003). Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and Escherichia coli strain O157 shedding of finishing beef steers. *Journal of animal science*, *81*(11), 2686–2698. https://doi.org/10.2527/2003.81112686x

FAO. (2016). Probiotics in animal nutrition – Production, impact and regulation by Yadav S. Bajagai, Athol V. Klieve, Peter J. Dart and Wayne L. Bryden. Editor Harinder P.S. Makkar. FAO Animal Production and Health Paper No. 179. Rome.

FAO/WHO. (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. *Report from FAO/WHO Expert Consultation*, 1-4.

Foschi, C., Laghi, L., Parolin, C., Giordani, B., Compri, M., Cevenini, R., Marangoni, A., & Vitali, B. (2017). Novel approaches for the taxonomic and metabolic characterization of lactobacilli: Integration of 16S rRNA gene sequencing with MALDI-TOF MS and 1H-NMR. *PLOS ONE*, *12*(2), e0172483. https://doi.org/10.1371/journal.pone.0172483

Gao, H., Li, X., Chen, X., Hai, D., Wei, C., Zhang, L., & Li, P. (2022). The Functional Roles of *Lactobacillus acidophilus* in Different Physiological and Pathological Processes. *Journal of Microbiology and Biotechnology*, *32*(10), 1226–1233. https://doi.org/10.4014/jmb.2205.05041

Ghazanfar, S., Anjum, M. I., Azim, A., & Ahmed, I. (2015). Effects of dietary supplementation of yeast (*Saccharomyces cerevisiae*) culture on growth performance, blood parameters, nutrient digestibility and fecal flora of dairy heifers. *J. Anim. Plant Sci.*

Ghazanfar, S., Khalid, N., Ahmed, I., & Imran, M. (2017). Probiotic Yeast: Mode of Action and Its Effects on Ruminant Nutrition. In A. Morata & I. Loira (Eds.), *Yeast—Industrial Applications*. InTech. https://doi.org/10.5772/intechopen.70778

Greenquist, M. A., Dicke, B., Erickson, G. E., Klopfenstein, T. J., & Drouillard, J. S. (2004). Effects of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* on growth performance and carcass characteristics of finishing beef cattle. *Kansas Agricultural Experiment Station Research Reports*, *1*, 71–74. https://doi.org/10.4148/2378-5977.1621

Hernández, J., Benedito, J. L., Abuelo, A., & Castillo, C. (2014). Ruminal Acidosis in Feedlot: From Aetiology to Prevention. *The Scientific World Journal*, *2014*, 1–8. https://doi.org/10.1155/2014/702572

Hernandez, J. D., Scott, P. T., Shephard, R. W., & Al Jassim, R. A. M. (2008). The characterization of lactic acid producing bacteria from the rumen of dairy cattle grazing on improved pasture supplemented with wheat and barley grain. *Journal of Applied Microbiology*, *104*(6), 1754–1763. https://doi.org/10.1111/j.1365-2672.2007.03696.x

Hoffmann, D. E., Fraser, C. M., Palumbo, F., Ravel, J., Rowthorn, V., & Schwartz, J. (2018). *Probiotics: Achieving a Better Regulatory Fit*. 37.

Huck, G. L., Kreikemeier, K. K., & Ducharme, G. A. (2000). Effects of feeding two microbial additives in sequence on growth performance and carcass characteristics of finishing heifers. *Kansas Agricultural Experiment Station Research Reports*, *1*, 32–34. https://doi.org/10.4148/2378-5977.1788

Ismail, Y., Yulvizar, C., & Mazhitov, B. (2018). Characterization of lactic acid bacteria from local cow´s milk kefir. *IOP Conference Series: Earth and Environmental Science*, *130*, 012019. https://doi.org/10.1088/1755-1315/130/1/012019

Kelly, W. J., Cookson, A. L., Altermann, E., Lambie, S. C., Perry, R., Teh, K. H., Otter, D. E., Shapiro, N., Woyke, T., & Leahy, S. C. (2016). Genomic analysis of three Bifidobacterium species isolated from the calf gastrointestinal tract. *Scientific Reports*, *6*, 30768. https://doi.org/10.1038/srep30768

Khafipour, E., Li, S., Tun, H. M., Derakhshani, H., Moossavi, S., & Plaizier, J. C. (2016). Effects of grain feeding on microbiota in the digestive tract of cattle. *Animal Frontiers*, *6*(2), 13–19. https://doi.org/10.2527/af.2016-0018

Krehbiel, C. R., Rust, S. R., Zhang, G., & Gilliland, S. E. (2003). *Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action1,2*. 15.

Leska, A., Nowak, A., & Motyl, I. (2022). Isolation and Some Basic Characteristics of Lactic Acid Bacteria from Honeybee (Apis mellifera L.) Environment—A Preliminary Study. *Agriculture*, *12*(10), 1562. https://doi.org/10.3390/agriculture12101562

Lesmeister, K. E., Heinrichs, A. J., & Gabler, M. T. (2004). Effects of Supplemental Yeast (Saccharomyces cerevisiae) Culture on Rumen Development, Growth Characteristics, and Blood Parameters in Neonatal Dairy Calves. *Journal of Dairy Science*, *87*(6), 1832–1839. https://doi.org/10.3168/jds.S0022-0302(04)73340-8

Liu, B., Wang, C., Huasai, S., Han, A., Zhang, J., He, L., & Aorigele, C. (2022). Compound Probiotics Improve the Diarrhea Rate and Intestinal Microbiota of Newborn Calves. *Animals*, *12*(3), 322. https://doi.org/10.3390/ani12030322

Mackey, S. J., Cooke, R. F., Colombo, E. A., Pickett, A. T., Batista, L. F. D., Block, E., & Brandâo, A. P. (2023). Supplementing pre- and probiotic ingredients to feedlot steers: Effects on health, growth performance, and physiological responses. *Animal*, *17*(4), 100770. https://doi.org/10.1016/j.animal.2023.100770

Molloy, E. M., Hill, C., Cotter, P. D., & Ross, R. P. (2011). BACTERIOCINS. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences (Second Edition)* (Second Edition, pp. 420–429). Academic Press. https://doi.org/10.1016/B978-0-12-374407-4.00049-2

Mansilla, F. I., Miranda, M. H., Uezen, J. D., Maldonado, N. C., D'Urso Villar, M. A., Merino, L. A., Vignolo, G. M., & Nader-Macias, M. E. F. (2023). Effect of probiotic lactobacilli supplementation on growth parameters, blood profile, productive performance, and fecal microbiology in feedlot cattle. *Research in Veterinary Science*, *155*, 76–87. https://doi.org/10.1016/j.rvsc.2023.01.003

Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens*, *10*(1), 21. https://doi.org/10.1186/s13099-018-0250-0

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W., & Cotter, P. D. (2019). The rumen microbiome: A crucial consideration when optimizing milk and meat production and nitrogen utilization efficiency. *Gut Microbes*, *10*(2), 115–132. https://doi.org/10.1080/19490976.2018.1505176

McAllister, T. A., Beauchemin, K. A., Alazzeh, A. Y., Baah, J., Teather, R. M., & Stanford, K. (2011). Review: The use of direct fed microbials to mitigate pathogens and enhance production in cattle. *Canadian Journal of Animal Science*, *91*(2), 193–211. https://doi.org/10.4141/cjas10047

Muzaffar, K. (n.d.). *Chapter 25 Commercially Available Probiotics and Prebiotics Used in Human and Animal Nutrition*.

Nalla, K., Manda, N. K., Dhillon, H. S., Kanade, S. R., Rokana, N., Hess, M., & Puniya, A. K. (2022). Impact of Probiotics on Dairy Production Efficiency. *Frontiers in Microbiology*, *13*, 805963. https://doi.org/10.3389/fmicb.2022.805963

Nami, Y., Vaseghi Bakhshayesh, R., Mohammadzadeh Jalaly, H., Lotfi, H., Eslami, S., & Hejazi, M. A. (2019). Probiotic Properties of Enterococcus Isolated From Artisanal Dairy Products. *Frontiers in Microbiology*, *10*, 300. https://doi.org/10.3389/fmicb.2019.00300

Nocek, J. E., & Kautz, W. P. (2006). Direct-Fed Microbial Supplementation on Ruminal Digestion, Health, and Performance of Pre- and Postpartum Dairy Cattle. *Journal of Dairy Science*, *89*(1), 260–266. https://doi.org/10.3168/jds.S0022-0302(06)72090-2

O'Toole, P. W., & Cooney, J. C. (2008). Probiotic Bacteria Influence the Composition and Function of the Intestinal Microbiota. *Interdisciplinary Perspectives on Infectious Diseases*, *2008*, 1–9. https://doi.org/10.1155/2008/175285

Pukrop, J. (2017). *Inclusion of antibiotic alternatives in feedlot cattle diets: Impact on health status, cattle performance and meat quality*.

Raabis, S., Li, W., & Cersosimo, L. (2019). Effects and immune responses of probiotic treatment in ruminants. *Veterinary Immunology and Immunopathology*, *208*, 58–66. https://doi.org/10.1016/j.vetimm.2018.12.006

Rezaee, P., kasra kermanshahi, R., & Falsafi, T. (2019). Antibacterial activity of lactobacilli probiotics on clinical strains of Helicobacter pylori. *Iranian Journal of Basic Medical Sciences*, *22*(10). https://doi.org/10.22038/ijbms.2019.33321.7953

Rodriguez-Palacios, A., Staempfli, H. R., Duffield, T., & Weese, J. S. (2009). Isolation of bovine intestinal *Lactobacillus plantarum* and *Pediococcus acidilactici* with inhibitory activity against *Escherichia coli* O157 and F5. *Journal of Applied Microbiology*, *106*(2), 393–401. https://doi.org/10.1111/j.1365-2672.2008.03959.x

Sanders, M. E., & Levy, D. D. (2011). The science and regulations of probiotic food and supplement product labeling: Sanders & Levy. *Annals of the New York Academy of Sciences*, *1219*, E1–E23. https://doi.org/10.1111/j.1749-6632.2010.05956.x

Senz, M., van Lengerich, B., Bader, J., & Stahl, U. (2015). Control of cell morphology of probiotic *Lactobacillus acidophilus* for enhanced cell stability during industrial processing. *International Journal of Food Microbiology*, *192*, 34–42. https://doi.org/10.1016/j.ijfoodmicro.2014.09.015

Thieszen, J., Van Bibber, C. L., Axman, J. E., & Drouillard, J. S. (2015). Lactipro (*Megasphaera elsdenii*) Increases Ruminal pH and Alters Volatile Fatty Acids and Lactate During Transition to an 80% Concentrate Diet. *Kansas Agricultural Experiment Station Research Reports*, *1*(1). https://doi.org/10.4148/2378-5977.1036

Thrune, M., Bach, A., Ruiz-Moreno, M., Stern, M. D., & Linn, J. G. (2009). Effects of Saccharomyces cerevisiae on ruminal pH and microbial fermentation in dairy cows. *Livestock Science*, *124*(1–3), 261–265. https://doi.org/10.1016/j.livsci.2009.02.007

Timmerman, H. M., Mulder, L., Everts, H., Van Espen, D. C., Van Der Wal, E., Klaassen, G., Rouwers, S. M. G., Hartemink, R., Rombouts, F. M., & Beynen, A. C. (2005). Health and Growth of Veal Calves Fed Milk Replacers With or Without Probiotics. *Journal of Dairy Science*, *88*(6), 2154–2165. https://doi.org/10.3168/jds.S0022-0302(05)72891-5

Touza-Otero, L., Landin, M., & Diaz-Rodriguez, P. (2024). Fighting antibiotic resistance in the local management of bovine mastitis. *Biomedicine & Pharmacotherapy*, *170*, 115967. https://doi.org/10.1016/j.biopha.2023.115967

US-FDA. 2015. “CPG Sec. 689.100 Direct-Fed Microbial Products.” U.S. Food and Drug Administration, FDA, www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-689100-direct-fed-microbial-products. Accessed Nov. 5, 2024.

Uyeno, Y., Shigemori, S., & Shimosato, T. (2015). Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes and Environments*, *30*(2), 126–132. https://doi.org/10.1264/jsme2.ME14176

Van Koevering, M. T., Secrist, D. S., and Owens, F. N. (1994). Cobactin II for feedlot steers. *1994 Animal Science Research Report, Oklahoma State University*.

Wallace, R. J., Sasson, G., Garnsworthy, P. C., Tapio, I., Gregson, E., Bani, P., Huhtanen, P., Bayat, A. R., Strozzi, F., Biscarini, F., Snelling, T. J., Saunders, N., Potterton, S. L., Craigon, J., Minuti, A., Trevisi, E., Callegari, M. L., Cappelli, F. P., Cabezas-Garcia, E. H., … Mizrahi, I. (2019). A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. *Science Advances*, *5*(7), eaav8391. https://doi.org/10.1126/sciadv.aav8391

Wang, L., Sun, H., Gao, H., Xia, Y., Zan, L., & Zhao, C. (2023). A meta-analysis on the effects of probiotics on the performance of pre-weaning dairy calves. *Journal of Animal Science and Biotechnology*, *14*(1), 3. https://doi.org/10.1186/s40104-022-00806-z

Weinberg, Z. G., Muck, R. E., Weimer, P. J., Chen, Y., & Gamburg, M. (2004). Lactic Acid Bacteria Used in Inoculants for Silage as Probiotics for Ruminants. *Applied Biochemistry and Biotechnology*, *118*(1–3), 001–010. https://doi.org/10.1385/ABAB:118:1-3:001

Zhu, K., Hölzel, C. S., Cui, Y., Mayer, R., Wang, Y., Dietrich, R., Didier, A., Bassitta, R., Märtlbauer, E., & Ding, S. (2016). Probiotic Bacillus cereus Strains, a Potential Risk for Public Health in China. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00718

Chapter 2 - Isolation of ruminal lactobacilli with antibacterial activities against pathogens implicated in liver abscesses of feedlot cattle

Abstract

Liver abscesses, prevalent in cattle fed high-concentrate diets, contribute to significant economic losses in the beef cattle industry. Traditionally, in-feed antimicrobials have been used to mitigate abscess formation by reducing *Fusobacterium necrophorum* populations in the rumen. Yet, liver abscesses still have significant economic impacts in beef cattle production and rising concerns over public health implications of antimicrobial resistance, thus necessitating alternative interventions. This study aimed to investigate the inhibitory potential of lactobacilli, a major group of lactic acid bacteria (LAB), isolated from ruminal contents of liver-abscess-free cattle on key liver abscess pathogens: *F. necrophorum, Trueperella pyogenes,* and *Salmonella enteric*a serovar Lubbock. Using agar-well diffusion assays, we assessed the antimicrobial activity of both pH-adjusted and unadjusted LAB supernatants against these pathogens. Results indicated that while neutral pH supernatants showed no inhibitory effects, unadjusted LAB supernatants demonstrated notable inhibition, particularly against *F. necrophorum*, with a subset of isolates also active against *T. pyogenes* and *S. enterica*. Findings suggest that LAB may suppress liver abscess pathogens via pH-dependent mechanisms and/or other organic compounds, warranting further exploration of LAB as direct-fed microbial candidates. Future research should focus on identifying and characterizing specific LAB strains with potential applications in antimicrobial-free livestock management.

Introduction

Liver abscesses occur in cattle enrolled in rigorous feeding programs and are a common welfare and economic issue for livestock producers and packers. Economic losses for producers occur from reduced performance, such as decreased feed intake and weight gain. For beef packers, economic losses occur in the form of liver condemnation, reduced carcass yield, reduced carcass grade. and therefore, reduced carcass value. Liver abscesses account for 67% of all liver abnormalities, followed by cirrhosis, distoma, and telangiectasis (Brown and Lawrence, 2010). Hepatic abscesses are purulent, encapsulated lesions in the liver that develop from bacterial infections, with bacteria entering via portal circulation (Nagaraja and Chengappa, 1998). It is generally accepted that in cattle, the rumen wall, damaged from chronic acidotic conditions, becomes susceptible to invasion and colonization of ruminal microorganisms (Nagaraja and Chengappa, 1998; Amachawadi and Nagaraja, 2016). This susceptibility leads to rumenitis, consequently allowing liver abscess pathogens to migrate into portal circulation and infect the liver. (Rezac et al., 2014).

Prevalence of liver abscesses is highly variable and can range from 12-32% (Amachawadi and Nagaraja, 2016). Harris et al. (2018) found that 17.8% of steers and heifers and 20.7% of cows and bulls exhibited liver abscesses. Cattle with liver abscesses show little to no clinical signs and diagnosis is not made until slaughter or necropsy (Nagaraja and Chengappa, 1998; Doré, et al., 2007).

Fusobacterium necrophorum originates in the rumen and is part of the normal flora. The bacterium is a Gram-negative, rod-shaped anaerobe (Langworth, 1977; Tan et al., 1994). The primary function of the bacterial species in the rumen is to metabolize lactic acid, and breakdown protein and amino acids (Tadepalli et al., 2009). *Fusobacterium necrophorum* is the major etiologic agent of liver abscesses with *F. necrophorum* subspecies *necrophorum* more frequently isolated than subspecies *funduliforme* (Scanlan and Hathcock, 1983; Tadepalli et al., 2009). The two subspecies differ in their abscess-causing abilities, colony and morphologic characteristics, growth patterns in broth, and virulence factors (Scanlan and Hathcock, 1983). The primary virulence factor for subsp. *necrophorum* is leukotoxin, encoded by gene *lktA*, followed by endotoxic lipopolysaccharide (LPS) (Tadepalli et al., 2009; Deepti et al., 2019). The cytotoxic effects of leukotoxin on ruminal cells aid in penetration and colonization of the rumen wall overcoming phagocytosis from any neutrophilic response (Nagaraja et al., 2005).

Trueperella pyogenes is also part of the normal rumen ecology and originates from the ruminal wall. It is the second most frequently isolated pathogen in liver abscesses and has pathogenic synergy with *F. necrophorum*. *Trueperella pyogenes* has several virulence factors, but its primary virulence factor is a hemolysin, called pyolysin. Leukotoxin from *F. necrophorum* is necessary for the development of abscesses by *T. pyogenes*. This suggests that *T. pyogenes* is a helper organism in the development of liver abscesses in cattle. *Salmonella enterica* serovar Lubbock is commonly found in the lymph nodes and enters the liver via lymphatic drainage or portal blood circulation. This species has been most recently reported in the occurrence of liver abscesses (Amachawadi et al., 2016; Bugarel et al., 2016).

Traditionally, nutritional management has been used reduce the incidence of ruminal acidosis. This includes increased roughage content in the diet, gradual diet step-ups in feed concentration, and uniform feeding intervals and amounts. Traditionally, controlling ruminal acidosis has been reliant on antimicrobial use. In 2017, the Veterinary Feed Directive (VFD) was implemented by the Food and Drug Administration (FDA) to move away from using antimicrobials as growth promoters and requires antimicrobials to be administered under veterinary supervision in beef cattle production. Tylosin (Tylan, Elanco Animal Health, Greenfield, IN) is an in-feed macrolide used in the feedlot industry to prevent liver abscesses. Because tylosin is labeled for reducing the incidence of liver abscesses and not for growth promotion, it is permitted under the VFD. Tylosin has been shown to reduce the *F. necrophorum* population in the rumen, therefore reducing the incidence of liver abscesses up to 50% and decreasing the risk from 30% to 8% (Wileman et al., 2009; Theurer and Amachawadi, 2022). However, inclusion of in-feed tylosin does not eliminate the problem entirely. Additionally, tylosin belongs in the same drug class as erythromycin, a widely used drug in human medicine. With growing awareness around antimicrobial use in food animals, there are concerns regarding emergence and dissemination of gut bacteria harboring resistance genes (Agga et al., 2023; Cazer et al., 2020). Therefore, a more effective and less medically important intervention is desirable.

Probiotics, or Direct-Fed Microbials, are defined by the United States Food & Drug Administration (FDA) as "products that are purported to contain live (viable) micro-organisms (bacteria and/or yeast)". In other words, DFM are feed additives containing viable microbial species. These microbes are labeled by the FDA as "Generally Regarded As Safe" (or GRAS). This means they are considered non-pathogenic, safe, and beneficial to the environment of the gastrointestinal tract and the host animal. Various responses have been reported with the use of DFM in ruminant diets, such as increased milk yield, improved feed efficiency, reduction of ruminal acidosis, and improved immune function (Adjei-Fremah et al., 2018; Desnoyers et al., 2009; Nalla et al., 2022). However, results can be variable between researchers and the mode of action is still debated (Molloy et al., 2011; Ban and Guan, 2021; Lawrence et al., 2020) In the production cattle industry, DFM are used to target the rumen microbial population to positively affect fermentation of feedstuffs, specifically digestibility and degradability. Additional benefits include stabilizing pH and lactate levels, and consequently performance outcomes.

Direct Fed Microbials predominantly utilize single or mixed cultures of bacteria like *Lactobacillus*, *Enterococcus*, and *Bifidobacterium*, and/or yeasts like *Saccharomyces* and *Candida* (Krehbiel et al., 2003; Anee et al., 2021). These species are chosen for their acid tolerance and based on their ability to produce or utilize lactate. It is thought that the increase in lactic acid in the rumen will stimulate lactic acid utilizers, increasing the stability of the rumen pH (Lawrence et al., 2020). Since lactic acid bacteria (LAB) are facultative anaerobes, the rumen is an ideal habitat for these organisms. For supplementation in cattle, probiotics are commonly formulated into the animal's diet as a feed additive. Improvement in performance is often attributed to increased nutrient digestibility and health outcomes (Ban and Guan, 2021). While the exact mode of action between probiotic species is still investigated, it has been shown that some species of lactobacilli possess antimicrobial activities against both Gram-positive and Gram-negative bacteria. These antimicrobial effects are due to production of metabolites like organic acids, hydrogen peroxide, and bacteriocins, and because of these attributes, have been identified as potential probiotic candidates (Touza-Otero et al., 2023; Muzaffar et al., 2021).

We hypothesize that cattle free of liver abscesses possibly harbor bacteria that inhibit *Fusobacterium necrophorum* in the rumen, lowering the population density. Therefore, the aim of this study was to isolate species of *Lactobacillus* from ruminal contents of liver-abscess-free cattle possessing anti-*F. necrophorum*, anti-*T. pyogenes*, and anti-*S. enterica* activities, to potentially reduce *F. necrophorum* in the rumen and subsequently prevent or reduce liver abscess in feedlot cattle. The objective of our study is to investigate the presence of *Lactobacilli* strains with anti-*Fusobacterium* activity in the ruminal fluid of slaughtered cattle. Additionally, we are also investigating the inhibitory activity of *Lactobacilli* strains against *Trueperella pyogenes* and *Salmonella enterica* serovar Lubbock strains, which are reported to be isolated from liver abscess of cattle.

Materials and methods

Ruminal content collection

Cattle with healthy livers were selected at slaughter to collect ruminal content samples for the isolation of lactic acid bacteria, which were then evaluated for inhibitory activity against major liver abscess pathogens – *F. necrophorum*, *T. pyogenes,* and *S. enterica* serovar Lubbock. Ruminal contents from 36 feedlot cattle originating from 18 different feedlots were collected at a slaughter plant in Kansas from July 1 to August 26, 2019. An additional 20 samples were collected at a slaughter plant in Nebraska from December 5, 2019 to February 26, 2020. After evisceration, the rumen was opened with a knife, and whole rumen contents (WRC) representing liquids and solids were collected and transferred into 50 mL tubes. The tubes were filled completely to maximize anaerobiosis, capped and placed in a cooler with ice and transported to the anaerobe laboratory.

Isolation of lactic acid bacteria from ruminal contents

Ruminal content samples were individually strained through 4 layers of cheese cloth and pH was recorded. Ten-fold serial dilutions of the strained ruminal fluid (SRF) in anaerobic buffered peptone water (BPW) was performed (10^{-1} and 10^{-2}). One hundred microliters of undiluted SRF, 10^{-1}, and 10^{-2} dilutions were spread-plated onto anaerobic modified De Man, Rogosa and Sharpe (MRS) agar plates (Thermo Fisher Scientific, Waltham, MA). Plates were incubated for 24h at 37˚C in an anaerobic glove box (80% N2, 10% H2, 10% CO2; Forma Scientific Inc., Marietta, OH). After incubation, morphologically distinct colonies (up to 2 colonies/dilution) were selected and inoculated into 10 ml anaerobic MRS broth and incubated overnight at 37˚C.

Enrichment of Strained Ruminal Fluid

Strained Ruminal Fluid (5 mL) was incubated in 5 ml of anaerobic BPW with 100 mg dextrose at 37˚C for 12h. After incubation the pH was recorded, and ten-fold serial dilutions of the culture was performed (10^{-1} to 10^{-3}) using anaerobic BPW. One-hundred microliters of 10^{-2} and 10^{-3} dilutions were spread-plated onto anaerobic MRS agar and incubated for 24 h at 37˚C in an anaerobic glove box. After incubation, morphologically distinct colonies (up to 2 colonies/dilution) were selected, inoculated into anaerobic MRS broth and incubated overnight at 37˚C.

Preparation of cell-free bacterial culture supernatant

The pH of the lactic acid bacterial (LAB) cultures prepared above were recorded. The culture was centrifuged at 4,000 rpm for 10 minutes and the supernatant was split into two aliquots. The pH of one aliquot was adjusted to 7.0 (± 0.3) with 0.1N NaOH, and the other was left as-is. The aliquots were filtered using 0.22 µm membrane filters.

Qualitative determination of antimicrobial activities of the cultures on Fusobacterium necrophorum

A strain of *F. necrophorum*, previously isolated from liver abscesses and stored at -70° C, was streaked onto Sheep Blood Agar (SBA) plates (Remel, Inc., Lenexa, KS) and incubated for 48 h at 37° C in anaerobic glove box. A single colony from the blood agar plates were then inoculated into 10 mL of pre-reduced (with 0.05% cysteine-HCl) sterile anaerobic brain heart infusion (BHI) broth (PRAS-BHI; Becton Dickinson, Sparks, MD) and incubated for 10 h at 37˚ C. Using a 4 mm cork borer, 4 wells were aseptically punched into the SBA plates and the bottom sealed with melted agar. The plate was then inoculated with the *Fusobacterium* culture so that a uniform bacterial lawn appeared after overnight incubation. One hundred microliters of each of the unadjusted and pH-adjusted supernatants of the LAB cultures were pipetted into each well. The plates were incubated for 24 h at 37˚ C in the anaerobic glove box. Results were recorded as inhibition or no zone of inhibition. If a zone was present, the diameter was recorded in millimeters.

Qualitative determination of antimicrobial activities of the cultures on Trueperella pyogenes

A strain of *T. pyogenes* previously isolated from liver abscesses and stored at -70° C, was streaked onto SBA plates and incubated for 48 h at 37˚C in a 5% CO_2 incubator. A single colony was inoculated into 10 mL of Tryptic Soy Broth (TSB; Becton, Dickinson Co.) and incubated for 12 h at 37° C. Using a 4 mm cork borer, 4 wells were aseptically punched into Mueller-Hinton blood agar plates (Remel, Inc.) and the bottom sealed with melted agar. The plates were inoculated with the *Trueperella* culture so that a uniform bacterial lawn appears after overnight incubation. One hundred microliters of the unadjusted and pH-adjusted supernatant of LAB cultures were pipetted into each well. The plates were incubated in 5% CO_2 incubator for 24h at 37˚C. Results were recorded as inhibition or no zone of inhibition. If a zone was present, the diameter was recorded in millimeters.

***Qualitative determination of antimicrobial activities of the cultures on Salmonella enterica* serovar Lubbock**

A strain of *Salmonella enterica* serovar Lubbock previously isolated from liver abscesses and stored at -70° C, was streaked onto SBA plates and incubated for 24 h at 37˚C. A single colony was inoculated into 10 mL of Tryptic Soy Broth (TSB) and incubated for 10 h at 37° C. Using a 4 mm cork borer, 4 wells were aseptically punched into BHI agar plates and the bottom sealed with melted agar. The plates were inoculated with the *Salmonella* culture so that a uniform bacterial lawn appears after overnight incubation. One hundred microliters of the unadjusted and pH-adjusted supernatant of LAB cultures were pipetted into each well. The plates were incubated for 24 h at 37˚C. Results were recorded as inhibition or no zone of inhibition. If a zone was present, the diameter was recorded in millimeters.

Results

Whole rumen content collection

A total of 560 lactic acid bacterial isolates were collected from the WRC samples. The average pH of strained ruminal fluid was 5.92 (range 5.18-7.55). Up to 10 colonies from the MRS agar plates spread plated with serial ten-fold dilutions of pre-enriched and enriched SRF (Figure 1.) were selected for use. The average pH of the enriched SRF was 4.87 (range 4.21-5.46) and the average pH of the LAB supernatant was 5.03 (range 5.35-6.30).

Agar-well diffusion assays

Of the 560 challenged LAB supernatants that were pH adjusted, there was no evidence of antimicrobial activity against *Trueperella pyogenes*, *Salmonella enterica* serovar Lubbock, or *Fusobacterium necrophorum* subsp. *necrophorum* (Table 1.) However, there were zones of inhibition present in some of the non-pH adjusted LAB supernatants. Forty-three LAB isolates originating from 14 rumen samples showed inhibitory activity against *T. pyogenes* (Fig 2) with zones of inhibition ranging from 10-13 mm. One-hundred and twenty-eight LAB isolates originating from 18 samples, showed inhibitory activity against *Salmonella* (Fig 3) with zones of inhibition ranging from 10-16 mm. One-hundred and sixty-three LAB isolates originating from 31 samples showed inhibitory activity against *F. necrophorum* (Fig 4), with zones of inhibition ranging from 11-17 mm. A total of 14 LAB isolates showed inhibition against all three pathogens (Table 2.) and only 1 showing inhibition of 17 mm (Table 3.).

Of the 14 that displayed inhibition against *F. necrophorum, S. enterica* and *T. pyogenes*, 10 were chosen to be experimentally repeated based on initial size of inhibitory zone. The pH of each culture and results against all three pathogens were variable between experiments. Additionally, 10 isolates of the 163 that displayed inhibition against only *F. necrophorum* were chosen to be experimentally repeated based on size of initial inhibitory zone. Again, the pH of each culture and results against *F. necrophorum* were also variable between experiments, indicating that initial inhibitions are likely influenced by LAB culture pH (Tables 4 and 5.)

Discussion

Liver abscesses are a common sequela to high-concentrate diets aimed at increasing animal production in feedlot and dairy cattle (Broadway et al., 2024). Liver abscesses have predominantly been mitigated by in-feed antimicrobials like tylosin, chlortetracycline, and oxytetracycline. Although tylosin has been shown to be the most effective at reducing the incidence of liver abscesses by 40-70%, there is still room in the animal health industry for improvement (Nagaraja and Chengappa, 1998; Theurer and Amachawadi, 2022). This is important not just for animal health perspective but economically too as liver abscesses are estimated to cost $400 million annually (Broadway et al., 2024). Additionally, as awareness grows around antimicrobial stewardship and concerns regarding antimicrobial resistance, the need for alternative therapies grows as well (Uyeno et al., 2015).

Our findings suggest that native ruminal lactic acid bacteria have potential to exhibit antimicrobial affects against *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella enterica* serovar Lubbock – prevalent liver abscess pathogens. Additionally, our results indicate that pH likely plays a role in pathogen survivability *in vitro*. It is not clear whether supernatant pH is a predominant factor in inhibition or if pH is working synergistically with other organic compounds the LAB could be producing. Several studies have shown inhibition of other Gram-negative pathogens like *E. coli* and *S. enterica*, and *Campylobacter* by lactic acid bacteria (Bertin et al., 2017; Mansilla et al., 2023; Brashears et al., 2003; Chen et al., 2021; Rodriguez-Palacios et al., 2009). Our findings in combination with previous studies further support that *Lactobacillus* species could be useful for inhibiting Gram-negative pathogenic bacteria in cattle. Additionally, the use of LAB species as probiotics in food animals may play a promising role in the era of limited antimicrobial therapy options and rapid progression of drug resistance.

While these results are hopeful, there are several limitations to this study. First, since there was not any characterization of the LAB supernatant, it is unclear if organic compounds or bacteriocins were produced that could have aided in pathogen suppression. Second, because there was not further identification and characterization of the ruminal isolates, it is unknown if these LAB harbor antimicrobial resistance genes or if any of our isolates were duplicate species. Overall, this study demonstrates how probiotics have potential to reduce liver abscess pathogens and can be used to develop antimicrobial alternatives. This study also supports the need for further research to be conducted on direct-fed microbial effects.

Conclusion

Our data demonstrated that the investigated lactic acid bacteria exhibited some antibacterial activity on major liver abscess pathogens. *Fusobacterium necrophorum* had the greatest zone of inhibition from the LAB isolates as well as the highest number of isolates to suppress lawn growth. Some variability between results is likely due to pH levels in the supernatant. This is supported when comparing each experimental result to their respective supernatant pH. The selective inhibition and variability between bacterial pathogens are interesting observations that need to further investigation. Our study supports additional testing on these ruminal isolates, particularly pertaining to identification and characterization for there to be potential for utilization in probiotic or direct-fed microbial products.

Literature cited

Adjei-Fremah, S., Ekwemalor, K., Worku, M., & Ibrahim, S. (2018). Probiotics and Ruminant Health. In S. Enany (Ed.), *Probiotics—Current Knowledge and Future Prospects*. InTech. https://doi.org/10.5772/intechopen.72846

Agga, G. E., Galloway, H. O., Appala, K., Mahmoudi, F., Kasumba, J., Loughrin, J. H., & Conte, E. (2023). Effect of continuous in-feed administration of tylosin to feedlot cattle on macrolide and tetracycline resistant enterococci in a randomized field trial. *Preventive Veterinary Medicine*, *215*, 105930. https://doi.org/10.1016/j.prevetmed.2023.105930

Amachawadi, R. G., & Nagaraja, T. G. (2016). Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle12. *Journal of Animal Science*, *94*(4), 1620–1632. https://doi.org/10.2527/jas.2015-0261

Amachawadi, R. G., Thomas, M., Nagaraja, T. G., & Scaria, J. (2016). Genome Sequences of Salmonella enterica subsp. *Enterica* Serovar Lubbock Strains Isolated from Liver Abscesses of Feedlot Cattle. *Genome Announcements*, *4*(3), e00319-16. https://doi.org/10.1128/genomeA.00319-16

Anee, I. J., Alam, S., Begum, R. A., Shahjahan, R. M., & Khandaker, A. M. (2021). The role of probiotics on animal health and nutrition. *The Journal of Basic and Applied Zoology*, *82*(1), 52. https://doi.org/10.1186/s41936-021-00250-x

Ban, Y., & Guan, L. L. (2021). Implication and challenges of direct-fed microbial supplementation to improve ruminant production and health. *Journal of Animal Science and Biotechnology*, *12*(1), 109. https://doi.org/10.1186/s40104-021-00630-x

Bertin, Y., Habouzit, C., Dunière, L., Laurier, M., Durand, A., Duchez, D., Segura, A., Thévenot-Sergentet, D., Baruzzi, F., Chaucheyras-Durand, F., & Forano, E. (2017). Lactobacillus reuteri suppresses E. coli O157:H7 in bovine ruminal fluid: Toward a pre-slaughter strategy to improve food safety? *PLOS ONE*, *12*(11), e0187229. https://doi.org/10.1371/journal.pone.0187229

Brashears, M. M., Jaroni, D., & Trimble, J. (2003). Isolation, Selection, and Characterization of Lactic Acid Bacteria for a Competitive Exclusion Product To Reduce Shedding of Escherichia coli O157:H7 in Cattle. *Journal of Food Protection*, *66*(3), 355–363. https://doi.org/10.4315/0362-028X-66.3.355

Broadway, P. R., Nagaraja, T. G., Lawrence, T. E., Galyean, M. L., & Hales, K. E. (2024). Liver abscesses—New perspectives on a historic fed-cattle issue*†‡. *Applied Animal Science*, *40*(3), 237–243. https://doi.org/10.15232/aas.2023-02498

Brown, T. R., & Lawrence, T. E. (2010). Association of liver abnormalities with carcass grading performance and value. *Journal of Animal Science*, *88*(12), 4037–4043. https://doi.org/10.2527/jas.2010-3219

Bugarel, M., Den Bakker, H. C., Nightingale, K. K., Brichta-Harhay, D. M., Edrington, T. S., & Loneragan, G. H. (2015). Two Draft Genome Sequences of a New Serovar of Salmonella enterica, Serovar Lubbock. *Genome Announcements*, *3*(2), e00215-15. https://doi.org/10.1128/genomeA.00215-15

Cazer, C. L., Eldermire, E. R. B., Lhermie, G., Murray, S. A., Scott, H. M., & Gröhn, Y. T. (2020). The effect of tylosin on antimicrobial resistance in beef cattle enteric bacteria: A systematic review and meta-analysis. *Preventive Veterinary Medicine*, *176*, 104934. https://doi.org/10.1016/j.prevetmed.2020.104934

Chen, C.-C., Lai, C.-C., Huang, H.-L., Su, Y.-T., Chiu, Y.-H., Toh, H.-S., Chiang, S.-R., Chuang, Y.-C., Lu, Y.-C., & Tang, H.-J. (2021). Antimicrobial ability and mechanism analysis of Lactobacillus species against carbapenemase-producing Enterobacteriaceae. *Journal of Microbiology, Immunology and Infection*, *54*(3), 447–456. https://doi.org/10.1016/j.jmii.2020.01.005

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C., & Sauvant, D. (2009). Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. *Journal of Dairy Science*, *92*(4), 1620–1632. https://doi.org/10.3168/jds.2008-1414

Doré, E., Fecteau, G., Hélie, P., & Francoz, D. (2007). Liver Abscesses in Holstein Dairy Cattle: 18 Cases (1992–2003). *Journal of Veterinary Internal Medicine*, *21*(4), 853–856. https://doi.org/10.1111/j.1939-1676.2007.tb03031.x

Harris, M. K., Eastwood, L. C., Boykin, C. A., Arnold, A. N., Gehring, K. B., Hale, D. S., Kerth, C. R., Griffin, D. B., Savell, J. W., Belk, K. E., Woerner, D. R., Hasty, J. D., Delmore, R. J., Martin, J. N., Lawrence, T. E., McEvers, T. J., VanOverbeke, D. L., Mafi, G. G., Pfeiffer, M. M., … Stelzleni, A. M. (2018). National Beef Quality Audit–2016: Assessment of cattle hide characteristics, offal condemnations, and carcass traits to determine the quality status of the market cow and bull beef industry. *Translational Animal Science*, *2*(1), 37–49. https://doi.org/10.1093/tas/txx002

Krehbiel, C. R., Rust, S. R., Zhang, G., & Gilliland, S. E. (2003). *Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action1,2*. 15.

Langworth, B. F. (1977). Fusobacterium necrophorum: Its Characteristics and Role as an Animal Pathogen. *BACTERIOL. REV.*, *41*.

Lawrence, M., Polukis, S., Barnard, A. M., Miller, M. A., Kung, L., & Gressley, T. F. (2021). Evaluating the effects of Lactobacillus animalis and Propionibacterium freudenreichii on performance and rumen and fecal measures in lactating dairy cows. *Journal of Dairy Science*, *104*(4), 4119–4133. https://doi.org/10.3168/jds.2020-19291

Mansilla, F. I., Miranda, M. H., Uezen, J. D., Maldonado, N. C., D'Urso Villar, M. A., Merino, L. A., Vignolo, G. M., & Nader-Macias, M. E. F. (2023). Effect of probiotic lactobacilli supplementation on growth parameters, blood profile, productive performance, and fecal microbiology in feedlot cattle. *Research in Veterinary Science*, *155*, 76–87. https://doi.org/10.1016/j.rvsc.2023.01.003

Muzaffar, K., Jan R., Bhat N. A., Gani A., & Shagoo M. A. (2021). *Chapter 25 Commercially Available Probiotics and Prebiotics Used in Human and Animal Nutrition*.

Nagaraja, T. G., & Chengappa, M. M. (1998). Liver abscesses in feedlot cattle: A review. *Journal of Animal Science*, *76*(1), 287. https://doi.org/10.2527/1998.761287x

Nagaraja, T. G., S. K. Narayanan, G. C. Stewart, and M. M. Chengappa. 2005. *Fusobacterium necorphorum* infections in animals: Pathogenesis and pathogenic mechanisms. Anaerobe 11:239-246.

Nalla, K., Manda, N. K., Dhillon, H. S., Kanade, S. R., Rokana, N., Hess, M., & Puniya, A. K. (2022). Impact of Probiotics on Dairy Production Efficiency. *Frontiers in Microbiology*, *13*, 805963. https://doi.org/10.3389/fmicb.2022.805963

Pillai, D. K., Amachawadi, R. G., Baca, G., Narayanan, S., & Nagaraja, T. G. (2019). Leukotoxic activity of Fusobacterium necrophorum of cattle origin. *Anaerobe*, *56*, 51–56. https://doi.org/10.1016/j.anaerobe.2019.02.009

Rezac, D. J., Thomson, D. U., Bartle, S. J., Osterstock, J. B., Prouty, F. L., & Reinhardt, C. D. (2014). Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle1. *Journal of Animal Science*, *92*(6), 2595–2602. https://doi.org/10.2527/jas.2013-7222

Rodriguez-Palacios, A., Staempfli, H. R., Duffield, T., & Weese, J. S. (2009). Isolation of bovine intestinal *Lactobacillus plantarum* and *Pediococcus acidilactici* with inhibitory activity against *Escherichia coli* O157 and F5. *Journal of Applied Microbiology*, *106*(2), 393–401. https://doi.org/10.1111/j.1365-2672.2008.03959.x

Scanlan, C. M., & Hathcock, T. L. (1983.). *BOVINE RUMENITIS - LIVER ABSCESS COMPLEX: A BACTERIOLOGICAL REVIEW*.

Tadepalli, S., Narayanan, S. K., Stewart, G. C., Chengappa, M. M., & Nagaraja, T. G. (2009). Fusobacterium necrophorum: A ruminal bacterium that invades liver to cause abscesses in cattle. *Anaerobe*, *15*(1–2), 36–43. https://doi.org/10.1016/j.anaerobe.2008.05.005

Tan, Z. L., Nagaraja, T. G., & Chengappa, M. M. (1994). Selective enumeration of Fusobacterium necrophorum from the bovine rumen. *Applied and Environmental Microbiology*, *60*(4), 1387–1389. https://doi.org/10.1128/aem.60.4.1387-1389.1994

Theurer, M. E., & Amachawadi, R. G. (2022). Antimicrobial and Biological Methods to Control Liver Abscesses. *Veterinary Clinics of North America: Food Animal Practice*, *38*(3), 383–394. https://doi.org/10.1016/j.cvfa.2022.07.001

Uyeno, Y., Shigemori, S., & Shimosato, T. (2015). Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes and Environments*, *30*(2), 126–132. https://doi.org/10.1264/jsme2.ME14176

Wileman, B. W., Thomson, D. U., Reinhardt, C. D., & Renter, D. G. (2009). Analysis of modern technologies commonly used in beef cattle production: Conventional beef production versus nonconventional production using meta-analysis. *Journal of Animal Science*, *87*(10), 3418–3426. https://doi.org/10.2527/jas.2009-1778

Figures

Figure 1. *Lactobacillus* colonies on MRS agar

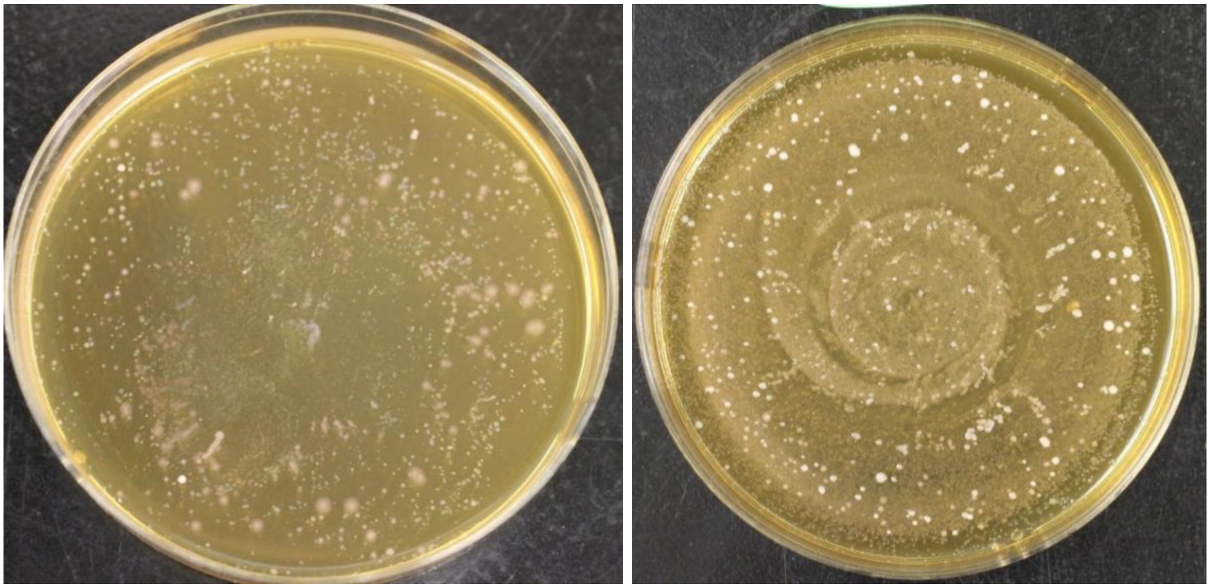

Figure 2. Antimicrobial activity of *Lactobacillus* cultures on *Trueperella pyogenes*

Figure 3. Antimicrobial activity of *Lactobacillus* cultures on *Salmonella enterica* serovar Lubbock

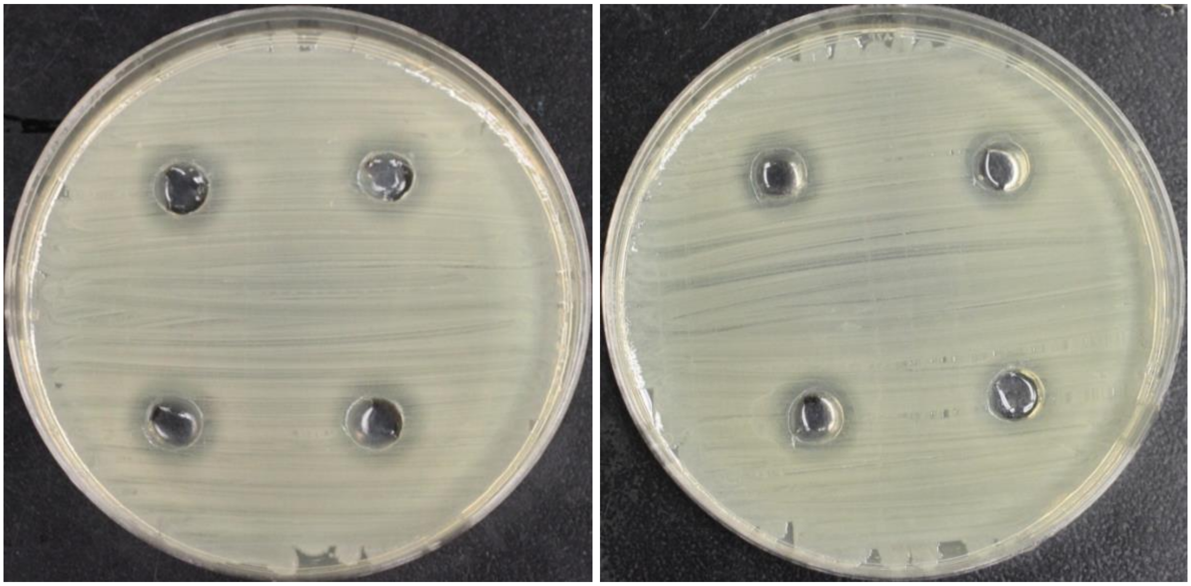

Figure 4. Antimicrobial activity of *Lactobacillus* cultures on *Fusobacterium necrophorum*

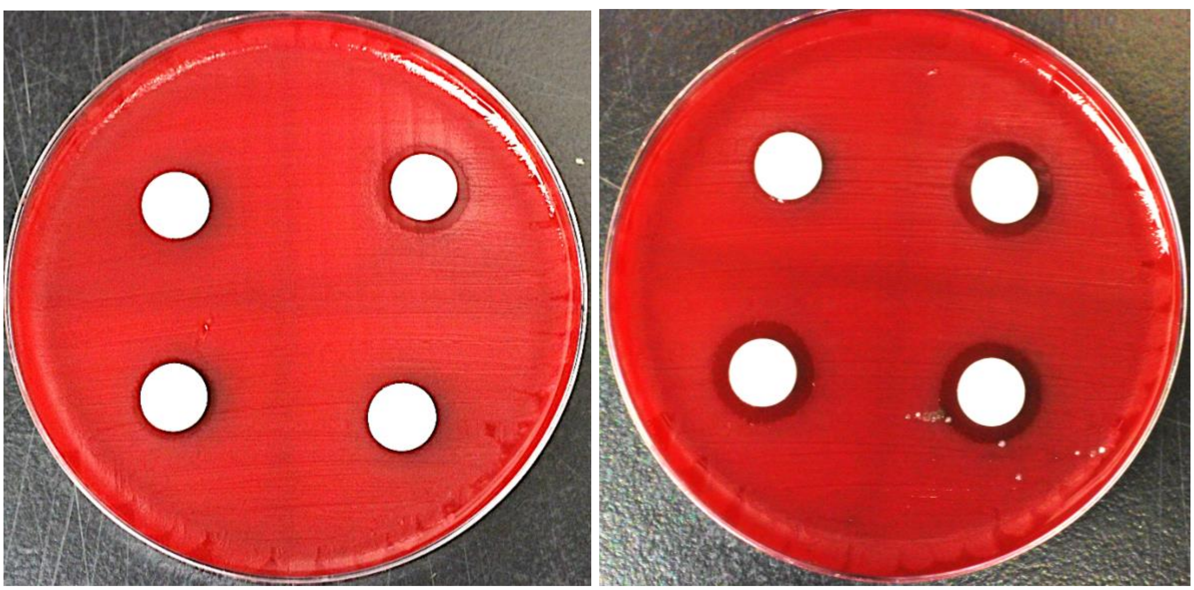

Figure 5. Negative control plate

Tables

Table 1. pH values of strained rumen fluid, enriched cultures, and Lactobacillus supernatants

pH Values				
Rumen Samples	**# Isolates**	Average pH (Range)		
		Strained Rumen Fluid	**Enriched Culture**	**LAB Supernatant**
1-6	60	6.20 (5.26-7.55)	4.97 (4.78-5.25)	4.97 (4.65-5.31)
7-16	100	5.58 (5.32-6.00)	4.85 (4.71-5.07)	4.81 (4.35-5.70)
17-26	100	5.46 (5.18-5.86)	4.77 (4.59-4.98)	4.82 (4.47-5.47)
27-36	100	4.49 (5.38-6.47)	4.87 (4.62-5.46)	5.49 (5.18-6.30)
37-46	100	6.73 (5.78-7.51)	4.94 (4.72-5.31)	5.10 (4.67-5.70)
47-56	100	7.07 (6.52-7.34)	4.83 (4.21-5.42)	4.97 (4.71-5.72)
Mean (Range)		**5.92 (5.18-7.55)**	**4.87 (4.21-5.46)**	**5.03 (5.35-6.30)**

Table 2. Inhibition of *Lactobacillus* cultures on *F. necrophorum*, *T. pyogenes*, and *S. enterica*

Zone of inhibition (mm)							
Rumen Samples	**# Isolates**	Number of positive isolates (Zone diameter range in millimeters)					
		Fusobacterium necrophorum		***Trueperella pyogenes***		***Salmonella enterica***	
		Non-pH Adjusted	**pH Adjusted**	**Non-pH Adjusted**	**pH Adjusted**	**Non-pH Adjusted**	**pH Adjusted**
1-6	60	26 (11-17)	0 (0)	7 (11-13)	0 (0)	25 (11-13)	0 (0)
7-16	100	64 (11-16)	0 (0)	0 (0)	0 (0)	6 (11-12)	0 (0)
17-26	100	28 (11-15)	0 (0)	0 (0)	0 (0)	11 (10-12)	0 (0)
27-36	100	22 (11-15)	0 (0)	34 (10-13)	0 (0)	79 (11-16)	0 (0)
37-46	100	10 (11-14)	0 (0)	2 (10-10)	0 (0)	2 (10-11)	0 (0)
47-56	100	13 (12-15)	0 (0)	0 (0)	0 (0)	5 (10-12)	0 (0)
Total (Range)		**163 (11-17)**	**0 (0)**	**43 (10-13)**	**0 (0)**	**128 (10-16)**	**0 (0)**

Table 3. Total number of isolates of *Lactobacillus* that showed inhibition (n=334)

Pathogen	Total # isolates
Fusobacterium necrophorum	163
Trueperella pyogenes	43
Salmonella enterica	128
F. necrophorum & T. pyogenes	21
F. necrophorum & S. enterica	41
F. necrophorum, T. pyogenes, & S. enterica	14

Table 4. Zone diameters with the number of *Lactobacillus* isolates that showed inhibition for each experimental group

Zone diameter (mm), No. of isolates positive				
Pathogen	**(11-12mm)**	**(13-14 mm)**	**(15-16 mm)**	**(17+ mm)**
Fusobacterium necrophorum	76	60	26	1
Trueperella pyogenes	41	2	0	0
Salmonella enterica	70	49	9	0

Table 5. Ten lactic acid bacterial (LAB) isolates chosen for their inhibition of all three bacterial species, *Fusobacterium necrophorum* (F), *Trueperella pyogenes* (T), and *Salmonella enterica* (S)

Ten isolates selected for greater inhibition of *F. necrophorum*																	
LAB	**SRF[1]/E[2]**	**pH**	Zone (mm)			**pH**	Zone (mm)			**pH**	Zone (mm)			**pH**	Zone (mm)		
			F	**T**	**S**		**F**	**T**	**S**		**F**	**T**	**S**		**F**	**T**	**S**
1-A1	SRF	4.9	17	0	0	5.54	14	11	12	ND[3]	ND[3]	ND[3]	ND[3]	4.62	15	11	13
1-C2	SRF	5.03	16	0	0	4.61	13	0	11	ND[3]	ND[3]	ND[3]	ND[3]	5.27	0	0	0
7-B2	SRF	4.76	16	0	0	4.6	13	0	11	4.71	13	0	12	5.46	0	0	0
12-B2	SRF	4.95	16	0	0	4.89	0	0	0	ND[3]	ND[3]	ND[3]	ND[3]	5.4	0	0	0
14-A2	SRF	4.56	16	0	0	4.61	12	0	0	ND[3]	ND[3]	ND[3]	ND[3]	4.97	14	0	14
15-B1	SRF	4.38	15	0	0	4.64	14	11	13	4.64	14	0	12	5.06	13	0	13
18-A2	SRF	4.48	15	0	0	4.53	14	12	12	4.73	14	11	12	5.55	0	0	0
19-B2	SRF	4.52	15	0	10	4.56	14	12	12	4.5	15	11	12	5.22	0	0	0
21-A1	SRF	4.5	15	0	0	4.57	14	12	11	4.58	14	11	11	4.87	13	0	14
27-E2	E	6.02	15	0	0	4.77	12	0	0	ND[3]	ND[3]	ND[3]	ND[3]	4.65	15	11	14

[1]SRF = Strained rumen fluid

[2]E = Enriched strained rumen fluid

[3]ND = No data available

Table 6. Ten lactic acid bacterial isolates (LAB) chosen for their inhibition F. necrophorum only

Ten isolates selected for inhibition of all three pathogens																	
LAB	**SRF[1]/E[2]**	pH	Zone (mm)			pH	Zone (mm)			pH	Zone (mm)			pH	Zone (mm)		
			F	**T**	**S**		**F**	**T**	**S**		**F**	**T**	**S**		**F**	**T**	**S**
3-D1	E	4.77	13	11	11	4.79	12	0	11	5.65	13	0	11	4.67	14	0	14
3-E1	E	4.65	12	13	12	4.91	11	0	11	ND[3]	ND[3]	ND[3]	ND[3]	4.81	15	0	13
29-A1	SRF	5.18	14	13	14	4.87	11	0	11	ND[3]	ND[3]	ND[3]	ND[3]	5.05	0	0	13
31-C1	SRF	5.34	13	12	15	4.95	0	0	12	4.88	11	0	12	4.99	11	0	13
33-C2	SRF	5.37	13	11	14	4.92	0	0	11	ND[3]	ND[3]	ND[3]	ND[3]	5.07	12	0	13
34-B1	SRF	5.23	14	15	14	4.6	14	11	12	4.68	14	12	12	5.06	13	0	14
34-C1	SRF	5.27	15	12	13	4.54	15	11	13	4.54	14	12	12	5.38	0	0	12
34-D1	E	5.2	13	12	13	4.55	13	12	12	ND[3]	ND[3]	ND[3]	ND[3]	5.55	0	0	12
34-D2	E	5.18	15	12	14	4.71	13	0	13	4.7	14	0	12	5.36	0	0	13
36-B1	SRF	5.26	11	12	14	4.59	15	11	12	ND[3]	ND[3]	ND[3]	ND[3]	5.18	0	0	13

[1]SRF = Strained rumen fluid

[2]E = Enriched strained rumen fluid

[3]ND = No data available