The Role of Probiotics in Prevention of Ovine Enterotoxaemia

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Abstract

Ovine enterotoxaemia is caused by anaerobic gram-positive bacteria *Clostridium perfringens* type C and D which commonly inhabit in the sheep's gut flora. It results in toxemia and mortality due to intestinal epithelium absorbing epsilon toxin (ε). Due to intake of high starch diets an anaerobic and nutrient rich environment creates and as a result rapid bacterial proliferation occurs in the colon and release toxins which damages intestinal epithelium and furthur enter into the systemic circulation causing vascular damage, organ failure and death. In acute form of disease, pathogonomic signs are; diarrhea, stomach pain, severe shock with frigid extremities, opisthotonous and sporadic convulsions. Probiotics which are live microorganisms when administered in adequate amounts improve the gut health. They play crucial role in the inhibition of *C. perfringens* growth through production of bacteriocins and decreasing intestinal pH e.g *Lactobacillus, Bifidobacterium* and *Saccharomyces* species. Supplementation strategies of probiotics administration is through feed additives, water supplements or direct oral dosing but ensure consistent delivery for better efficacy. The benifits and limitation of probiotics have been discussed in this chapter.

Keywords: Enterotoxemia, *Clostridium perfringens*, Starch diets, Probiotics

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Introduction

1.1 Overview of Ovine Enterotoxaemia

The severe and acute disorder known as "pulpy kidney disease," or ovine enterotoxaemia, is mainly caused by *Clostridium perfringens* types C and D. Although these spore-forming, gram-positive bacteria are common in sheep's gut flora, they have the ability to grow rapidly and create toxins under the suitable circumstances. Its morbidity and mortality rates are significant, and it mostly affects the lambs and sheep that are on high energy diets (Uzal & Songer, 2019). Farmers suffer significant financial losses as result of the disease's high mortality rate and widespread incidence in sheep across the globe. It is primarily brought on by *Clostridium perfringens* type D and caused by the intestinal epithelium absorbing epsilon toxin, which results in toxemia and mortality. One of the main risk factors is excessive carbohydrate feeding along with poor management practices. The afflicted sheep exhibit the typical symptoms of pyrexia, which can result in shock and death, and diarrhea, with or without bloody diarrhea. Pulmonary edema and fibrinohemorrhagic enterocolitis are both associated with necrotic lesions. Histopathological abnormalities include cerebral white matter degeneration, small intestine necrotizing enteritis, and perivascular edema. Finding intestinal tissue and fluids that contain epsilon toxin is the goal of ultimate diagnosis. There are many more bacteriological and serological diagnostic tools available. Immunoprophylaxis and supportive therapy are two treatment strategies that are followed by appropriate care procedures. This chapter offers a thorough analysis of the various aspects of enterotoxemic in sheep (Rajamohan & Rajasekaran, 2025).

1.2 Current Challenges in Preventing Enterotoxaemia

Although *C. perfringens* toxin-targeting vaccines are successful, including inconsistent effectiveness and the need for booster shots. Furthermore, for large-scale operations, dietary changes to prevent excessive fermentation may not be feasible (Rodriguez-Palacios et al., 2013). The challenges underscore the need for alternative preventive measures. Ovine enterotoxaemia is difficult to diagnose, and many tests are frequently needed for the better sensitivity and specificity than a single test. The history, clinical symptoms, and the gross lesions observed during post-mortem examination are used to make a preliminary diagnosis of the enterotoxaemia in sheep and goats. However, a number of laboratory diagnostic methods are needed to confirm the diagnosis. The most widely recognized standard for the definitively diagnosing the enterotoxaemia in intestinal contents is the presence of toxins from *C. perfringens*. Additional diagnostic techniques include urine glucose test and intestinal mucosa gram-stained smears. If the aforementioned diagnostic tests are negative, it is imperative that enterotoxaemia cannot be ruled out (Pawaiya et al., 2020). However, some other important tests can also be used to diagnose the pulpy kidney disease in sheep and goats like PCR, Immuno-fluorescence, and mouse inoculation test (MIT). The most common cause of low vaccine efficacy in sheep and goats against enterotoxaemia is the high to moderate level of serum antibodies to protect the gastrointestinal complications. These are the most important factors that must be consider while making the control strategy for enterotoxaemia (Pawaiya et al., 2020).

1.3 The Emerging Role of Probiotics

Current innovative treatment option for ovine enterotoxaemia is the probiotics, which are live microorganisms that provide many health perks when administered to the animals in appropriate dose. To stop detrimental bacterial growth, boost immune system, and strengthen the gut barrier are the main functions of the probiotics that are performed by changing the gut microbiota (Hill et al., 2014). Probiotics are the safest recognized substitute for animal health. Probiotics remarkably play important role in increasing small ruminants' immunity, production and well-being when administered in appropriate amount in ration. Probiotics increased the overall growth performance of small ruminants by improving the feed conversion rate, microbial ecology, and feed digestibility. Along with these functions probiotics also play significant role in enhancing the rumen performance by increasing the production of VFAs, regulate rumen acidity, and promote lactic acid utilizing protozoa in rumen. Probiotic usage has also been shown to boost milk production and lower the risk of newborn diarrhea and death. The precise processes by which probiotics carry out these actions are unknown, though. The current review aims to address the possible effects of this class of feed additives on the health and productivity of small ruminants, given the paucity of studies on their use.

2. Understanding Ovine Enterotoxaemia

2.1 Etiology: Clostridium perfringens Types C and D

Clostridium perfringens is categorized into five toxinotypes (A-E) based on the production of major toxins. Types C and D are particularly pathogenic in sheep. Type C produces β-toxin, which causes hemorrhagic enteritis, while type D produces ε-toxin, a potent neurotoxin responsible for systemic effects (Songer, 2010). The Gram-positive, anaerobic rod-shaped bacteria *Clostridium perfringens* was initially isolated from a human cadaver in 1891 and was subsequently dubbed bacteria *welchii* and *Clostridium welchii* in honor of William Henry Welch. Probably the most well-known and pervasive anaerobic pathogen in the world is *C. perfringens* (Songer, 1996). In humans and other healthy warm-blooded animals, it is a typical part of the gut flora. Similar to other bacterial species, *C. perfringens* can be further classified into strains based on the outcomes of several typing techniques. For *C. perfringens* toxin genotyping, PCR-based genotyping is typically utilized (Meer & Songer, 1997).

2.2 Pathophysiology: Toxin Production and Disease Mechanism

The pathogenesis of enterotoxaemia begins with rapid bacterial proliferation, often triggered by high-starch diets that create an anaerobic, nutrient-rich environment. The resulting toxin production damages the intestinal epithelium, allowing toxins to enter systemic circulation. This leads to vascular damage, organ failure, and death (Uzal & Songer, 2008). Up to 30 possible toxins can be produced by *C. perfringens*, and strains are still traditionally categorized into five groups (A, B, C, D, and E) based on the production of four main toxins (α, β, ι, and ε). There are two main types of clostridial toxins like necrotic B-like toxin (NetB) and enterotoxin (CPE) (Rood et al., 2018). The enterotoxin causes *C. perfringens* type F, and necrotic B-like toxin causes *C. perfringens* type G. NetB is the main toxin that is involved in the pathophysiology of necrotic enteritis in birds (Keyburn et al., 2008). Bacteria can also produce other toxins in addition to the mentioned above toxins like sialidase, enterotoxins, and haemolysins (Amimoto et al., 2007). Ovine and caprine pulpy kidney disease is caused by the *Clostridial perfringens* type D, while *C. perfringens* type A is the main culprit of ovine enterotoxaemia (Uzal & Songer, 2008). Although it dies there faster than other *Clostridium* species, it can survive in soil and is ejected in feces (Karthik et al., 2017). Under optimum conditions, the organism's short generation period allows it to multiply rapidly in the colon and create toxins that infect the host and cause illness (Fahimeh et al., 2018).

2.3 Clinical Manifestations and Economic Impact

Clinically, afflicted sheep show neurological symptoms such ataxia and convulsions, which are frequently followed by abrupt death. Due to treatment expenses, lost productivity, and mortality, the economic burden is substantial (Hassanein et al., 2017). Epsilon toxin is believed to be the primary toxin involved, while the potential effects of other toxins remain unknown. Children are more likely to have a peracute form of enterotoxaemia that results in sudden death. The occurrence of four clinical forms, ranging from acute to chronic, clinical predominance of diarrhea, severe enterocolitis, the rarity of brain lesions at necropsy, and the frequent failure of the present vaccination strategy are the primary characteristics that set sheep apart. The intestinal tract's caprine-specific reaction, which results in fewer systemic effects of toxins, may be the reason for these variations, as the intestines of goats absorb epsilon toxin more slowly than those of sheep (Sumithra et al., 2013). Young animals are more likely to experience the peracute form. It is comparable to the illness that frequently results in lambs being discovered dead with no visible symptoms. Pyrexia (40.5 ºC), acute shock, agony, final convulsions, coma, and death within hours after commencement are the clinical symptoms. Recovery is uncommon, and the clinical course typically lasts less than 24 hours (Smith, 2016). The acute variant, which lasts 4–24 hours, is more common in adult goats. Death or recovery could be the result. If the animals are not given treatment, recovery is rare. Diarrhea, stomach pain, severe shock with frigid extremities, opisthotonous, and sporadic convulsions are the hallmarks of the acute type. At first, the diarrhea will be pasty and yellow-green, but it quickly turns watery or mucoid, containing blood and gut mucosa fragments. The main aggravating issues in this kind are typically dehydration and acidosis brought on by the diarrhea (Van Metre, 2009). Although fever is rarely described as a symptom of caprine enterotoxaemia, peracute instances have been reported to have a temperature of 40.5 °C (Ayaz et al., 2020). According to the results of the ELISA kit used for toxicogenic typing of *C. perfringens* in clinical samples, the most common kinds were type A (67.2%), type D (16.4%), type B (13.4%), and type C (3%). Using a polymerase chain reaction, further validation of the representative *C.*

perfringens isolated from animals with enterotoxaemia was carried out. At the animal and herd levels, the total prevalence of enterotoxaemia illness was 27.2% and 26.47%, respectively (Omer et al., 2020).

3. Probiotics: Mechanisms of Action

3.1 Definition and Types of Probiotics

Probiotics are defined by the Food and Agriculture Organization (FAO) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Reddy, 2021). Common probiotic strains include *Lactobacillus, Bifidobacterium*, and *Saccharomyces* species, each offering unique benefits. The concept of probiotics has changed over time, moving from a live, active culture that enhances the composition of the gut microbiota to specific benefits, particularly the immunomodulatory potential of distinct strains. The genera *Bifidobacterium* and *Lactobacillus* are most commonly home to the beneficial strains, which may be probiotic sources. Some of these strains have substantial anti-inflammatory qualities. In fact, studies on infectious, inflammatory, and allergy diseases in humans have shown interest in probiotic therapy. The well-documented illness that modifies the gut flora is childhood acute infectious diarrhea. The goal of current probiotic research is to prevent aberrant immune reactivity associated with inflammatory and allergy illnesses by providing a safe yet enough bacterial stimulation (Isolauri et al., 2004).

3.2 How Probiotics Modulate the Gut Microbiome

Probiotics enhance gut health by competing with pathogens for adhesion sites, producing antimicrobial substances, and altering gut pH. They also promote the growth of beneficial commensals, fostering a balanced microbiome (Hou et al., 2020). It is believed that since the mechanisms of action of medications are all well understood, probiotics should also be. Undoubtedly, by preventing the production of vitamin K-dependent clotting factors, medications such as warfarin, which are administered to lower the risk of cardiac arrest, restrict the body's capacity to produce blood clots. Studies on humans and animals provided this knowledge, which was potentially made simpler to demonstrate due to the particular mechanism of action. However, not all medications have this feature. Conflicting information exists about the use of aspirin, a well-researched medication whose actions are thought to be understood, to prevent cardiovascular disease. Therefore, demonstrating causation and effort is not easy (Halvorsen et al., 2014).

3.3 Immune Modulation by Probiotics

Probiotics stimulate mucosal immunity by enhancing the production of IgA and cytokines. They also modulate systemic immune responses, reducing inflammation and improving disease resistance (Revankar & Negi, 2024). In a study of patients with atopic dermatitis, 12 weeks of treatment with *Bifidobacterium* breve BR03 and *L. salivarius* LS01 reduced gut microbial translocation and triggered an immune response, as evidenced by improved Th1/Th2 ($p = 0.028$) and Thper cell (Th)17/regulatory T cell (Treg) ratios. Even if strain knock-outs were not evaluated, this undoubtedly pointed to mechanistic pathways. The idea that better barrier function prevents disease is supported by additional human research. It is challenging to assess and draw broad generalizations about how probiotics affect and regulate the immune system (Giamarellos-Bourboulis et al., 2009). Depending on the host and maybe the mode of delivery, the same strain may boost antibacterial activity and the Th1 response in addition to increasing the anti-inflammatory Th2 response or regulatory action (Sharma & Im, 2018).

4. Role of Probiotics in Prevention of Enterotoxaemia

4.1 Reduction of Pathogenic Clostridia

Probiotics inhibit the growth of *C. perfringens* by producing bacteriocins and lowering intestinal pH, creating a hostile environment for pathogens (Cull et al., 2022). *L. reuteri* SD2112 (ATCC 55730) and *L. reuteri* RC-14 are two bacterial strains that differ genetically and functionally. The former produces reuterin, which is thought to be crucial in inhibiting gut pathogens, while the latter produces biosurfactants that prevent uropathogen adhesion (Hill et al., 2014). This highlights the necessity to try to identify the strains rather than using the general term "probiotics" when discussing methods of action. Nevertheless, probiotics are considered to provide a number of general advantages, many of which share the mechanism of improving the gut environment and promoting a healthy immune system and digestive tract (Langa et al., 2014). High-quality meta-analyses and action against intestinal transit, antibiotic-associated diarrhea, infectious diarrhea, irritable bowel syndrome, and other illnesses served as the foundation for this result. This does not imply that specific processes have been established or that the mechanisms are the same for every circumstance (Velraeds et al., 1998). Although there aren't many attempts to do so in people due to the challenges of employing recombinant strains, a rat study effectively demonstrated that a bacteriocin generated by *L. salivarius* UCC118 was in charge of inhibiting *Listeria monocytogenes* (Mathipa, 2019).

4.2 Enhancement of Gut Barrier Function

Probiotics strengthen the gut epithelial barrier by upregulating tight junction proteins and reducing epithelial permeability. This prevents toxin translocation into systemic circulation (Runkle & Mu, 2013). In a study of patients with atopic dermatitis, 12 weeks of treatment with *Bifidobacterium* breve BR03 and *L. salivarius* LS01 reduced gut microbial translocation and triggered an immune response, as evidenced by improved Th1/Th2 ($P=0.028$) and Thper cell (Th)17/regulatory T cell (Treg) ratios. Even if strain knock-outs were not evaluated, this undoubtedly pointed to mechanistic pathways. The idea that better barrier function prevents disease is supported by additional human research. The capacity of certain probiotic strains to have effects that are far from the site of administration is an interesting feature. The organisms may really move from the gut to the mammary glands of nursing animals, for instance, to cause this (Arroyo et al., 2010).

4.3 Neutralization of Toxins

Certain probiotics can bind and neutralize bacterial toxins directly, mitigating their effects. For example, *Lactobacillus* species have shown efficacy in neutralizing ε-toxin *in-vitro* (La Ragione & Woodward, 2003). It might result from the synthesis of molecules that are adsorbed throughout the intestine or that have a direct or indirect impact on host chemicals. The latter include compounds that promote heart re-modeling after cardiac ligation by raising the adiponectin/leptin ratio (45) and colostrum, which may reduce the incidence of gestational diabetes mellitus (Luoto et al., 2012). Another example is the reduction of blood cholesterol, which can be achieved through a variety of mechanisms, such as the assimilation of cholesterol, the production of compounds that inhibit 3-hydroxy-3-methylglutaryl coenzyme A, the deconjugation caused by bile salt hydrolase activity, and the reduction of intestinal levels of deoxycholic acid (DCA). Probiotic strains' capacity to lessen the intensity and length of respiratory tract infections is an example of a remote site impact that is most likely caused by an increase in immune mediators (Öner et al., 2014).

5. Application of Probiotics in Sheep Husbandry

5.1 Probiotic Supplementation Strategies

Probiotic administration can be achieved through feed additives, water supplements, or direct oral dosing. Ensuring consistent delivery is critical for efficacy (Yunus, 2016). The substances that probiotics make, such neurochemicals, or that they drive the host to produce seem to reach the brain at least through the vagus nerve system, even in cases when the probiotic itself cannot travel to remote locations like the brain (Lyte, 2011). Given that the organ regulates a large portion of our actions, this may be the most fascinating mechanistic field of probiotic study. It appears likely that drastically altering the gut microbiota by transplanting or administering probiotic strains that produce particular components could affect not only our mood and cognition but also what we eat, when we eat it, and how quickly or slowly pathological changes occur (Reid et al., 2015).

5.2 Integration into Feeding Programs

Integrating probiotics into existing feeding regimes requires consideration of factors like diet composition, environmental conditions, and animal health status. Customization based on flock needs enhances outcomes (Chaucheyras-Durand & Durand, 2010). The strain(s), product formulation, and mechanisms of action must all be thoroughly documented before probiotics can be used as therapy or as a preventative measure. It will be feasible to discover how probiotics function at various locations if computer-guided sampling can be carried out inside the gut in real time, without material evacuation, and RNA analysis is performed. The compounds mediating effects can then be found by combined proteome and metabolomic investigations. Our toolkit for patient care will be further enhanced by the capacity to cultivate now unculturable organisms and modify them via recombination. Therefore, in the future, more microbial species that target a range of ailments will be added to food and administered as supplements and pharmaceuticals (Reid, 2016).

5.3 Considerations for Large-Scale Implementation

Scaling the use of probiotics requires addressing concerns like cost, storage stability, and regulatory approval. The trustworthy distribution means must be developed for widespread implementation (McFarland, 2015). While introducing the probiotics into animal feeding programs some important factors must be consider like longevity and effectiveness of probiotics. The most important factor that must be take on serious note is the cost of the product available in market for farmers (Sangild et al., 2014). Along with that probiotics shelf life and stability are the significant aspect to consider as improper handling and storage decrease the efficacy of probiotics (Lohita & Srijaya, 2024). The maintenance during storage and transport are also crucial factors to gain desired results when given in appropriate amount to animals. Because of the species difference as well as health status, age, and ecological aspects tailored the administration methods accordingly (Krysiak et al., 2021). To ensure the quality control, transparency, and safety proper regulations and monitoring channels are necessary at large-scale operations. Although probiotics are facing a lot of challenges in field implementation but along with these problems' probiotics may reduce the load of antibiotics, improve animal well-being, and promote sustainable agriculture practices (Sangild et al., 2014).

6. Limitations and Future Directions

Stomach pH, humidity and temperature are the important factors that affect the quality of probiotics. To improve their quality and stability, innovative formulation strategies and encapsulation can help (de Vos & de Vos, 2012). Environmental factors and geographical differences in gut microbiota can impact the probiotics efficacy (Patel et al., 2023). Strain-specific processes, conducting real time field experiments, and improving dosing regimens should be the future study objectives to gain proper health benefits of probiotics (Sanders et al., 2019). Although probiotics have shown a lot of perks in animal health, along with there is a lot of issues need to be resolved before unlocking their full potential. Age, health status of animal, species, and individual variability are significant drawbacks that related with their effectiveness (Amenyogbe et al., 2020). Additionally, more research is needed to identify the standard formulations for individual species (Chase et al. 2019). The lack of standard protocols for administration and dosage are also challenges that could lead to false results in field (Matzke et al., 2011). Probiotics are lacking in long term effects on animals specially in gut flora stability and resistance to potential infections (Kouhounde et al. 2022). Improving delivery system, encapsulation techniques and broader therapeutic potential should be the focus of future research on probiotics. An innovative advancement in veterinary care is the recognition of link between probiotics and gut microbiota of animals (Abouelela et al., 2024).

Conclusion

In concluding remarks, pulpy kidney disease, a detrimental disease of sheep and goat that is caused by *Clostridial perfringens* type C, D, and administration of probiotics in animals ration can avoided this illness. Probiotics play significant role in minimizing the ovine enterotoxaemia by controlling gut flora, enhancing the gut barrier, and counteracting the clostridial's toxins. Because of their ability to strengthen animals' immune system and reduce the burden of gut microbiota, probiotics are excellent alternative to traditional preventive measures. Despite of a number of benefits, probiotics are facing many challenges in field like stability, longevity, shelf life, strain-specificity and effective distribution system. A recent study found that probiotics are beneficial for animals, but further research is needed to gain the complete understanding of their long-term impacts on sheep production and optimize supplementation alternatives. If these problems are overcome and research, formulation, and administration techniques, probiotics in sheep husbandry have a promising future. A comprehensive strategy that incorporates probiotics with other managemental techniques would eventually encourage strong, healthy animal populations, reduce antibiotic load, and advance sustainable sheep husbandry. The role of probiotics in preventing ovine enterotoxaemia, which emphasizes the necessity of ongoing innovation in animal health procedures. Probiotics offer a comprehensive strategy to prevent ovine enterotoxaemia by modifying immune responses, improving gut barrier function, and suppressing the growth of dangerous microbiota. Sheep husbandry techniques that incorporate probiotics can enhance animal health, reduce the need of antibiotics, and sustainable livestock production system.

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