

An investigation into the effect of
probiotic supplementation on the
prevalence and severity of
Cryptosporidium infections in
young calves

BY

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Abstract

Cryptosporidiosis causes substantial health and economic issues for the livestock industry, particularly affecting newborn calves. This study investigates the effectiveness of probiotic supplementation in reducing the prevalence and severity of *Cryptosporidium* infections in young calves in a commercial setting. Probiotics, which are known for their benefits on gastrointestinal health, have been suggested to offer therapeutic therapy against *Cryptosporidium* due to their impact on gut microbiota and immune response.

The study, which was conducted over 6 weeks on a commercial dairy farm, involved 50 calves systematically separated into treatment and control groups. Probiotic supplementation was administered to the treatment group following manufacturer guidelines. Data collecting methodologies included faecal sample testing, used to confirm the presence of *Cryptosporidium*, as well as to confirm that *Cryptosporidium* was the causative agent of diarrhoea amongst the calves. Scour scoring, body weight measurements, and body temperature were also monitored and recorded to compare the severity of symptoms amongst groups.

Results indicated a significant decrease in *Cryptosporidium* prevalence in the treatment group, as well as significant impacts on milk consumption, temperature regulation and weight gain ($p < 0.05$). Probiotic supplementation resulted in greater morning milk consumption (Estimate = 0.327 litres, SE = 0.046 litres, t-value = 7.145, $p < 0.0001$) and evening milk consumption (Estimate = 0.1847 litres, SE = 0.0446 litres, t-value = 4.139, $p < 0.0001$). These findings demonstrate the positive impact of probiotic supplementation on feeding behaviour.

Temperature regulation in treated calves was notably improved, as evidenced by lower temperatures compared to the control group (Estimate = -0.36°C , SE = 0.09°C , $p = 0.0052$). Additionally, the analysis of scour scores revealed a significant effect of the treatment on reducing scour severity (Estimate = -1.83 , $z = -8.25$, $p < 0.001$). Despite variations in breed, sex, and pen, the probiotic supplementation consistently resulted in lower scour scores, indicating improved gastrointestinal health and reduced disease severity. Treatment effects varied with age, suggesting dynamic responses over the study period. Post-hoc comparisons identified significant temperature changes at ages 7, 8, 9, 10, 11, and 12 days old ($p < 0.05$), indicating the course of infection, and how symptoms vary over time.

This research highlights the potential of probiotic supplementation as a viable option for reducing *Cryptosporidium* infections in young calves, opening up new possibilities for improved livestock health and economic sustainability in the agricultural industry. Further research into probiotic mechanisms and long-term effects is needed to optimise their use in cattle management methods.

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List Of Abbreviations

CLMM - Cumulative Link Mixed Model

QQ plots - Quantile-Quantile plots

SD – Standard deviation

SE – Standard error

REML - Restricted maximum likelihood

LMEM – Linear mixed-effect model

1. Introduction

1.1 Background

Cryptosporidiosis, a parasitic infection; caused by the protozoan parasite *Cryptosporidium parvum*, causes intestinal disorders such as diarrhoea, dehydration, weight loss and potential death. Such infections compromise the health of new-born calves' and in so doing, the economic viability of the livestock industry. Due to the sensitivity of young calves to *Cryptosporidium* and the possibility of zoonotic transmission, comprehensive control measures are required to protect both animal welfare and public health.

Cryptosporidiosis has a substantial economic impact, e.g., reduced growth rates in infected animals, which leads to significant financial losses for farmers. As a result, veterinarian strategies to reduce the burden of *Cryptosporidium* infections in calves are intensively pursued. However, relying on drug-based strategies for treatment and control has drawbacks, including the emergence of drug resistance.

The growing concern regarding drug resistance necessitates the investigation of alternative medicines, such as probiotic supplements, to enhance or replace conventional drug-based treatments. Probiotics offer promising avenues for reducing *Cryptosporidium* infections in calves while reducing the possibility of contributing to antibiotic resistance. Research efforts focusing on discovering effective probiotic formulations and elucidating their mechanisms of action are crucial to developing sustainable solutions for combating *Cryptosporidiosis* in livestock, safeguarding both animal and human health.

1.2 Rationale for the study

Probiotics, defined as live microorganisms that provide health benefits to the host, have attracted interest for their potential to alleviate gastrointestinal infections, particularly as a strategy for combating *Cryptosporidium* infections in calves. Probiotics influence the gut microbiota, improve mucosal barrier function, and produce antimicrobial substances, including short-chain fatty acids (SCFA), organic acids, hydrogen peroxide, and bacteriocins, all of which may influence the dynamics of *Cryptosporidium* infections.

While previous research has investigated the benefits of probiotics in a variety of livestock contexts, the specific application of probiotics to reduce *Cryptosporidium* infections in calves under commercial conditions is worthy of further examination. The purpose of this literature review is to consolidate the existing knowledge on the subject while also analysing the efficacy of probiotic supplementation and understanding the mechanisms by which probiotics may alter the prevalence and severity of *Cryptosporidium* in calves.

2. Literature Review

2.1 Objectives of the literature review

The primary objectives of this literature review are as follows:

1. To offer a complete overview of *Cryptosporidium* infections in calves, with a focus on the parasite's impact on young animals, the resulting economic ramifications of infection, and the potential of zoonotic transmission to humans and other livestock.

2. To summarise the fundamental concepts of using probiotics, including their properties, modes of action in the gastrointestinal system, and prior applications in cattle health.
3. To review and analyse available research on the impact of probiotic supplementation on reducing the frequency and severity of *Cryptosporidium* infections in calves.
4. To study the mechanisms via which probiotics may exert their protective benefits against *Cryptosporidium* infections, leveraging insights from relevant studies.
5. To examine the issues related to probiotic therapies, potential remedies, and future research directions.

2.2 Cryptosporidium in calves

2.2.1 Overview of Cryptosporidium parvum

Cryptosporidium parvum, a protozoan parasite belonging to the phylum *Apicomplexa* (Morrisette and Sibley, 2002), is recognized as a significant causative agent of enteric disease in various animal species, including bovines (Tzipori, 1983). The life cycle of *Cryptosporidium parvum* involves six stages, with oocysts being the infectious form as described by Bouzid et al, (2013). Once ingested, oocysts release sporozoites, initiating the infection within the host's gastrointestinal tract (Figure 1).

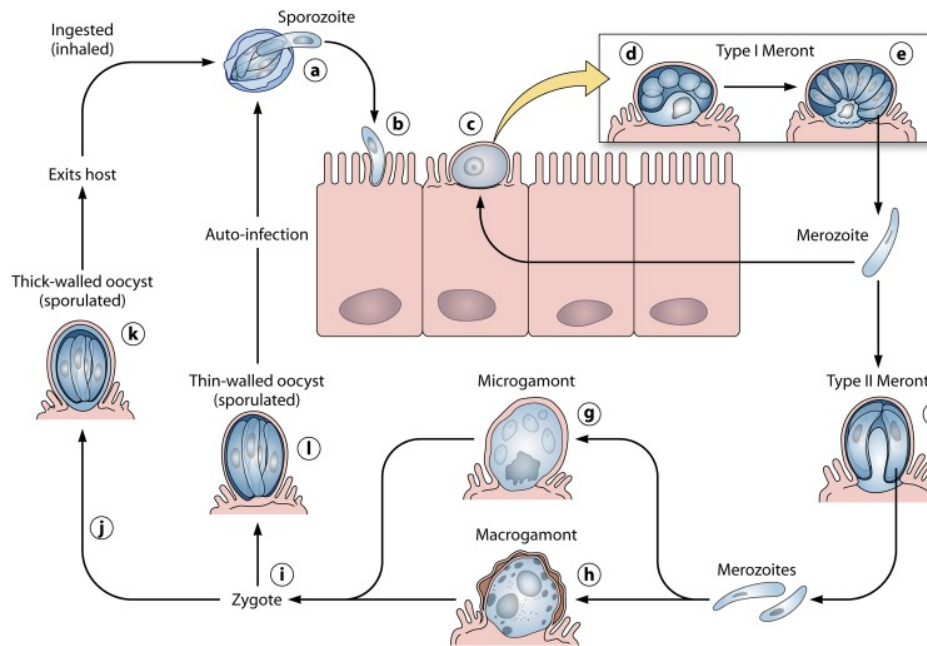


Figure 1. Depicted illustration of the life cycle of *Cryptosporidium parvum* (American Society for Microbiology, 2013)

In young calves, *Cryptosporidium* infections commonly manifest as diarrhoea, which can range from mild and self-limiting to severe and persistent (Leitch and He, 2012). The parasite primarily targets the epithelial cells lining the small intestine, leading to malabsorption of nutrients and subsequent weight loss (Costa et al., 2011). The severity of *Cryptosporidiosis* is further compounded by the potential for dehydration, electrolyte imbalance, and compromised immune function, particularly in neonatal calves (Berchtold, 2009).

2.2.2 Impact on young calves

Young calves are vulnerable to *Cryptosporidium* as they have an underdeveloped and unchallenged immune system (Helmy and Hafez, 2022). Neonatal calves are especially vulnerable, and infections are common in the first few weeks of life (Niine et al., 2018).

The repercussions of *Cryptosporidiosis* go beyond clinical signs, affecting the animal's overall health and growth. Reduced production increased veterinary costs and increased mortality rates – all of which have an economic impact on cattle farming, making effective control methods critical for sustainable agricultural practices (de Graaf et al., 1999).

2.2.3 Economic and zoonotic implications

Cryptosporidium parvum causes economic issues for the cattle business because of the potential negative impact on calf health. Prevention is better than a cure. Infection incurs treatment costs and possible productivity losses highlighting the importance of taking preventative measures. Furthermore, *Cryptosporidium's* zoonotic potential raises implications for human health, particularly in those with impaired immune systems. These include, young children, immunocompromised persons, and immunodeficiency syndrome patients, especially those with HIV/AIDS (Pumipuntu and Piratae, 2018).

Understanding the lifespan, impact, and economic implications of *Cryptosporidium* infections in calves is useful for evaluating alternative interventions, such as probiotic supplementation, as a way to minimise the effects of this parasitic disease.

2.3 Probiotics and gastrointestinal health

2.3.1 Definition and characteristics of probiotics

Probiotics are live bacteria that provide health advantages to the host when administered in sufficient concentrations (Kechagia et al., 2013). These microorganisms, primarily bacteria and yeast, play an important role in balancing the gut microbiota, which regulates different aspects of gastrointestinal health (Wang et al., 2022). *Lactobacillus*,

Bifidobacterium, and *Saccharomyces* are some of the most studied probiotic strains (Fijan, 2014).

Probiotics operate in a variety of ways, including the competitive exclusion of pathogenic bacteria, mucosal barrier strengthening, and host immune response modulation (Bermudez-Brito et al., 2012; Figure2).

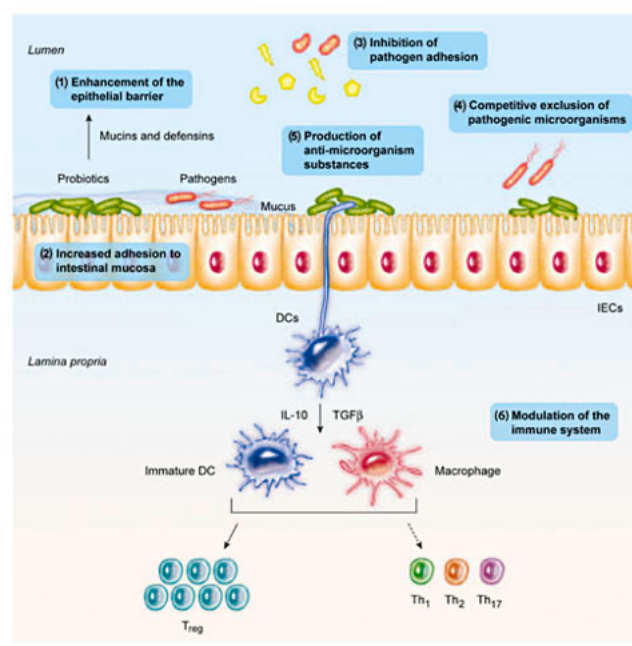


Figure 2. Probiotics' main mechanisms of action (Bermudez-Brito et al., 2012)

Probiotics' can survive in the harsh environments of the gastrointestinal tract. They adhere to intestinal epithelial cells which in turn, contributes to their therapeutic potential in the treatment of numerous gastrointestinal illnesses (Amara and Shibl, 2015).

2.3.2 Mechanisms of action in the gastrointestinal tract

In the context of gastrointestinal health, probiotics contribute to the balance of gut microbiota by competing with pathogenic bacteria for nutrients and adhesion sites. Furthermore, probiotics produce a variety of metabolites themselves, including short-chain fatty acids, bacteriocins, and organic acids, which create an unfavourable environment for pathogenic microbes (Markowiak and Śliżewska, 2017). Probiotics maintain the mucosal barrier by improving tight connections between intestinal epithelial cells, preventing bacteria and their toxins from crossing the gut lining (Gou et al., 2022).

Probiotics also have an important function in influencing the host's immunological response. They interact with immune cells in the gut-associated lymphoid tissue, affecting cytokine (small proteins that regulate the growth of all blood cells and other cells that help the body's immune and inflammation responses) and other immune mediator syntheses (Mazziotta et al., 2023). This immunomodulatory function contributes to the maintenance of a balanced immune response by limiting excessive inflammation and encouraging tolerance to commensal bacteria (Guo and Lv, 2023).

2.3.3 Previous research on probiotics in livestock

The use of probiotics has been studied in livestock in a variety of contexts, from growth enhancement and feed efficiency to disease prevention. Studies in swine and poultry have indicated that there are benefits to overall health, such as the reduced occurrence of diarrhoea and improved weight gain (Liao and Nyachoti, 2017; Kabir, 2009). While the use of probiotics in ruminants, particularly cattle, has received much less attention, there is rising interest in their ability to improve digestion and minimise gastrointestinal problems (Lambo et al, 2021), especially in the pre-ruminant calves.

Understanding the mode of probiotic action in the gastrointestinal tract is fundamental to understanding their possible applicability in preventing *Cryptosporidium* infections in calves. Although *Cryptosporidium* is a protozoan parasite, recent research suggests possible interactions between certain probiotic bacteria and protozoan pathogens (Berrilli et al., 2012). This connection underscores the importance of investigating whether probiotics could mitigate the impact of *Cryptosporidium* infections.

2.4 Probiotics and *Cryptosporidium*

*2.4.1 Review of studies investigating probiotic effects on *Cryptosporidium**

As the ability of probiotics to control gastrointestinal health became known, researchers began looking into their involvement in preventing *Cryptosporidium* infections in calves. Several studies have looked into the effects of probiotic supplementation on the prevalence and severity of *Cryptosporidium*.

*2.4.1.1 Effects of Mannan-Oligosaccharide and *Bacillus subtilis* supplementation on preweaning Holstein dairy heifers*

The study conducted one of the first large-scale field trials from a commercial dairy in a comprehensive investigation examining the effects of probiotic, prebiotic, and symbiotic supplementation on 1,801 preweaning Holstein dairy heifers. At 14 days, the probiotic treatment with *Bacillus subtilis* revealed considerable results, including a 100-fold reduction in faecal shedding of *Cryptosporidium* oocysts. While the probiotic treatment alone did not affect weight gain, the symbiotic treatment, which included prebiotic (mannan-oligosaccharide) and probiotic, resulted in a significant 19 g rise in the average daily weight gain compared to the control group from 7 to 56 days. Furthermore, the

group being administered probiotics shed fewer *Cryptosporidium* oocysts, suggesting potential benefits in lessening the overall burden of infections in preweaning dairy calves. This reduction affects not only the individual animal but also implies a potential reduction in environmental contamination, which benefits herd health and management.

2.4.1.2 Effect of Lactobacillus and Bifidobacterium on Cryptosporidium parvum oocyst viability

Probiotics were investigated to identify if they had a beneficial impact on the infective stage of *Cryptosporidium parvum*. Four common food-fermenting bacteria—*Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Bifidobacterium breve*, and *Bifidobacterium longum*—were investigated for their in vitro effects on the survivability of *C. parvum* oocysts. This was measured using a novel flow cytometric viability assay. *Lactobacillus* supernatants dramatically reduced oocyst viability by up to 81%, while *Bifidobacterium* supernatants reduced it by 10-37%. *L. acidophilus* and *L. reuteri* supernatants, in particular, significantly increased the proportion of nonviable oocysts, implying the presence anti-parasitic compounds with anti-*C. parvum* activity. While *Bifidobacterium* supernatants performed less well, especially when compared to *Lactobacillus*, undiluted *Bifidobacterium longum* supernatant revealed a significant reduction in oocyst viability, indicating possible therapeutic usage.

2.4.1.3 Field testing of prophylactic measures against Cryptosporidium parvum infection in calves in a California dairy herd

The objective of this field study on a dairy farm, where *Cryptosporidiosis* was endemic, was to evaluate the effectiveness of two preventive measures, oral vaccination with a

Cryptosporidium parvum vaccine and probiotic treatment with lactic acid-producing bacteria. A total of 134 calves were assigned to one of three groups at random:

1. The vaccine group, which received an oral *C. parvum* vaccine shortly after birth
2. The bacteria group, which received daily oral doses of lactic acid-producing bacteria for the first 10 days after birth
3. The control group, which received no specific treatment.

Over three weeks, no significant differences in the incidence of diarrhoea or faecal shedding of *C. parvum* oocysts were found among the three groups. The study confirms that neither vaccination nor probiotic treatment was successful in preventing *C. parvum* infection in calves under field conditions. The excessive prevalence of *C. parvum* in the environment may have offset any possible benefits of these regimens. While this conclusion is supported by the available data, additional empirical measurements are needed to precisely quantify the impact of *C. parvum* prevalence on regimen efficacy. These findings highlight the need for additional studies to create more effective preventive methods against *C. parvum* in the field.

2.5 Mechanisms of probiotic action of Cryptosporidium

2.5.1 Competition for adhesion sites

The concept of competition for adhesion sites inside the gastrointestinal tract is one intriguing feature of the probiotic-*Cryptosporidium* interaction. Adhesion is a critical first step in the *Cryptosporidium* lifecycle when the parasite adheres to intestinal epithelial cells to start infection (O'Hara and Chen, 2011). Probiotics, when appropriately delivered into the gut ecology, may compete for these adhesion sites (Wang, et al., 2021).

The principle of a probiotic microorganism is to occupy and cover the surfaces of the intestinal epithelium where *Cryptosporidium* would normally adhere (Oliveira and Widmer, 2018). Probiotics thus generate a barrier effect, preventing *Cryptosporidium* from attaching and colonising successfully (Travers et al., 2011). This competition for adhesion sites is thought to decrease the parasite's capacity to establish a foothold in the gut, resulting in less *Cryptosporidium* oocyst shedding in faeces (Travers et al., 2011).

While the specific mechanics of this competition are still being studied, the hypothesis holds promise to aid further understanding on how probiotics can help reduce *Cryptosporidium* shedding in calves. More research into the specific processes, strains, and settings that optimise this competition for attachment sites may provide light on probiotics' potential use as a preventive therapy against *Cryptosporidium* infections.

This aspect of probiotic action highlights the intricate ecological competition occurring at the microscale within the gut environment and its potential impact on *Cryptosporidium* prevalence, laying the groundwork for further investigation into the nuanced interactions between probiotics and this opportunistic parasite.

2.5.2 Production of antimicrobial substances

A prominent aspect of the complex interplay between probiotics and the *Cryptosporidium* parasite is the synthesis of antibacterial compounds by probiotic microorganisms (Javanshir et al., 2021). This novel approach highlights the probiotic arsenal's ability to produce antimicrobial chemicals, which have a significant impact on *Cryptosporidium* parasite's survival and epidemiology.

By the study conducted by Lucey et al., 2021, the probiotic repertoire appears to include a wide range of antibacterial compounds. These may include organic acids, peptides, and other bioactive substances, all of which can inhibit *Cryptosporidium* development (Sharifi-Rad et al., 2020). The precise identity and specificity of these antimicrobial components necessitate careful investigation, as understanding their nature holds the key to optimising probiotic formulations for targeted efficacy.

Probiotics' antimicrobial onslaught is thought to occur within the intestinal environment, directly affecting the production of *Cryptosporidium* oocysts and potentially altering their lifecycle (Vitetta et al., 2016). Probiotics produce organic acids such as lactic acid and acetic acid which are thought to contribute to this antibacterial impact, making the environment less suitable for *Cryptosporidium* parasite survival (Javanshir et al., 2021).

This mechanism's consequences go beyond the rapid reduction in *Cryptosporidium* shedding. Probiotics regulate host innate and adaptive immune responses by modulating the functions of dendritic cells, macrophages, and T and B lymphocytes. Probiotic organisms producing antimicrobial compounds have the potential to reduce environmental contamination and public health problems connected with *Cryptosporidium* species (Amalaradjou and Bhunia, 2012). Understanding the subtleties of how probiotics orchestrate this biochemical defence against *Cryptosporidium* is key to understanding the application of probiotics in calf health care.

2.5.3 Modulation of host immune response

Probiotics, particularly *Lactobacillus* and *Bifidobacterium* strains, can influence the host immunological response. Probiotics boost the host's immunological defence against

Cryptosporidium (Isolauri et al., 2001). This improvement entails activating immune cells and increasing cytokine synthesis (Yan and Polk, 2011). The goal is to strengthen the host's defences, reducing susceptibility to *Cryptosporidium*-induced illnesses. Ongoing research intends to identify the precise mechanisms of this immunological modulation, providing insights for tailored probiotic therapies to strengthen the host's inherent defence against *Cryptosporidium* (Gill, 2003).

2.6 Challenges and future direction

2.6.1 Strain-specific effects

Understanding and utilising probiotic strain-specific effects is critical in optimising their use for *Cryptosporidium* management (Sāsāran et al., 2023). Given the wide variety of probiotic strains available, it is essential to identify those with higher efficacy in reducing *Cryptosporidium* shedding and improving calf health. Future research should identify the exact mechanisms via which various strains affect the gut environment, compete for adhesion sites, create antimicrobial compounds, and influence the host immune response. This focused investigation is critical for developing precise probiotic therapies and unlocking their full therapeutic potential in *Cryptosporidium* management.

2.6.2 Dosage optimization

Optimising probiotic dosage for successful *Cryptosporidium* management is worthy of extensive research. It is important to conduct systematic dose studies that consider aspects such as probiotic strain, calf age, and specified results. Setting exact dosing guidelines is critical for ensuring probiotic efficacy in minimising *Cryptosporidium* shedding and improving calf health. Different probiotic concentrations may produce different results in

terms of *Cryptosporidium* suppression, thus emphasising the importance of dosage standardisation.

2.6.3 Variability in calf susceptibility

The inherent diversity in calf *Cryptosporidium* sensitivity makes it harder to identify other contributory factors. Ongoing studies are required to identify crucial characteristics such as calf age, immunological health, and environmental parameters. For example, younger calves are more susceptible to *Cryptosporidium* shedding (Shaw et al., 2021), emphasising the significance of personalised probiotic therapies based on individual calf traits to maximise their efficiency in fighting *Cryptosporidium* infections.

2.6.4 Standardization of probiotic formulations

Standardising probiotic formulations entails determining the optimum concentrations, and administration techniques. According to a recent study, individual strains within probiotic formulations may have variable effects on *Cryptosporidium* (Gaber et al., 2022), emphasising the importance of thorough standardisation. Consistency in probiotic treatment promotes uniformity and allows for precise assessments of their impact on reducing *Cryptosporidium* shedding and enhancing calf health.

2.6.5 Optimal administration protocols

The development of optimum administration methods is imperative for the practical application of probiotics in *Cryptosporidium* management. The most effective time, frequency, and duration of probiotic delivery should be studied. Notably, current research suggests that providing probiotics during critical phases of calf growth may result in increased *Cryptosporidium* protection (Wang et al., 2023), emphasising the necessity of

accuracy in administration procedures. However, the specific key developmental milestones within these critical phases are not explicitly outlined in the cited study, warranting further investigation in future research

2.6.6 Long-term effects on calf health

Understanding the long-term impact of probiotic therapies on calf health is critical for developing long-term management methods. In-depth research should look into the long-term effects of *Cryptosporidium* control, considering issues including growth, development, and overall well-being. Recent longitudinal studies have shown that probiotic treatment during the preweaning period can improve not only *Cryptosporidium* shedding but also calf weight gain (Cangiano et al., 2020), indicating the potential for long-term benefits. The interrelation of these two factors raises important questions about the significance of weight gain for young calves. Weight gain in the early stages is crucial for the overall health and future productivity of the calf. Potential consequences of inadequate weight gain may include delayed age at first calving or, in the case of beef cattle, delayed finishing. Examining long-term effects informs comprehensive strategies for incorporating probiotics into calf health management over time.

2.7 Summary of key findings

It has been suggested that probiotics may inhibit infection through excretion of substances harmful to one of the parasite's developmental stages and possibly offer new therapeutic agents for the treatment of *Cryptosporidiosis*. These studies revealed a significant reduction in *Cryptosporidium* shedding, highlighting the potential of probiotics in moderating the effects of this parasite illness.

Competition for adhesion sites, generation of antimicrobial compounds, and modification of the host immune response are the processes underpinning probiotic efficacy against *Cryptosporidium*. Probiotics generate a barrier effect in the gastrointestinal system by competing for adhesion sites, preventing *Cryptosporidium* attachment and colonisation. Probiotics' production of antimicrobial substances, such as organic acids and peptides, adds another layer of defence against *Cryptosporidium*, impacting its lifespan and infectivity. Furthermore, probiotics, particularly *Lactobacillus* and *Bifidobacterium* strains, boost the immune response of the host, providing a comprehensive defence plan against *Cryptosporidium* infections.

2.7.1 Implications for practical application

Current findings have far-reaching implications for the practical use of probiotics in *Cryptosporidium* care in calves. Because of the competition for attachment sites and the synthesis of antimicrobial compounds, probiotics may have an important role in lowering *Cryptosporidium*-related environmental contamination and public health hazards. Furthermore, the regulation of the host immune response indicates the potential for probiotics to improve calves' overall health and resilience to *Cryptosporidium*-induced diseases.

2.7.2 Recommendations for future research

In light of the existing literature, which primarily indicates the efficacy of probiotics in a controlled environment, there is a compelling need for future research to bridge the gap between controlled settings and real-world, commercial environments. This study aims to address this crucial gap by conducting research in a commercial setting in the UK, offering an opportunity for repeatability and validation of findings. The literature review

underscores the significance of focusing on key aspects such as strain-specific effects, dosage optimization, and variability in calf susceptibility. These critical areas require thorough investigation, particularly in the context of commercial settings where external factors may influence outcomes. Standardizing probiotic formulations, determining optimal dosing procedures, and comprehending the long-term impacts on calf health become pivotal in the development of precise and effective probiotic therapy. The guidelines outlined here emphasize the importance of conducting comprehensive and targeted research, aligning with the research goals of understanding the practical implications and applications of probiotics in preventing and controlling *Cryptosporidium* infections in calves within a commercial environment.

3 Aim And Objectives

3.1 Aim

To investigate the impact of probiotic supplementation on the prevalence and severity of *Cryptosporidium* infections in calves within a commercial environment.

3.2 Objectives

- To assess the prevalence of *Cryptosporidium* infections in calves within the study population.
- To determine the severity of *Cryptosporidium* in the infected calves, considering factors such as clinical symptoms and disease progression.
- To investigate the impact of probiotic supplementation on reducing the prevalence of *Cryptosporidium* infections in calves.

- To evaluate the effect of probiotic supplementation on mitigating the severity of *Cryptosporidium* infections in infected calves.

3.3 Hypothesis

Relative to the aim, the following hypothesis will be assessed:

- H0: Probiotic supplementation has no effect on the prevalence or severity of *Cryptosporidium* infections in calves within the study population.
- H1: Probiotic supplementation reduces the prevalence and severity of *Cryptosporidium* infections in calves within the study population.

4 Methods

4.1 Study location

The study was conducted on Roadhead Farm, Quathquan, Biggar, ML12 6ND.

4.2 Study duration

The study was conducted over 6 weeks, starting from 26/06/2023 to 7/082023.

4.3 Sample size

A total of 50 calves participated in the study, with 25 calves in both the treatment group and control group.

4.4 Allocation

The allocation of calves to treatment and control groups was performed systematically using a pen-stratified approach. Calves were assigned to groups in a sequential approach, with the first five calves born being dedicated to the control group, the next five allocated to the treatment group, and this alternating pattern continued. The stratification by pens ensured that experimental conditions were distributed evenly throughout the many environmental factors linked with each pen, improving the study's internal validity.

4.5 Treatment group

The treatment group received the probiotic supplementation (Precision Microbe's calf probiotic).

4.6 Control group

The control group received no supplement and will serve as a baseline comparison.

4.7 Housing

Calves were housed indoors, inappropriately sized pens of 5 calves, for 14 days. The treatment allocation was blocked for the pen position (Figure 3).

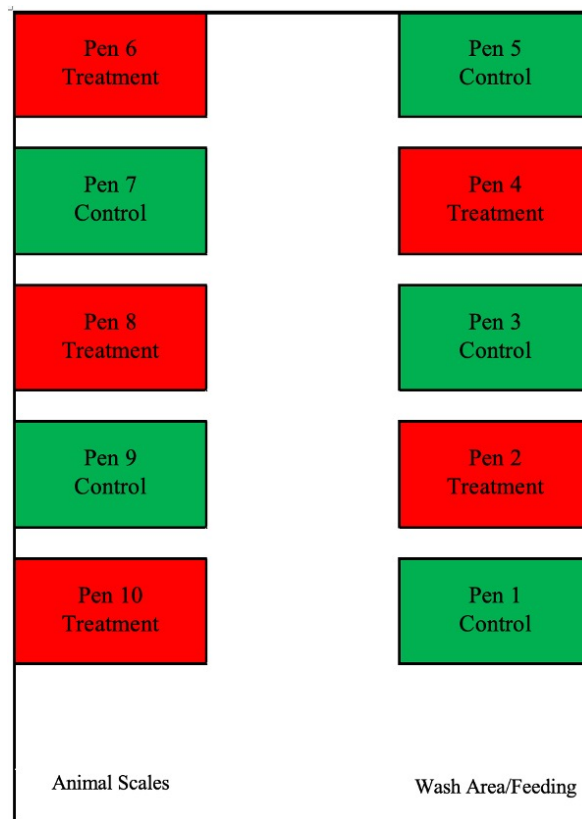


Figure 3. The layout of indoor calf housing, with control (green) and treatment (red) groups blocked for pen position

4.8 Pen management

Before introducing calves, the shed in which the calves were housed was thoroughly disinfected. Calves were bedded daily using straws to ensure comfort and hygiene standards were met.

4.9 Environmental conditions

Pens were maintained under similar environmental conditions, which included temperature, humidity, and ventilation, to minimize potential confounding factors. The shed doors were kept shut and heaters and fans running along the roof of the shed were used appropriately.

4.10 Sanitation practices

Strict sanitation practices were followed by all members of staff, this included using foot baths before and after entering the shed as well as regular cleaning and disinfectant of equipment (water buckets, feeders, milk mixer). This helped to minimize the risk of cross-contamination between pens and groups.

4.11 Supplement preparation

Following the manufacturer-recommended guidelines, the supplement was shaken before use.

4.12 Administration

Following the manufacturer-recommended guidelines, calves were administered 30ml of probiotic, orally (mixed into milk), once daily (AM), for the first 14 days of life.

4.13 Therapeutic intervention protocol

In the event of *Cryptosporidium* infection manifestations or indications of decreased fluid intake, affected calves were administered Avital, an electrolyte solution via milk or tube feeding. In addition, for severe cases of *Cryptosporidium* infection that necessitate relief from potential discomfort, 2ml Meloxidyl, a pain medicine, was administered as part of the therapeutic intervention protocol.

4.14 Data collection

4.14.1 Daily Health Monitoring

Calves were visually examined daily for signs of diarrhoea; this includes faecal consistency and the presence of scour. Any observed clinic signs were recorded.

4.14.2 Faecal sample collection

Faecal samples were collected every second day from each calf using sterile containers which were labelled accordingly with the calf's identification number. Prior to sample collection, hands were thoroughly washed, and gloves were worn at all times. Calves were monitored closely to ensure a fresh sample could be collected after defecation, taking care to avoid contamination from bedding or other sources, and weighed between 5-10 grams. Once the sample was collected, gloves were put into a bin and hands were washed before collecting the next sample.

4.14.3 Faecal testing

Faecal samples were tested on-site using Actus rapid detection tests specifically designed to detect *Cryptosporidium*, *Rotavirus*, *E. coli* and *Salmonella*. These tests were compared to commercially available ELISA tests (Table 1). All test results were recorded.

Test performance	Rota	Corona	E. coli	Crypto-sporidien
Sensitivity	96,43 %	100,00 %	88,89 %	100,00 %
Specificity	95,45 %	100,00 %	100,00 %	95,85 %
PPV	96,43 %	100,00 %	100,00 %	96,30 %
NPV	95,45 %	100,00 %	97,62 %	100,00 %
Total	96,00 %	100,00 %	98,00 %	98,00 %

Table 1. Rapid diarrhoea testing kit performance in comparison to commercially available ELISA

4.14.4 Scour score

Faecal consistency was assessed daily by trained individuals using the Alltech faecal scoring guide (Figure 4) . Results were recorded.

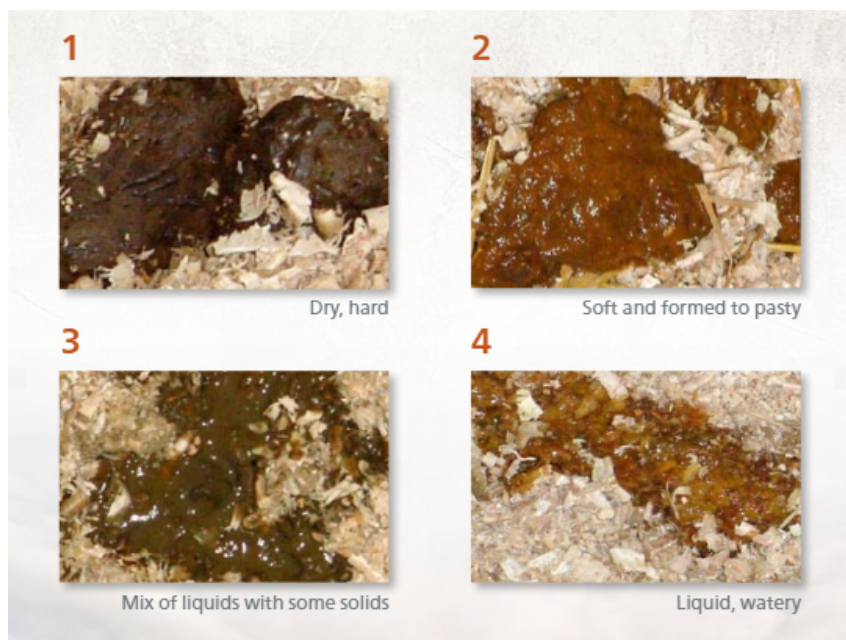


Figure 4. Alltech faecal scoring guide

4.14.5 Body weight measurements

Using a farm animal scale, calves were weighed at birth (once dry) and every day following at noon.

4.14.6 Body temperature

Calves body temperature was recorded, once daily (noon) using a non-contact infrared thermometer. This was performed by standing at the recommended distance, aiming at the calf's forehead or ear, pressing the trigger, and recording the temperature.

4.15 Ethical considerations

The study abided by ethical standards and laws governing animal welfare to protect the well-being of the research animals. Any adverse events or health issues were promptly addressed, and if required, suitable veterinary care was provided. The study has been approved by SRUC (Scotland's Rural College) AWERB under number BOR 2023-017 DAI A00 (7 June 2023).

4.16 Data analysis

The data was analysed using R statistical software, utilizing ggplot2 for visualisation. Histograms and quantile-quantile (QQ) plots were used to determine the normality of data distributions, and non-parametric tests were used to identify variables that deviate from normal. As calves were fed individually, the calf was the experimental unit.

With the variable of interest being ordinal, the relationship between parameters and *Cryptosporidium* prevalence was analysed using cumulative link mixed models (CLMM).

The relationship between scour score and predictive factors was also assessed using this model.

Linear mixed effect models (LMEM) were used to investigate temperature, milk intake and weight gain with age as a random factor. Fixed effects analysis assesses treatment significance, with post hoc comparisons identifying significant variations.

5 Results

5.1 Prevalence of disease

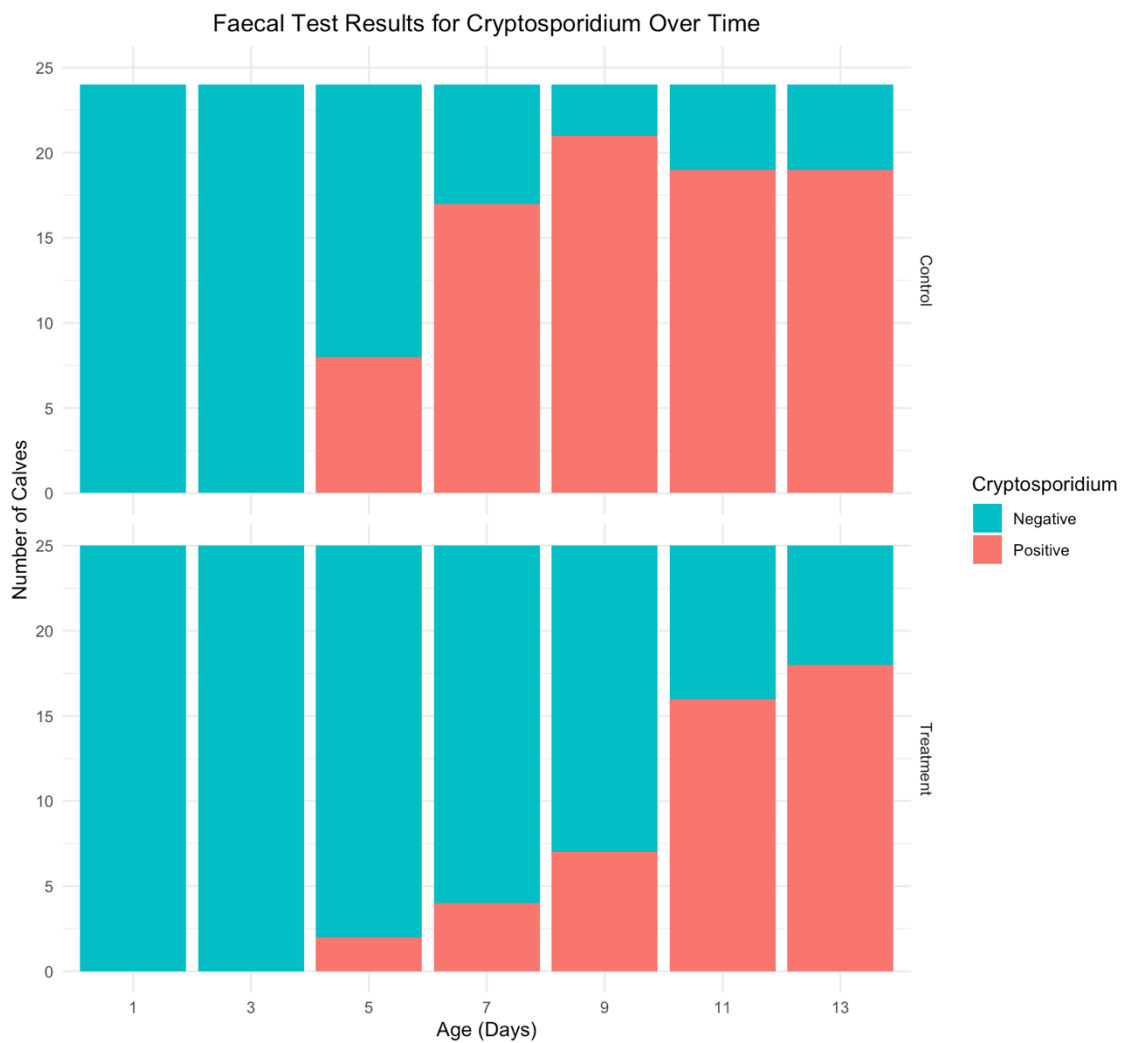


Figure 5. *Cryptosporidium faecal test results over time, with one calf removed from the control group due to death on day 1*

The CLMM applied to faecal test data provided insights into the association between several parameters and *Cryptosporidium* prevalence.

The model indicated a significant influence of treatment on *Cryptosporidium* prevalence (Estimate = -1.854446, $z = -3.932$, $p < 0.001$), indicating that calves receiving probiotics had a lower prevalence of *Cryptosporidium* than the control group. This suggests that for each unit increase in the treatment variable (e.g., receiving probiotics), the log odds of *Cryptosporidium* prevalence decreased by approximately 1.854446 units. However, no significant impacts were observed for breed, sex, or pen ($p > 0.05$).

"Random effects analysis found significant variability between individual animals (ear tags) and across different ages. The variance estimates for ear tag (Variance = 1.153, SD = 1.074) and age (Variance = 4.905, SD = 2.215) indicate substantial individual-level variability in the occurrence or persistence of *Cryptosporidium* infections. This underscores the importance of considering individual factors in understanding infection dynamics, as some animals may exhibit varying susceptibility or resilience to infection over time."

5.2 Severity of symptoms

5.2.1 Milk intake

The data was initially visualised (Figures 6 & 7) before being explored using histograms and QQ plots to assess the normality assumptions underlying the LMEL. However, these diagnostic plots did not show a clear normal distribution for the data. This observation may be attributed to the nature of the experimental setup, where the quantity of milk offered to subjects was not unrestricted ('ad libitum'). Consequently, the variability in milk consumption patterns among the subjects could have influenced the distribution of the data, leading to deviations from a typical normal distribution. This consideration underscores the importance of contextual factors in interpreting the distributional properties of the data.

To analyse the distribution of feed intake data between treatment and control groups, a Wilcoxon rank sum test with continuity correction was used. This non-parametric test found a significant difference in morning milk intake between the treatment and control groups ($W = 46885$, $p\text{-value} = 8.017e-09$) and evening milk intake ($W = 49478$, $p\text{-value} = 2.497e-06$). This finding shows that the treatment had impact on feed intake relative to the control group, regardless of distributional assumptions.

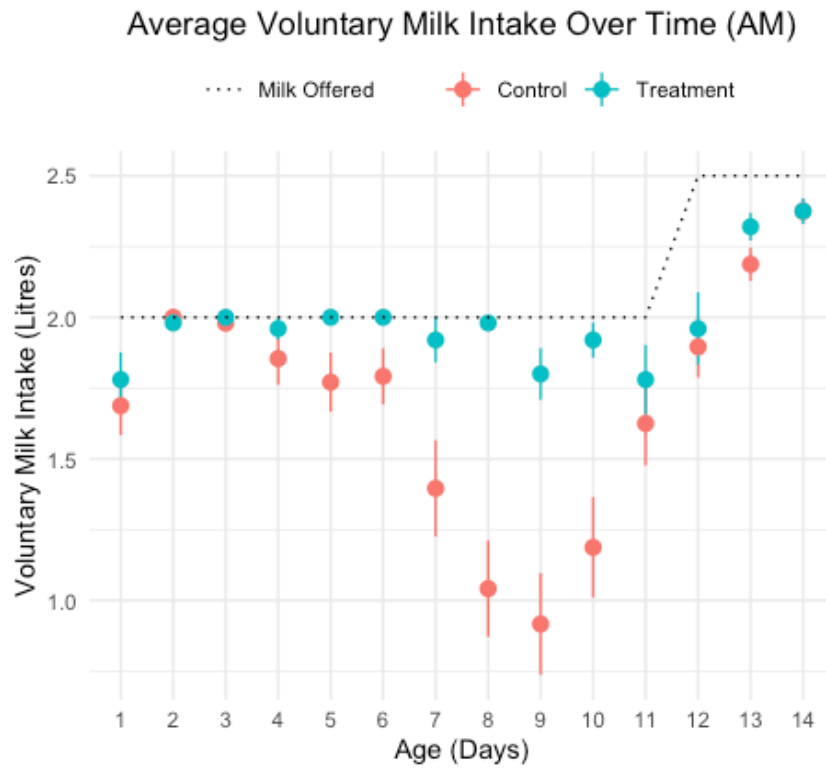


Figure 6. Average morning voluntary milk consumption over time

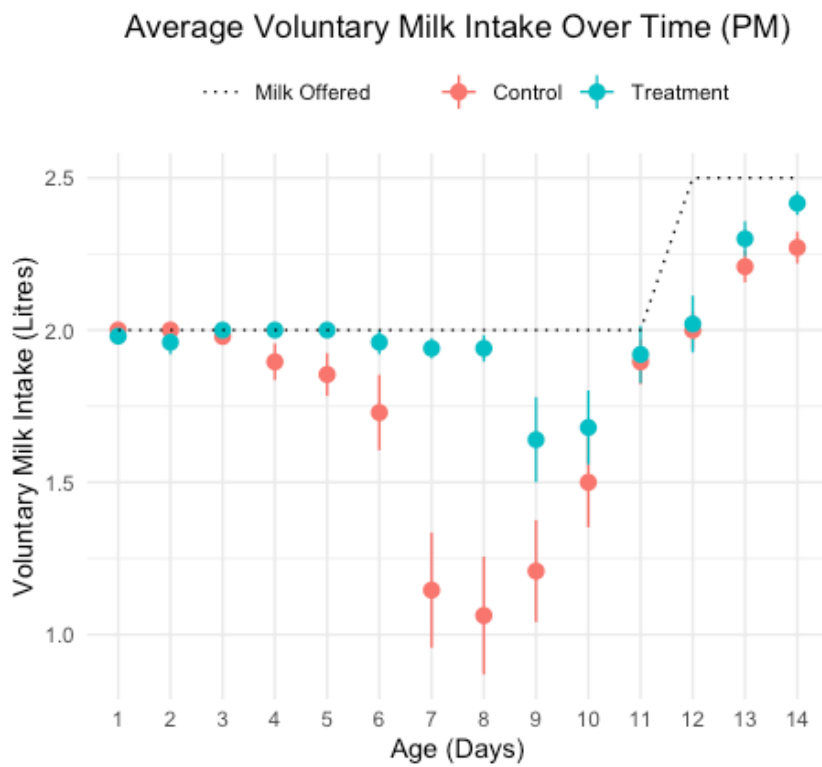


Figure 7. Average evening voluntary milk consumption over time

The analysis of feed intake in the morning indicated substantial differences between the treatment and control groups at various ages. Throughout the trial, the treatment group had a greater average milk consumption than the control group. Specifically, the treatment group had a relatively consistent pattern of milk intake, with just a minor decline detected between days 9 and 14. In comparison, the control group's milk intake remained steady until days 4 to 6, after which a sharp decline occurred, reaching its lowest point around day 9. Subsequently, milk intake gradually increased, although it never fully recovered to the level of milk offered.

An analysis of evening milk consumption revealed a similar pattern of responses in the treatment and control groups. The treatment group consumed less milk on day 9, followed by a gradual return to the offered milk level. In contrast, the control group's milk intake decreased from days 4 to 6, with a significant drop beginning on day 7 and reaching a point of lowest intake on day 8. Milk intake gradually increased, approaching the offered milk line by day 14.

The LMEM revealed that several independent variables had a substantial effect on milk consumption. The fixed effects analysis showed that the treatment group consumed considerably more milk than the control group in both the morning (Estimate = 0.327 litres, SE = 0.046, t-value = 7.145, $p < 0.0001$) and evening (Estimate = 0.1847 litres, SE = 0.0446, t-value = 4.139, $p < 0.0001$). However, breed (Holstein), sex (male), and pen had no statistically significant effects on milk consumption.

The more complex model, which included age as a random variable, provided more insight into how the treatment affected intake throughout the study. The contrast analysis revealed that treatment effects varied by age group. Significant effects were observed for age groups 7, 8, 9, and 10 ($p < 0.005$). However, for other age groups, the treatment effect did not reach statistical significance ($p > 0.05$).

5.2.2 Weight gain

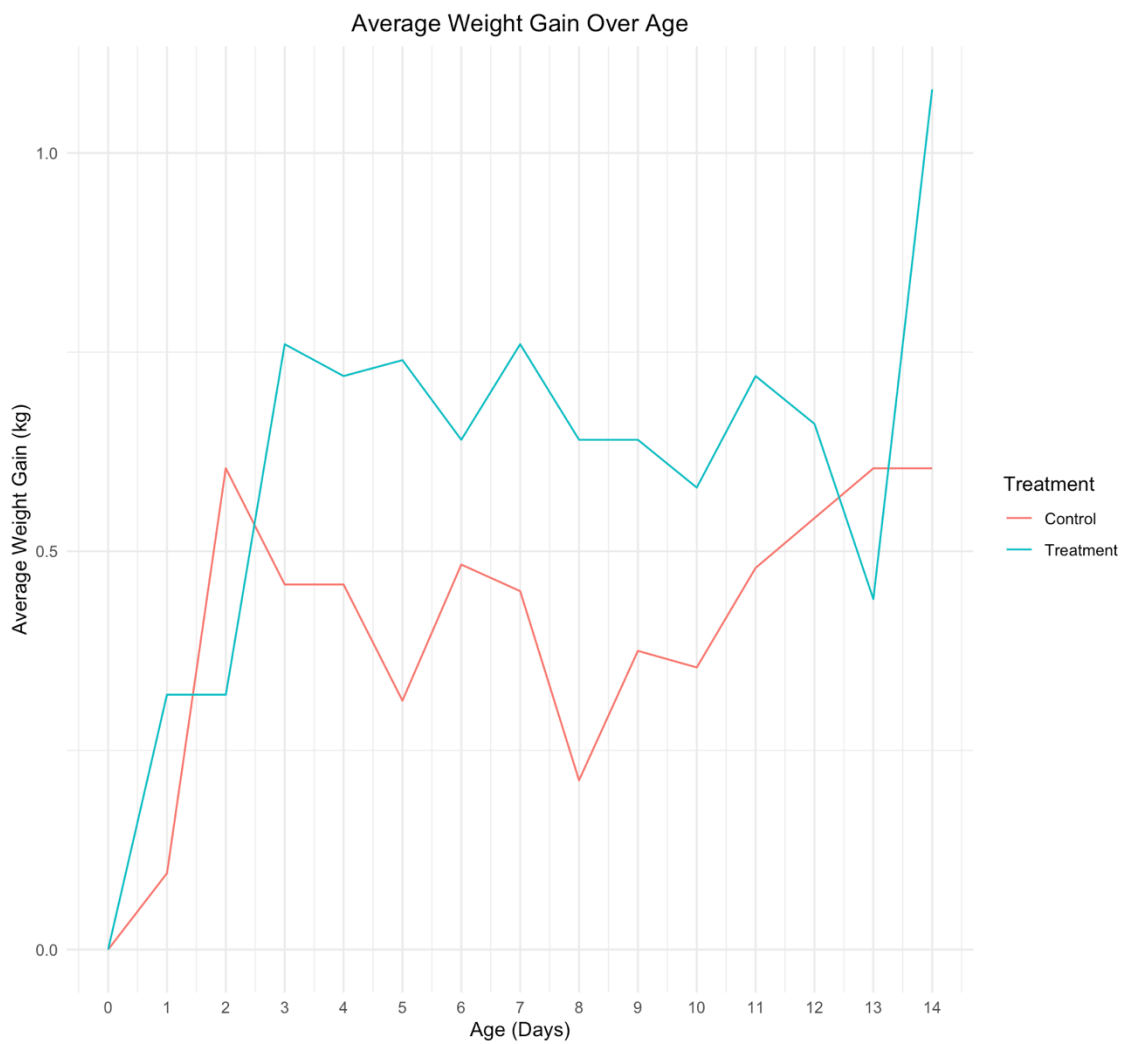


Figure 8. Average daily weight gain of treatment and control group

The LMEM fitted to the weight gain data indicates a significant effect of treatment on weight gain in dairy calves ($p < 0.001$). Calves that received the probiotic gained more weight on average than the control group. The calculated treatment effect was 0.239 kg (SE = 0.0597), showing a significant increase in weight gain due to the treatment. No significant impacts were found for breed, sex, or pen ($p > 0.05$).

Age-specific contrasts assisted in clarifying the differences in treatment effects across age groups. While the probiotic impact is consistent across most age groups, there are some differences, but not statistically significant. Notably, by day 14, calves receiving treatment had a considerably larger mean weight growth than the control group ($p = 0.0004$).

Random effects analysis indicates significant variation among individual animals and between ages. The variance estimates for ear tag and age suggest that weight growth varies significantly at the individual level, emphasising the need to take individual characteristics into account when determining weight gain dynamics.

5.2.3 Scour score

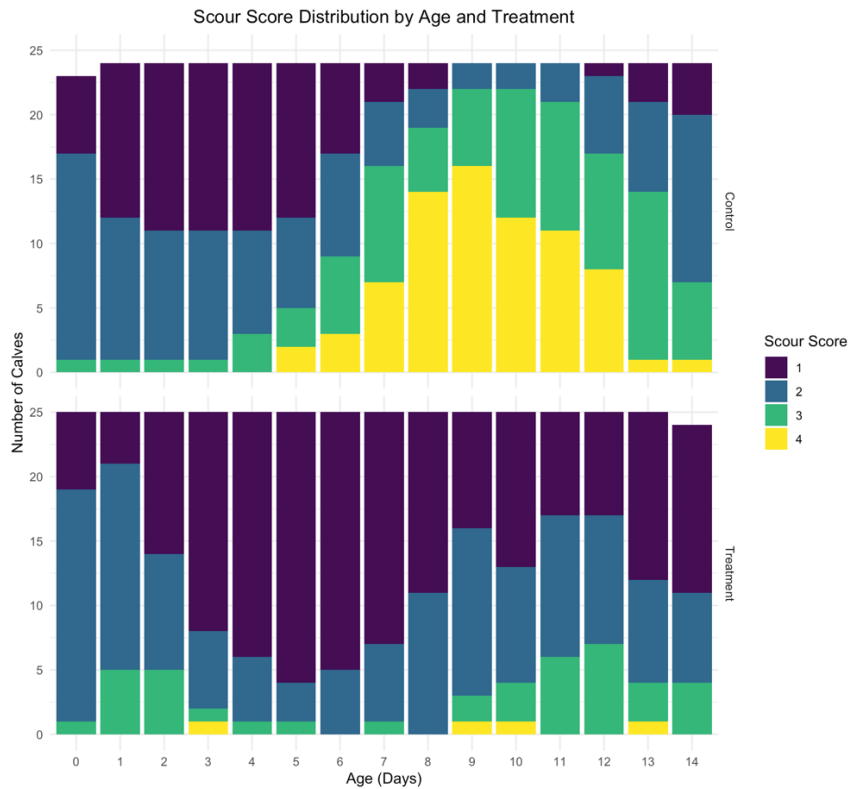


Figure 9. Scour score distribution over time, showing a significant increase in scour score in the control group from ages 4-14.

As scour score is an ordinal variable, CLMM was utilised to investigate the relationship between scour score and other predictor factors including treatment, breed, sex, and pen, while controlling for the random effects of age and calf ear tag. The model yielded a significant effect of treatment on scour score (Estimate = -1.82840, $z = -8.254$, $p < 0.001$), showing that the probiotic was effective, reducing the average scour score by 1.83 units compared to the control group. However, breed (Holstein vs. Aberdeen Angus), gender (male vs. female), and pen did not have a significant effect on scour score ($p > 0.05$).

The random effects analysis demonstrated intercept variability between ear tags (Variance = 0.2673, SD = 0.5170) and ages (Variance = 0.9446, SD = 0.9719), showing variability in scour scores between individuals and over time.

The threshold coefficients exhibited considerable variances between adjacent levels of scour score thresholds. The fluctuations from score 1 to score 2 (estimate = -1.7621, $z = -4.435$, $p < 0.001$) and score 3 to score 4 (estimate = 1.7786, $z = 4.440$, $p < 0.001$) were statistically significant.

5.2.4 Temperature trends over time

Temperature changes in both the control and treatment groups were systematically recorded throughout the trial:

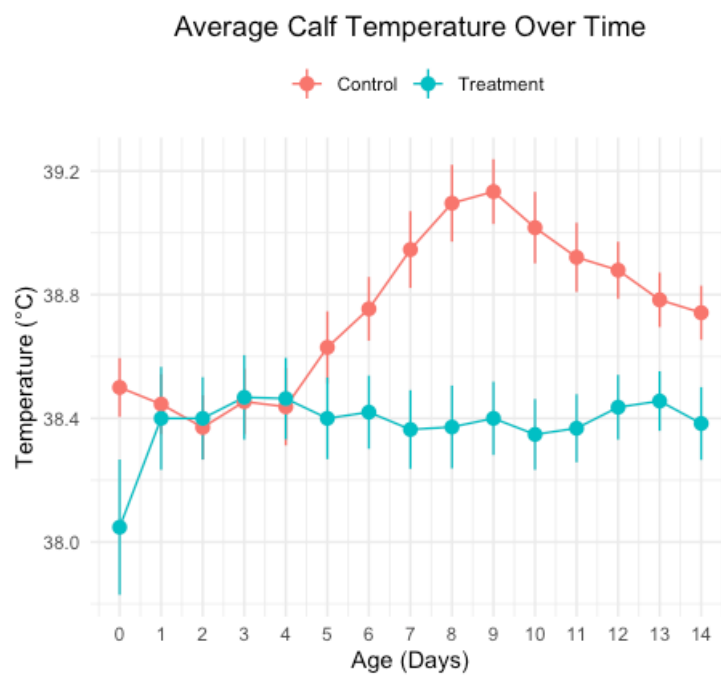


Figure 10. Average calf temperature over time

Initially, no significant differences were observed between each of these groups until approximately day 4. From this point forward, distinct trends developed. Temperatures in the control group increased significantly from day 4 to day 9, followed by a gradual

decline. Conversely, the treatment group demonstrated temperature measurements remained relatively consistent throughout the study.

Normality testing was conducted to evaluate the distribution of temperature data. The histogram revealed a relatively normal distribution (Figure 11). Additionally, a QQ plot was used to validate the normality assumption (Figure 12). The majority of data points closely aligned with the QQ line, indicating adherence to normal distribution assumptions.

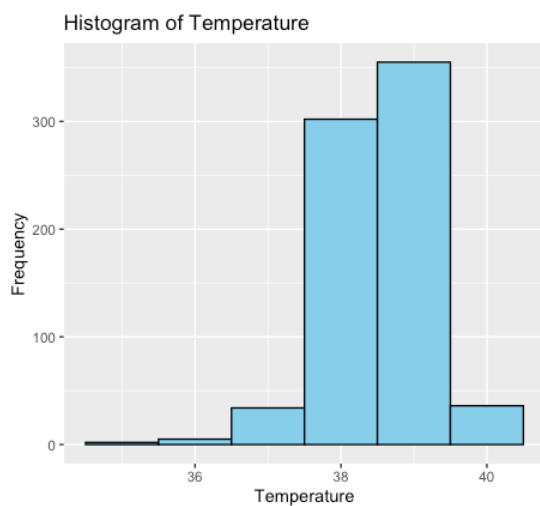


Figure 11. Histogram of temperature data

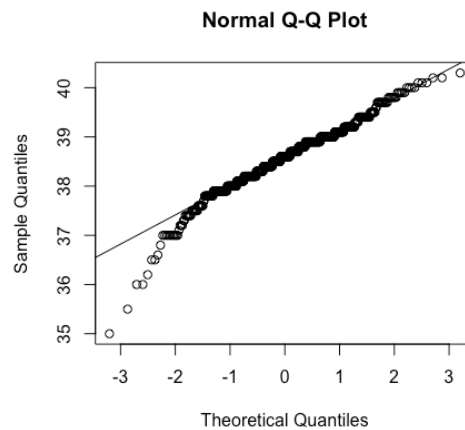


Figure 12. Q-Q plot of temperature data

LMEMs were used to further investigate the relationship between treatment and temperature while accounting for potential confounding factors. Using Restricted maximum likelihood (REML) estimation, the model was developed to incorporate a random intercept for ear tags, allowing for repeated measurements within individual calves, with a SD of 0.4106518. Focusing on fixed effects, specifically the influence of

the treatment group on calf temperature, the intercept, which represents the control group's baseline temperature, was estimated at 38.74°C with a SD of 0.0874.

The coefficient for the treatment group was -0.35888, indicating a decrease in temperature by approximately 0.36°C for calves receiving probiotics compared to the control group. The associated p-value was 0.0052, indicating statistical significance. The correlation between the intercept and treatment groups was estimated to be -0.714.

The standardized within-group residuals ranged from -5.36 to 3.94, with a median of -7.93e-05, suggesting that the model adequately captured the variability in temperature measurements.

To account for the longitudinal nature of the data and potential interactions with other variables such as breed, sex, and pen, an extended LMEM was then developed. This model included age as a random factor to account for variability between days.

Post-hoc comparisons were made at each age using the emmeans package., to determine the time points at which there were substantial variations in temperature between treatment groups. This identified that treatment groups showed significant temperature changes at ages 0, 7, 8, 9, 10, 11, and 12 ($p < 0.05$), indicating that treatment had a dynamic effect over time.

While no significant differences were identified at ages 1–6, the trend shifted at age 7 and continued through subsequent ages, reflecting a shift in treatment efficacy or physiological response at later stages of the study.

6 Discussion

This study aimed to investigate the effects of probiotic supplementation on the prevalence of *Cryptosporidium* infections in calves, as well as its impact on the severity of symptoms, such as temperature, weight gain, scour scores and milk intake.

6.1 Disease prevalence

The considerable reduction in positive *Cryptosporidium* tests among calves administered probiotics represents a promising improvement in disease management in calf-rearing operations. These notable findings imply that probiotics may effectively regulate the gut environment, potentially inhibiting *Cryptosporidium* growth. This aligns with a previous study investigating the impact of specific probiotic strains on *Cryptosporidium parvum* oocyst viability (Foster et al., 2003). In that study, *Lactobacillus acidophilus* and *Lactobacillus reuteri* supernatants significantly increased the proportion of nonviable oocysts, suggesting the presence of anti-parasitic compounds with anti-*C. parvum* activity. Similarly, our findings suggest that probiotics, when administered to calves, may exert beneficial effects on gut health and potentially reduce the prevalence of *Cryptosporidium* infections. Together, these studies contribute to our understanding of the therapeutic potential of probiotics in combating *Cryptosporidium* infections in livestock. A decline in positive test results suggests the possibility of a reduction in economic losses and animal welfare concerns related to *Cryptosporidiosis*, indicating success in disease control efforts for calf health.

While breed, sex, and pen did not emerge as important predictors in this analysis, their

inclusion in the model provides an enhanced understanding of the factors impacting *Cryptosporidium* prevalence. Additional research on potential interactions or confounding effects of these variables may shed light on their significance in *Cryptosporidium* transmission dynamics. However, the lack of breed and sex effects suggests that the findings could be universal.

The observed variability among individual animals and between ages highlights the heterogeneous nature of *Cryptosporidium* infections within commercial farm environments. Strategies for identifying and targeting individuals at risk, as well as employing age-specific management practices, may help to reduce the spread of *Cryptosporidium* and lessen its impact on calf health and production.

However, various considerations necessitate a cautious interpretation of these findings. First, while the decrease in prevalence is encouraging, it is critical to examine the extent of the effect. The period of the study might influence the observed prevalence rates. *Cryptosporidium* infections can exhibit seasonal fluctuations, with higher prevalence rates during certain periods of the year (Robertson et al., 2013). As a result, longer-term trials spanning multiple seasons would provide an enhanced understanding of the efficacy of probiotic administration in various environmental conditions.

Another factor to consider is the risk of cross-contamination between treatment groups, particularly when pens are used as the experimental unit. In this scenario, analysing data at the pen level could offer insights into how the physical layout and management of pens may influence the spread of *Cryptosporidium* oocysts between groups. Despite efforts to maintain strict hygiene regulations the proximity of calves in a shared environment could

facilitate the spread of *Cryptosporidium* oocysts between groups, thereby skewing the results. Implementing steps to reduce cross-contamination, such as individual housing or stringent biosecurity rules, may aid future investigations.

The rapid scour testing kits used have the possibility of receiving false-positive or false-negative results, which could confound the interpretation of the findings. False-positive results may exaggerate the prevalence of *Cryptosporidium* infection, whereas false-negative results may understate it. Implementing quality control techniques, such as duplicate testing and independent validation, can help reduce the possibility of misclassification bias and improve test reliability. However, it is reasonable to assume that any test errors are consistent between treatment groups, meaning this has not invalidated the results.

6.2 *Enhanced milk intake*

The research undertaken in this study provides useful insights into the parameters influencing calf milk intake, with a particular emphasis on the effect of probiotic treatment on milk consumption. The findings not only demonstrate the efficacy of the probiotic intervention but also highlight the need to use a variety of statistical methods when confronted with non-normally distributed data.

The LMEL analysis identified numerous significant determinants of morning and evening milk consumption, including the probiotic treatment, breed, and sex. The treatment group demonstrated a substantial favourable effect, displaying those calves' receiving probiotics consumed more milk than their counterparts in the control group ($p < 0.0001$). This result supports our initial prediction, indicating that the probiotic treatment has a positive impact

on milk consumption, demonstrating the intervention's efficacy in improving nutritional absorption among young calves.

The emergence of positive *Cryptosporidium* tests around day 5 signals a critical turning point in the study, corresponding with significant changes in milk consumption patterns. While both the treatment and control groups had reduced milk intake, the magnitude of the decline varied significantly. The control group had a greater reduction in milk intake than the treatment group.

However, it is crucial to note that the assumption of normality underlying the LMEM was not entirely met, as evidenced by the histograms and QQ plots performed. As a result, further investigation into the distributional features of the data was required. To overcome such concerns, a Wilcoxon rank sum test, a non-parametric approach that does not require normality assumptions was used. The findings of this test indicated a significant difference in feed intake between the treatment and control groups, providing strong evidence for probiotics' effectiveness in increasing milk consumption.

The observed increase in feed intake in the probiotic-treated group has important implications for calf health and production, notably in the management of infectious diseases like *Cryptosporidiosis* which can manifest itself through reduced voluntary milk intake. Notably, the symptomatology of lower milk intake found in *Cryptosporidiosis* is consistent with our findings, as demonstrated by the significant difference in milk consumption between the probiotic-treated and control groups.

Probiotics may help counterbalance the unfavourable effects of low milk intake associated with *Cryptosporidiosis* by encouraging higher feed consumption, hence enhancing calf growth and development.

6.3 Increased weight gain

The findings on weight gain in calves provided convincing insights into the benefits of probiotic treatment. My analysis using LMEM found a substantial impact of treatment on weight gain ($p < 0.001$). Calves administered the probiotic showed a significant increase in weight compared to the control group. Specifically, the calculated treatment effect of 0.239 kg (SE = 0.0597) highlights the importance of probiotic supplementation in increasing weight gain in calves.

Interestingly, my analysis found no significant influences of breed, sex, or pen on weight increase ($p > 0.05$), implying that these variables had no discernible effect on the observed outcomes.

Further investigation using age-specific contrasts revealed subtle differences in treatment effects across age groups. While the probiotic's overall benefit looked constant across most age groups, some differences were identified, but not statistically significant. Among these findings is the significantly higher mean weight increase found in probiotic-treated calves compared to the control group by day 14 ($p = 0.0004$), showing an early commencement of positive effects due to the probiotic intervention.

The observed weight gain in probiotic-treated calves could be attributed to a variety of factors. Firstly, it is possible that these calves consumed more milk due to enhanced gastrointestinal health facilitated by probiotic supplementation. Furthermore, probiotics

may have improved nutrient absorption efficiency by promoting gut health and integrity. Specifically, the presence of probiotics may have mitigated the damage to intestinal villi caused by *Cryptosporidium* infection (Maradana et al., 2023), allowing for greater nutritional absorption and utilisation, resulting in enhanced weight gain.

Examining random effects revealed significant differences at both the individual animal and age levels. The variance estimates for ear tag and age revealed significant variations in weight gain dynamics across individual calves and between ages. These findings highlight the importance of considering individual characteristics and age-related factors into account when attempting to understand the intricacies of weight gain trends in calves.

In summary, my work provides compelling data supporting the usefulness of probiotic supplementation in improving weight development in dairy calves. The observed advantages, together with insights into age-related impacts and individual variability, help to provide a thorough picture of the mechanisms controlling weight gain dynamics in this population. These findings have significance for optimising management techniques that promote growth and production in calf-rearing procedures.

Furthermore, our findings are consistent with prior research, such as that conducted on the effects of Mannan-Oligosaccharide and *Bacillus subtilis* supplementation on preweaning Holstein dairy heifers. While our study showed a significant increase in weight gain as a result of probiotic treatment, the previous study found no direct impact on weight gain from probiotic treatment alone, but it did observe a significant increase in average daily weight gain with symbiotic treatment, which included both prebiotic and

probiotic components. Despite this discrepancy, both studies support the potential benefits of probiotic therapies in improving calf health and productivity.

6.4 Variations in scour score

The analysis, which employed a CLMM, systematically addressed the assumptions intrinsic to this modelling framework to verify the validity and reliability of my findings. While typical assumptions such as the normality of the response variable did not apply to CLMM, I extensively assessed other essential assumptions to enhance the robustness of the analysis.

The proportionate odds assumption, which is central to CLMM, was meticulously scrutinised to ensure the consistency of predictor effects across different levels of scour severity. I confirmed the stability of predictor effects using proportional odds ratio tests and a visual evaluation of the parallel line's assumption, revealing that variables such as treatment, breed, sex, and pen had a consistent influence on scour scores throughout increasing severity levels.

Furthermore, the random effects assumptions were thoroughly evaluated to confirm the validity of calculated random effects, particularly those related to ear tag and Age. The normal distribution of random effects was confirmed by an examination of model residuals, supporting the credibility of individual variability estimates and their impact on scour score.

In addition to model diagnostics, a comprehensive statistical evaluation was carried out to determine the precise impacts of treatment on calf scour score. The probiotic treatment

significantly reduced scour severity (Estimate = -1.82840, $z = -8.254$, $p < 0.001$) compared to untreated calves. This research emphasises the usefulness of probiotic interventions in minimising scouring episodes in calves exposed to *Cryptosporidium*.

The observed reduction in scour scores among probiotic-treated calves compared to the control group suggests that supplementation can relieve diarrhoea symptoms associated with *Cryptosporidium* infection. This finding implies that probiotics may have an anti-diarrheal influence by altering gut microbiota composition, improving mucosal barrier function, and decreasing pathogen colonisation in the gastrointestinal tract. The strong therapeutic effect emphasises the clinical importance of probiotic supplementation in controlling *Cryptosporidium*-induced diarrhoea in calves.

While the treatment effect on scour scores is significant it is critical to address any confounding variables that could influence the reported results. Factors like breed, gender, and pen environment may have an impact on calf health and susceptibility to *Cryptosporidium* infection, potentially complicating therapy. The lack of significant impacts for these variables shows that probiotic supplementation provides consistent benefits across varied demographic groups, increasing the generalizability of the findings.

However, while our study controlled for measured variables, such as treatment group and certain demographic factors, it's important to acknowledge that residual confounding by unmeasured variables, such as variations in management practices or environmental conditions, may still exist. Notably, our trial did not investigate a range of environmental or management conditions that could potentially influence the effectiveness of probiotics. Therefore, future research should aim to address these possible confounders by

employing rigorous study designs and comprehensive data collection techniques. This would allow for a more thorough examination of the efficiency of probiotics under different settings, seasons, management practices, and other relevant factors, ultimately enhancing the validity and generalizability of the results.

6.5 Temperature

The initial LMEM compared temperature across control and treatment groups, revealing the impact of probiotic supplementation on temperature regulation. The fixed effects coefficients indicate a substantial temperature difference between the control and treatment groups. The intercept reflects the estimated mean temperature for the control group, which was around 38.74°C. In contrast, the coefficient for the treatment group suggests a temperature decrease of about 0.36°C relative to the control group. The treatment coefficient has a significant p-value ($p = 0.0052$), indicating that this difference was unlikely to arise by coincidence.

To investigate the association between temperature and numerous independent variables, an expanded LMEM was constructed, which included additional covariates such as breed, sex, and pen conditions. The inclusion of these variables allows for a more thorough analysis, considering potential confounding factors that could influence temperature dynamics.

Even after correcting for other model factors, the treatment coefficient remains significant, demonstrating that probiotic supplementation has a measurable influence on temperature regulation. The negative coefficient indicates that calves receiving probiotics had somewhat lower temperatures than those in the control group.

Breed coefficients (e.g., Holstein) represent the predicted change in temperature associated with various breeds when compared to a reference group. However, in this model, the coefficient for breed was not statistically significant ($p > 0.05$), implying that breed may not have a major effect on temperature regulation in this study. Similarly, the sex coefficient (e.g., male) shows the estimated change in temperature associated with various sexes when compared to a reference category (e.g., female). In this model, the sex coefficient was not statistically significant ($p > 0.05$), implying that sex may not be a relevant predictor of temperature variability. The coefficient for pen conditions shows the expected temperature change associated with various pen settings. In this model, the coefficient for pen number was statistically significant ($p < 0.05$), indicating that pen conditions may influence temperature dynamics in calves. While all calves were housed indoors, ventilation, insulation, and temperature management within the pens could all vary and affect calf temperature regulation. For example, pens with insufficient ventilation may result in poor air circulation and heat build-up, whereas pens with efficient temperature management systems may provide optimal thermal comfort for the calves. Furthermore, changes in pen design or layout may alter the distribution of heat in the environment, thereby impacting calf temperature dynamics. Thus, despite being indoors, changes in pen conditions may still influence temperature trends in calves. Additional research might investigate deeper into the specific features of indoor pen conditions that contribute to temperature variations, providing insights into effective ways for optimising calf comfort and welfare.

The significant effects identified in the expanded model highlight the necessity of considering a variety of parameters when analysing calves' temperature data. While

probiotic supplementation is associated with temperature regulation, other factors, such as pen conditions, may also have an impact on temperature dynamics. These findings illustrate the complexities of temperature regulation in cattle, emphasising the importance of comprehensive management strategies that consider many aspects influencing animal health and wellbeing.

The reported effects of probiotics on temperature regulation have important implications for managing *Cryptosporidium*. *Cryptosporidiosis* frequently leads to physiological stress and changes in body temperature (Ramirez, Ward and Sreevatsan, 2004), which may exacerbate illness severity and compromise calf health. Probiotics may help alleviate the symptoms of *Cryptosporidium* infection by stabilising temperature dynamics, lowering the disease load and enhancing calf welfare in commercial settings.

6.6 Reflections on research procedure and recommendations for future studies

Reflecting on the research procedure, my study took a comprehensive approach that included methodical allocation of calves to treatment groups, meticulous data gathering, and extensive statistical analysis. By following ethical guidelines and performing the study on a commercial farm, I hoped to ensure that my findings were relevant and applicable to real-world cattle management techniques. However, it is important to note that my data was not balanced in terms of breed and sex, which could introduce potential biases. Therefore, future research should aim to explore the effect of these variables more comprehensively to better understand their influence on the efficacy of probiotic supplementation against *Cryptosporidium* infections.

Additional investigation into the probiotic mechanisms behind their efficacy against *Cryptosporidium*, as well as longitudinal studies to determine the long-term impact of probiotic supplementation on calf health and productivity, should also be considered for future research. Furthermore, research into appropriate probiotic formulations, doses, and administration techniques may improve the efficacy of probiotic therapies in livestock settings.

7 Conclusion

In conclusion, the findings of this study give compelling evidence that probiotic supplementation reduces the prevalence and severity of *Cryptosporidium* infections in young calves. The primary research question on the effect of probiotics on *Cryptosporidium* infections has been unequivocally answered, with significant decreases in both prevalence and severity found in treated calves compared to controls.

This research contributes to our understanding of probiotic supplementation as a potential strategy to manage *Cryptosporidium* infections in young calves. My study, which showed considerable decreases in both infection prevalence and severity, provides practical insights for livestock producers looking for efficient disease control measures while also emphasising the significance of taking age-associated variations in treatment efficacy into account.

This research paper contributes to an existing body of knowledge in veterinary medicine and livestock management by explaining the role of probiotics in the prevention of *Cryptosporidium* infections in young calves. The findings highlight the potential of

probiotic supplementation as a long-term and cost-effective way to enhance animal health and well-being in the cattle industry, with important implications for both research and practice. Further research into breed and sex-related effects will improve the applicability and generalizability of these findings.

8 References

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