Gastrointestinal permeability in calves: Validation of indigestible sugar markers and effects of dietary starch inclusion

by

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B.S., Kansas State University, 2020

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Veterinary Biomedical Sciences
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2026

Approved by:

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Abstract

Gastrointestinal (GI) permeability refers to an animal's ability to regulate the passage and absorption of nutrients, toxins, and even pathogens from the lumen of the gut into the bloodstream. During episodes of increased GI permeability, animals can experience increased systemic inflammation, decreased performance, and it has been hypothesized that increased GI permeability can lead to severe conditions such as liver abscesses (LA). Liver abscesses are topics of concern in feedlot animals, especially beef-dairy (BD) cross animals. Overall, increased GI permeability can negatively affect animal performance, health, and welfare, as well as farm productivity, making it an increasingly interesting issue in production animals. Currently, there are several gaps in our knowledge of the causes and consequences of increased GI permeability in cattle. Therefore, the objective of this thesis was to identify current knowledge and knowledge gaps and to validate methods of diagnosis of GI permeability, as well as evaluate the effect of starch inclusion level in starter feed.

For the first chapter, a literature search was performed to identify published studies that provided valuable information on current knowledge of etiological risk factors, consequences, diagnostic methods, and mitigation strategies of increased GI permeability. The literature search resulted in 69 publications that were scanned for relevant information, with 37 being retained for the literature review. The primary etiological risk factors identified throughout the literature were nutrition, physiological stressors (e.g., weaning and heat stress), environmental, illness, and inflammation. Each of these etiological risk factors includes multiple mechanisms that can impair GI intestinal barrier function and contribute to increased GI permeability in cattle. Consequences of increased GI permeability were also identified with the main consequences negatively effecting animal performance, exacerbating inflammation, and contribution to the development of

other disease conditions. These consequences are being studied through diagnostic methods for research settings that help study the GI permeability including quantifying inflammatory markers, using exogenous tracers, and evaluating tight junction gene expression. These diagnostic methods are only for research settings, therefore, identifying increased GI permeability in production settings is difficult and mitigation strategies are employed to reduce the effects of altered epithelial integrity. Throughout the literature, mitigation strategies revolved around animal management and nutrition. Primary mitigation strategies include nutritional management and stress reduction for animals at higher risk for exhibiting hyperpermeability in the GIT. Overall, GI permeability in cattle remains a topic requiring further study to better understand the impacts and possible mitigation strategies in improving animal health, performance, and welfare for animals in production systems.

Objectives of the first study were to assess the suitability of lactulose and sucralose, a novel sugar, as tracers of GI permeability and to determine appropriate blood sampling time for peak concentrations in functioning ruminants. Three yearling Holstein steers (290 ± 7.86 kg) were individually fed standard grass hay, *ad libitum,* before being restricted to 25% of their *ad libitum* intake to induce gastrointestinal permeability. An oral dose of lactulose (0.4 mg/ kg of body weight) and sucralose (0.4 mg/ kg of body weight) was administered and blood sampled at time 0 and every 3 hours until 36 hours post dosing. Serum was extracted and analyzed for sugar concentration by gas chromatography-tandem mass spectrometry. Arithmetic mean concentrations were 4.05 µg/mL for sucralose and 2.78 µg/mL for lactulose from 3-36 hours post dosing. The predicted probabilities revealed that at three hours both sugars had high probabilities of exceeding LOQ (Lactulose = 0.805, Sucralose = 0.905). In addition, the probability of sucralose being detected above LOQ was higher over the entire time period from 3-36 hours compared to

lactulose (Lactulose = 0.857, Sucralose = 0.901). To conclude this study, the probability of sucralose concentration exceeding LOQ was consistently higher than lactulose indicating sucralose could be used as a more quantifiable and effective biomarker than lactulose with time points between 3 and 33 hours being equally acceptable time points for blood sampling.

Objectives for the second study were to assess the effect of starch inclusion level in calf starter, fed during the hutch phase, on growth performance, rumen pH, GI morphology, and GI permeability during weaning and the finishing transition periods. Twenty beef-dairy (BD) cross calves, one week of age, were randomized to either a high (HS) or low starch (LS) calf starter diet, fed only during the hutch phase. During weaning rumen pH boluses were placed in four animals (n=4; HS=2, LS=2) and continuously measured rumen pH for a period of time. Dry matter intake and BW were collected and used to calculate ADG and G:F in each phase of the study. After the weaning period 10 animals (n=10, HS=5, LS=5) continued on to the grower phase, where they were fed a common grower diet for 131 days until moving on to the finishing transition. At weaning and finishing transition, GI permeability was assessed in all animals by orally dosing two indigestible sugars (lactulose and sucralose) and evaluating serum-sugar concentration at 3 separate timepoints in each phase. At the end of both hutch and finishing phases 10 animals (n=10, Hs=5, LS=5) were harvested and GI contents and tissues were collected from the ventral sac of the rumen, duodenum, jejunum, ileum, and spiral colon. During the hutch phase, pre-weaning BW increased over time from day 0 to day 54 and the LS calves, on average weighed more than the HS calves (P=0.034), whereas, post-weaning BW showed no differences between treatments. Similarly, pre-and post-weaning ADG, DMI, and G:F revealed no treatment differences or interactions (P>0.05). Hutch phase GI morphology and GI permeability was also similar between treatments. Finishing phase performance was minimally affected by hutch phase

treatment diets. Rumen morphology in the finishing phase was different between treatments, as papilla width (0.049) was greater in the HS group than in the LS group and there were tendencies for other rumen histologic measurements to be greater in the HS group ($P \leq 0.1$). In the finishing phase, there were no other significant effects for GI permeability and GI morphology. Overall, starch inclusion level in calf starter diets had minimal effects on animal performance, GI permeability, and GI morphology in the hutch phase and minimal carryover effects in the finishing transition.

These projects emphasize the difficulties and unknowns of researching, diagnosing, and mitigating GI permeability in cattle. Utilizing exogenous tracers and proven methods of inducing permeability as useful research approaches can provide insights that help bridge current knowledge gaps. This work establishes a foundation for future studies aimed at improving the GI health, performance, and welfare of animals at higher risk of exhibiting increased GI permeability.

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Acknowledgements

I am sincerely grateful to the faculty, staff, and graduate students of the Beef Cattle Institute at Kansas State University, past and present, for their invaluable contributions to this research. This project would not have been possible without the dedication, collaboration, and support of everyone involved in the live animal trials.

I am especially thankful to my major professor, Dr. Phillip Lancaster, whose guidance, mentorship, and patience have shaped both my research and my growth as a scientist. I also extend heartfelt thanks to my committee members, Dr. Billy Brown and Dr. Bob Larson, for their steady support, insight, and encouragement throughout this journey. Their influence has helped me grow personally and professionally, preparing me for my future in veterinary medicine.

The friendships I've gained in the BCI office have meant more to me than I can fully express. They turned long days into meaningful ones and reminded me that the best part of any work is the people you share it with. I will carry those friendships with me always.

To my family Eva and Ruben Rivas and Richard and Lisa Craine, Hayden Kunnary and all those who have been part of my world, thank you for being my foundation. Even my pets, Jed, Duck, and Teeny, have offered comfort in their own sweet ways. Each of you has helped me feel supported, grounded, and loved.

Your unconditional love and encouragement have carried me through every step of this journey. You've celebrated my victories, lifted me through challenges, and reminded me of my strength when I couldn't see it myself. I am endlessly grateful for your faith in me, and I share this milestone with you.

Dedication

This work is dedicated to my parents, Eva and Ruben Rivas Jr. and my grandparents, Rick and Lisa Craine. Thank you for encouraging me in all of my endeavors to pursue veterinary school and for only encouraging me more when I decided to pursue large animal veterinary medicine. Your belief in me has been a constant source of strength and motivation, especially during the challenges and accomplishments of graduate school. I would not be where I am today without their sacrifices, patience, and confidence in my ability to achieve my goals. This accomplishment is just as much theirs's as it is mine.

Chapter 1 - Current understanding of gastrointestinal permeability in cattle: mechanisms, diagnosis, and management

INTRODUCTION

The gastrointestinal tract (GIT) plays a significant role in regulating nutrient absorption, immune function, and productivity in cattle. Even though the primary function of the GIT is nutrient digestion and absorption, it is a critical component of the animal's innate immune system, as it acts as a selective barrier regulating movement of nutrients, toxins, pathogens, and antigens from the lumen of the intestine to the animal's systemic circulation. The intestinal barrier is maintained through the coordinated function of tight junction proteins, epithelial cells, mucus production, and other regulatory mechanisms. The integrity of the intestinal barrier can become compromised, a condition known as gastrointestinal (GI) permeability or "leaky gut." When increased permeability occurs, substances that are normally restricted, such as endotoxins, pathogens, and inflammatory compounds, may enter the blood stream and contribute to further disease processes within the animal.

In cattle, GI permeability has become an area of increasing interest, due to its potential implications in animal health, performance, production efficiency, and sustainability. Gastrointestinal permeability is commonly associated with enteric disease states such as parasite infestations or pathogen exposure. However, numerous additional risk factors and stressors have been implicated including nutritional such as low forage inclusion rate (Pereira et al., 2023b; Pereira et al., 2023a), environmental such as heat stress (Fontoura et al., 2022), and physiological factors such as calving (Abuajamieh et al., 2016). Each of these stressors can cause alterations within the GIT such as decreased pH, shifts in microbial populations, excessive fermentation, or reduced fermentation. Alterations in GIT function may disrupt the tight junction proteins responsible for

regulating permeability, resulting in translocation of endotoxins and inflammatory compounds, and decreased nutrient utilization. Increased GI permeability may negatively impact metabolic processes, animal performance, and inflammatory responses (Kvidera et al., 2017a).

Although interest in GI permeability in cattle has increased, considerable knowledge gaps remain regarding diagnostic measures, pathogenesis, and long-term effects. Furthermore, assessing GI permeability *in vivo* is increasingly challenging due to the inconsistencies among measurement methods and the lack of standardized biomarkers. Therefore, the objective of this literature review is to summarize the current understanding of GI permeability in cattle including the normal physiology of the GI barrier, etiological and risk factors that predispose cattle to increased permeability, methods to diagnosis and mitigate GI permeability, associated consequences, and current knowledge gaps and future research directions.

Literature in this narrative review was identified through searches conducted in PubMed, AGRICOLA, and CABI between 1982 and 2026. Using a combination of key words related to gastrointestinal permeability in cattle, the primary search string was:
((((((((((cattle) OR (cow)) OR (cows)) OR (heifer)) OR (heifers)) OR (steer)) OR (steers)) OR (bull)) OR (bulls)) OR ("bos taurus")) OR (bovine)) AND (("gastrointestinal permeability") OR ("leaky gut"))

The search yielded 69 publications. Articles were screened for relevance to GI barrier function, GI permeability, risk factors, etiology, diagnostic methods, and mitigation strategies in cattle. Additional relevant publications were included to provide foundational context for the review.

PHYSIOLOGY

Under normal physiology, the GIT is responsible for nutrient and water absorption, acting as a protective barrier against toxins and pathogens, immune regulation, and maintenance of microbial homeostasis. Selective permeability is present under normal conditions, where the GI barrier allows for the absorption of water, electrolytes, and other nutrients, but also prevents the translocation of pathogens, endotoxins, and inflammatory markers from the lumen of the GIT to systemic circulation. In healthy cattle, there is a single layer of columnar epithelial cells lining the small and large intestine serving as the primary physical barrier. Rapid cell turnover and regeneration help maintain intestinal integrity and barrier function. Furthermore, tight junction proteins connect adjacent columnar epithelial cells regulating paracellular transport and maintaining impermeability between cells. There are three primary tight junction proteins that act as an extra layer of protection, including claudins, occludins, and zonula occluden proteins (Briggs et al., 2020). Lastly, there is a mucus layer primarily produced by goblet cells in the small intestine and colon. This mucus layer protects the epithelium from mechanical damage while also trapping pathogens and debris and providing lubrication for digesta to move through the GIT (Horst et al., 2020).

Aside from the physical barrier, the GI system also plays a critical role in immune regulation and host defense (Foster et al., 2026). Throughout the GIT, gut associated lymphoid tissue is responsible for regulating inflammatory responses, preventing excessive immune activation, and secreting of protective antibodies. Protective compounds secreted from lymphoid tissue include immunoglobulins, particularly IgA, cytokines, inflammatory markers associated with acute phase protein production (APP), including haptoglobin, serum amyloid A (SAA), and lipopolysaccharide binding protein (LBP) (Abuajamieh et al., 2016). These immune responses can be triggered

by alterations in tight junction activity where substances such an endotoxins or pathogens may cross the intestinal barrier into the blood stream (Silva et al., 2023). Additionally, interactions exist between the microbiota and immune signaling, where commensal bacteria regulate immune development, microbial metabolites can influence signaling, and a healthy, balanced GI microbial community supports proper immune responses. Overall, the immune component of the GIT can significantly influence epithelial integrity and, when disrupted, contribute to increased permeability and systemic inflammation (Cangiano et al., 2022).

While the lower portions of the GIT rely primarily on a columnar epithelial barrier and associated immune defenses to maintain intestinal integrity, the rumen possesses distinct structural and physiological characteristics that also contribute to barrier function and overall GI homeostasis in cattle. The rumen is the largest compartment of the ruminant forestomach and is the primary site of microbial fermentation functioning to breakdown macromolecules prior to enzymatic digestion in lower parts of the GIT in cattle. The normal rumen environment is oxygen free as the microbes are anaerobes and helps maintain both host and microbial homeostasis. Unlike the small intestine, large intestine, and colon, the rumen does not contain a columnar epithelial layer. Instead, it consists of four layers of stratified squamous cells with papillae covering the epithelial surface. Rumen papillae increase surface area for absorption of volatile fatty acids (VFAs), water, and electrolytes. The size and density of the papillae is heavily influenced by diet and fermentation end products. The rumen also contains a substantial microbial population that functions to break down feed stuffs and utilize the fermentation products, and similar to papillae development, they are highly influenced by diet composition. Additionally, normal rumen functions include muscular contractions that mix and distribute digesta and aid in passage to the

lower digestive tract. Furthermore, saliva production and other ruminal pH buffering mechanisms play a significant role in maintaining rumen health and supporting proper fermentation.

The rumen relies on several physiological mechanisms to maintain homeostasis, regulate fermentation, and preserve overall GI health. As a fermentation vat, the rumen normally maintains a pH ranging from 5.8-7.0, although ruminal pH is heavily influenced by diet composition and feeding frequency. Stabilization of ruminal pH through various mechanisms reduces the risk of metabolic disorders that can cause further systemic inflammation (Pereira et al., 2023a). Furthermore, volatile fatty acids (VFAs) are absorbed into the blood stream which also aids in reducing acid accumulation and stabilization of ruminal pH.

The ruminal epithelial layer functions similarly to the epithelial layer in the lower digestive tract by preventing excessive movement of bacteria and toxins, while tight junctions between cells regulate paracellular movement (Briggs et al., 2020) and are selectively permeable (Horst et al., 2020). Additionally, the integrity of the epithelium is maintained through continuous cell turnover and regeneration. Overall, homeostasis is sustained through balancing fermentation products, absorption of those products, pH buffering capacity, rumen motility, and microbial populations. A stable rumen microbial ecosystem supports efficient fermentation, optimal GI health, and improved animal performance; however, altered epithelial integrity may increase GI permeability and induce systemic inflammation responses (Sanz-Fernandez et al., 2024).

ETIOLOGY AND RISK FACTORS

Increased gastrointestinal permeability is considered a multifactorial condition as there are a variety of nutritional, environmental, physiological, and infectious factors that can cause an animal to experience hyperpermeability. There is no one causative factor that can lead to an animal with increased GI permeability; there are multiple chronic and acute stressors that could be

impacting an animal's ability to regulate GIT permeability. Each of these etiologic factors ultimately alter tight junction function, epithelial integrity, microbial homeostasis, or inflammatory signaling within the GIT. Often, stressors occur simultaneously, leading to additive effects increasing GI permeability. The etiologic factors this review explores are nutritional, environmental, physiological, and infectious.

Nutrition Risk Factors

A main etiologic factor for increased GI permeability is nutrition. There are many aspects of nutrition that can cause increased GI permeability which includes high starch inclusion (Abeyta et al., 2023c), fiber source and inclusion rate (Pereira et al., 2023b), and feed restriction (Pisoni et al., 2022).

High starch inclusion in cattle diets has been thought to be a main factor contributing to increased permeability as high concentrates lead to a decrease in ruminal pH and a condition of prolonged low pH with increased VFA production known as ruminal acidosis. Furthermore, increased concentrates in the diet increase the amount of fermentable carbohydrates that flow from the rumen to the hindgut (Sanz-Fernandez et al., 2024). The increased flow of starch from the rumen to the hindgut, similar to the rumen, can lead to incidences of hindgut acidosis which has been studied and found to increase permeability, induce damage to the hindgut epithelium, and increased inflammatory biomarkers (Abeyta et al., 2023b; Abeyta et al., 2023c; Sanz-Fernandez et al., 2024). Regardless of the location of acidosis, there can be disruption to the integrity of the epithelial barrier resulting in increased permeability, and the translocation of inflammatory substances to systemic circulation, having the potential to cause systemic inflammation (Kvidera et al., 2017a).

Another nutritional aspect that can negatively affect GI permeability in cattle includes fiber inclusion rate and fiber source. There have been multiple studies evaluating the effect of different types of fiber and fiber inclusion levels on GI permeability, ruminal pH, and inflammatory factors (Pereira et al., 2023b; Pereira et al., 2023a). A main finding is that an increased amount of both physically effective neutral detergent fiber (peNDF) and undigestible neutral detergent fiber (uNDF), can increase ruminal pH, increase ruminal contractions, and decrease GI permeability, but also dampen systemic inflammatory responses and decrease inflammatory markers (Pereira et al., 2023b; Pereira et al., 2023a). The goal of dietary fiber formulation is to balance physically and effective neutral detergent fiber (peNDF) and undigested neutral detergent fiber (uNDF) to promote optimal rumen function, fermentation, and animal health. Inclusion rate of both starch and fiber in cattle diets is extremely important in GI health and function, and are important etiological factors leading to GI permeability.

Another nutritional aspect that can impact GI permeability is feed restriction. Feed restriction may not be deliberate; however, it can happen when animals are transported for an extended amount of time or when animals decrease their feed intake dramatically due to overcrowding or illness. Regardless of the mechanism of feed restriction in production settings, it is used as a reliable method to induce permeability in cattle in research settings (Zhang et al., 2013a; Horst et al., 2020; Coleman et al., 2023). Furthermore, research assessing the effects of the degree of feed intake restriction on GI permeability have shown that a higher degree of feed restriction increases the severity of GI permeability which could be due to decreases in morphological measurements of structures within the intestines such as mucosal area, villi height, and goblet cells counts (Zhang et al., 2013a; Kvidera et al., 2017a). Although the exact mechanism through which feed restriction alters epithelial barrier integrity is unknown, feed restriction is

used as a research method to assess GI permeability and is a contributing factor to further disease progression and GI function.

There are also several risk factors that overlap substantially with etiological factors of GI permeability, some of which are more management practices, but related to nutrition. Feedlot cattle or lactating dairy cows fed high-starch, low fiber diets maybe at an increased risk for developing increased permeability, particularly when diets are fed for extended periods of time. Additionally, animals that undergo a lot of transportation such as beef animals that are transported to several facilities throughout their life cycle, can exhibit a higher risk for increased GI permeability due to the stress and feed restriction during these periods (Kvidera et al., 2017b).

Physiology Risk Factors

In addition to nutritional factors, an animal's physiological state such as calving, lactation, and age can impact an animal's ability to regulate GI permeability and can significantly affect GI health and function. During these physiological events, there are substantial immune, endocrine, and metabolic changes that occur that can negatively affect nutrient partitioning, energy balance, and immune function. These alterations can ultimately result in altered epithelial barrier integrity and increased GI permeability or decreased GI health and function.

An animal's age has a significant impact on an animal's ability to regulate the passage and absorption of substances across the GI barrier. Calves, for example, have very little immune function and therefore cannot regulate GI permeability like healthy functioning adult ruminants. Over time an animal's GIT develops as well as its immune function, and its ability to regulate GI permeability increases, leading to increased barrier function. However, the stress of weaning can also increase GI permeability in a calf, although the exact mechanism of this is not known (Wood et al., 2015). Furthermore, as the calf ages and consumes increasing amounts of dry feed,

the rest of the GIT develops, and GI function improves (Wood et al., 2015). Improved GI function may be due to an alteration in the microbial community in the rumen and hindgut, allowing for improved GI health and metabolism (Wei et al., 2012). Collectively, these studies emphasize the importance and impact of an animal's physiological age and stage of life on GI health and function.

In addition to age, other physiologic stressors such as calving and early lactation can negatively impact GI health and function for specific periods of time. During calving and early lactation, an animal's nutrients are partitioned from calf development toward milk production, however, when inflammation associated with calving and milk production are present, productivity can be disrupted impacting multiple organ systems in the body including the GIT (Loor et al., 2005; Bertoni et al., 2008). Prior to calving, dairy cows can experience a decrease in feed intake, similar to feed restriction, which has been shown to increase GI permeability (Zhang et al., 2013a; Abuajamieh et al., 2016). Furthermore, mastitis, an infection of the mammary gland, has been shown to increase systemic inflammation and decrease dry matter intake, which can subsequently cause hyperpermeability of the GIT (Bertens et al., 2026). Ketosis is another physiological disorder that can cause a decrease in feed intake and an increase in systemic inflammation, increasing GI permeability in cows after calving (Abuajamieh et al., 2016). Overall, the physiological and metabolic demands associated with calving and early lactation may create conditions that predispose cattle to decreased epithelial barrier function and GI health.

There are various substantial physiologic risk factors that may predispose animals to compromised GIT health and increased permeability many of which overlap with the established etiological factors. Young calves and neonates are considered particularly susceptible due to the ongoing development of both the GIT and immune system. Management practices that induce

weaning related stress may further the risk of GI barrier dysfunction during this critical developmental period. Furthermore, cattle approaching parturition, particularly dairy cows, may be at greater risk for increased GI permeability due to the physiological and metabolic demands associated with calving, as well as the occurrence of transition-related disorders such as ketosis and mastitis. Other factors that may increase susceptibility to GI dysfunction include stressors that negatively affect feed intake, rumination behavior, and overall GI function. Collectively, these physiological risk factors may contribute to impaired GI barrier integrity and increase the likelihood of permeability-related disturbances.

Environmental Risk Factors

As previously discussed, stressors of various origins may act as risk factors that predispose cattle to increased GI permeability. Among these, environmental stressors are of significance due to their widespread occurrence in cattle production systems and their ability to elicit physiological, metabolic, and immune responses that can compromise GI barrier integrity. Environmental changes such as heat stress and transportation have been associated with disruptions in the homeostasis of the animal leading to alterations in feed intake, immune responses and nutrient utilization, all of which may contribute to hyperpermeability.

Heat stress, a common stressor for cattle in warm locations, has been researched significantly, assessing the effects it has on GI permeability, milk production, inflammatory markers, and various other performance measures. Through this research, an association has been identified between heat stress and increased GI permeability (Baumgard and Jr, 2013). The hypothesized mechanism for this is when heat stress occurs; blood is shunted to the skin or periphery to maximize heat dissipation from the animal. Therefore, there is less oxygen and nutrient supply to

the GIT, leading to hypoxia and oxidative damage, and disruption of the tight junction complexes (Opgenorth et al., 2021). Increased permeability results from disruption and alterations in tight junctions. These observations support the hypothesis that heat stress is an important environmental factor capable of altering gastrointestinal barrier function and promoting increased permeability in cattle.

In addition to heat stress, stress associated with transportation to new facilities has also been associated with disruption in the function of the GI barrier. Transportation can cause excess stress from overcrowding, feed and water restriction, physical injuries, handling stress, and comingling (González et al., 2012). Transportation stressors can cause an increase in cortisol, and other physiological stress markers such as acetylcholine, glucocorticoids from parasympathetic activation, reducing blood flow to the GIT. The reduction in blood flow results in hypoxia, oxidative damage, acidosis, and ATP depletion, leading to alterations in epithelial barrier integrity (Lambert, 2009). Furthermore, the increase in physiological stress markers can decrease feed intake causing change in tight junction proteins leading to increased permeability (Kvidera et al., 2017a), and feed and water restriction are common occurrences during transportation of cattle. There has been research done where transportation is used as a method to induce GI permeability (Silva et al., 2023), indicating transportation has a significant impact on GI health and function in cattle, although the mechanism through which alteration in GI integrity happens is unknown.

Environmental stressors such as heat stress and transportation may act as etiological risk factors that contribute to decreased GI barrier function and compromised GI health; however, several risk factors can increase an animal's exposure to these stressors. Animals raised in re-

gions where daytime temperatures are elevated, and nighttime temperatures do not decrease sufficiently to allow animals to dissipate heat properly may be at an increased risk for experiencing heat stress. Furthermore, black hided cattle, heavier animals, and cattle experiencing illness (Brown-Brandl et al., 2006) are all at higher risk for experiencing heat stress potentially resulting in increased susceptibility to increased GI permeability. Regarding transportation, native beef calves transported at weaning and dairy calves transported shortly after birth are at greater risk for incidences of increased GI permeability. Overall, environmental conditions that impose excessive stress on cattle may alter feeding behavior and other physiological processes, ultimately resulting in impaired GI barrier function and compromised GI health.

Illness and Inflammation

In addition to physiological, environmental, and nutritional factors, illness and inflammation are significant contributors to GI permeability in cattle. Both localized and systemic disease processes can alter GI function through immune mediated responses such as increased inflammatory markers and activation of inflammatory signaling. Various inflammatory conditions have been shown to elicit an immune mediated response that increases systemic inflammatory markers, ultimately impacting other organ systems including the GIT (Plaizier et al., 2012). This may occur when an infection originating in another organ system such as the uterus, lungs, or mammary gland is not adequately contained, and the pathogens, cytokines, and other inflammatory molecules enter systemic circulation. This can result in decreased feed intake, only worsening the immune response and inflammatory cascade, increasing GI permeability (Kvidera et al., 2017c) and susceptibility to other conditions (Horst et al., 2020).

Although much of the research conducted focuses on the impact of GI permeability on other systemic infections and inflammatory conditions, some studies have investigated the impact of induced mastitis (Bertens et al., 2026) or clinically diagnosed ketosis (Abuajamieh et al., 2016) on GI permeability. However, in observational studies, looking at diagnosed ketosis, it is unclear if ketosis occurred as a result of increased GI permeability, or if GI permeability occurred as a result of inflammation associated with ketosis. Collectively, these findings suggest a complex and potentially bidirectional relationship between systemic inflammation and GI barrier function.

There are several risk factors that can increase an animal's likelihood for contracting illness or developing inflammation potentially resulting in increased GI permeability like previously discussed risk factors. The main risk factors that may contribute to increased susceptibility to illness and inflammation include animals experiencing transportation-related stress where there is comingling, a new environment, or overcrowding. More risk factors include animals in more energy demanding physiological states such as calving and lactation. Lastly, animals that are housed in a chronic or hospital pen, that are already experiencing chronic illness, could be at a higher risk for developing GI permeability due to illness.

Together, these proposed nutritional, physiological, environmental, and illness related etiological risk factors for increased GI dysfunction and permeability highlight the importance of overall animal health, nutritional, and management practices in maintaining the GI health and reducing the likelihood of permeability related dysfunction. Because many of these etiological risk factors are common in commercial cattle production settings, understanding their effects of GI

health is important for minimizing the potential consequences associated with increased GI permeability. As a result, compromised barrier function may have implications that extend beyond the GI tract and affect overall animal health and productivity.

CONSEQUENCES

Compromised GI barrier integrity can have significant impacts on animal performance, exacerbate inflammation, and potentially contribute to the development of other disease conditions in cattle. The GIT is the one of the largest and most important components of the immune system, playing a critical role in regulating inflammatory responses and signaling (Klaenhammer et al., 2012). Under normal conditions, the GI barrier helps to maintain separation between luminal contents and systemic circulation; however, when GI health is compromised inflammatory mediators, endotoxins, and other luminal contents may translocate into circulation, potentially contributing to systemic inflammation and further impairing animal health.

Animal Performance

Regarding profitability and producer concerns, GI permeability negative effects animal performance in various ways. The effects of induced GI permeability on milk production, feed intake, and daily weight gain have been assessed, and GI permeability has been found to decrease feed intake and milk production, and milk components (Kvidera et al., 2017c). However, other research has assessed similar objectives and has found that GI permeability is not the sole cause of reduction in milk yield and alterations in milk components, but it is a contributor to these consequences (Fontoura et al., 2022; Ellett et al., 2024). Other than reduced performance characteristics, there are further health complications that can arise from compromised GI barrier integrity.

Inflammation

Inflammation may be both a primary etiological factor and a significant consequence of increased GI permeability. Inflammatory biomarkers such as lipopolysaccharide binding protein (LBP), serum amyloid A (SAA), and haptoglobin are acute phase proteins (APP) that interact with bacteria and initiate an inflammatory response where cytokines are released (Zweigner et al., 2006). It has been determined that during induced GI permeability, regardless of the method of induction, there is an increase in circulating LPS, SAA, and haptoglobin, as well as pro-inflammatory cytokines (Kvidera et al., 2017c; Cangiano et al., 2022; Goetz et al., 2024) as increased permeability allows for systemic circulation of these biomarkers. Systemic inflammation can result in fever, and a further decline in animal health and welfare.

Other Illness/ Infections

There have been several proposed conditions that can develop from increased GI permeability and decreased GI health in cattle. Two significant health conditions have been linked with increased permeability, particularly in the rumen, are liver abscesses (LA) and laminitis. These may develop when endotoxins, pathogenic bacteria, or antigens present in the GIT enter systemic circulation from increased GI permeability and become translocated to different areas of the body (Briggs et al., 2020). Currently, LA is a significant problem in the feedlot industry and the hypothesized pathogenesis for the development of LA is associated with increased GI permeability from high grain diets (Nagaraja and Lechtenberg, 2007a). Together, these observations suggest that GI barrier dysfunction may represent an important underlying factor in the pathogenesis of several systemic diseases affecting cattle.

The consequences of decreased GI barrier integrity can be detrimental to cow health and welfare, performance, and profitability. As discussed previously, increased GI permeability may

contribute to systemic inflammation, impaired nutrient utilization, reduced performance, and the development of other health conditions. Collectively, these outcomes highlight the importance of GI permeability and support the need for further research focused on improving the diagnosis, prevention, and management of GI permeability in cattle.

DIAGNOSIS

Currently the diagnosis methods for GI permeability are for research purposes as they may not be practical for cattle in production settings; however, there is ongoing research and an increased number of diagnosis methods in research settings that show promising results for production settings. Diagnosis methods include the presence of inflammatory markers, exogenous markers including indigestible sugars and metal tracers, and tight junction protein gene expression.

Inflammatory markers

The previously discussed inflammatory markers including LBP, serum amyloid A, and haptoglobin, can be used as physiological biomarkers to assess the presence of GI permeability as they are endogenous and a representation of the process of inflammation. There has been substantial research assessing the presence of inflammatory biomarkers such as LBP, SAA, and haptoglobin during induced permeability studies (Kvidera et al., 2017c; Horst et al., 2020; Goetz et al., 2024). Although these markers are exceptional at indicating inflammation, they do not necessarily give any information regarding the severity of GI permeability, and they are not specific to the GIT, as they can be released from other organs and locations of inflammation. Furthermore, these inflammatory markers can be present in plasma during any type of inflammation in any location such as inflammation associated with ketosis (Abuajamieh et al., 2016) and mastitis

(Bertens et al., 2026): therefore, if permeability is occurring concurrently with another inflammation process, these markers may not be helpful in determining if permeability is present, and other diagnostic methods would need to be considered.

Exogenous tracers

Exogenous markers or tracers are typically large molecules, that in a healthy animal should not cross the epithelial barrier and should theoretically only cross the intestinal barrier when the epithelial integrity is compromised. Currently, some of the most used and reliable tracers for assessing GI permeability include indigestible sugars and metal-based markers. Both sugar and metal-based tracers contain exogenous, indigestible components that can be quantified in serum or urine. Sugar markers including lactulose, mannitol, and sucralose have been used, although there is speculation about which is a more reliable tracer as there is potential for these sugars to be degraded in the rumen environment of cattle. Typically, lactulose is used in tandem with mannitol as the lactulose-mannitol test and is used primarily in pre-ruminant studies (Cangiano et al., 2022; Pisoni et al., 2022). Sucralose, on the other hand, has only been used in one study to our knowledge, as a more novel tracer (Ellett et al., 2024). Metal biomarkers such as Cr-EDTA and Co-EDTA are also widely used, mainly in functioning ruminants (Horst et al., 2020; Cangiano et al., 2022; Ellett et al., 2024; Bertens et al., 2026) as they are large molecules representative of generalized barrier permeability using the paracellular pathway to enter the blood stream; however, they are not a measure of endotoxin translocation (García-Lafuente et al., 2001; Zhang et al., 2013a). Furthermore, there are concerns regarding withdrawal times, tissue residues, and food safety in food animal settings. In contrast, sugar-based tracers generally present fewer food safety concerns and may offer greater practical applicability in commercial cattle settings. Although each tracer system has both advantages and disadvantages regarding their use to

accurately and effectively assess GI permeability, both have contributed substantially to the current understanding of GI barrier function in cattle.

Tight Junction Protein Gene Expression

Additionally, there are methods to assess GI permeability and morphology; however, many of these techniques are limited to research settings because they require tissue collection from harvested animals. Tight junction protein gene expression is one method utilized in numerous studies and has served as a reliable approach for evaluating indicators of compromised GI health and barrier integrity (Malmuthuge et al., 2013; Jiang et al., 2023; Welboren et al., 2023). This technique involves measuring the expression of genes associated with tight junction proteins, including claudins, occludin, and zonula occluden proteins which are responsible for regulating paracellular transport and maintaining epithelial barrier function. Alterations in the expressions of these proteins may indicate disruptions in barrier integrity and increased permeability. This method is performed using q-PCR, RC-PCR, or RNA sequencing, and results are typically interpreted alongside results from physiological permeability markers such as Cr-EDTA, Co-EDTA, or lactulose/mannitol (Welboren et al., 2023). Although alterations in tight junction gene expression may indicate compromised barrier integrity, this method does not directly measure GI permeability and is primarily used to identify potential mechanisms associated with barrier dysfunction.

Overall, substantial progress has been made in the development of methods to assess GI permeability in cattle; however, each diagnostic method possesses inherent limitations. While inflammatory biomarkers, exogenous tracers, and tissue-based assessments can provide valuable information regarding barrier integrity and GI health, many of these techniques remain primarily

confined to research settings due to limitations associated with specificity, practicality, and invasiveness, or cost. Given the challenges associated with accurately diagnosing GI permeability in live animals, considerable attention has been directed toward identifying management, nutritional, and therapeutic strategies to mitigate GI barrier dysfunction before substantial health and performance consequences occur.

MITIGATION

There is a variety of research done looking at methods to prevent or lessen the negative effects of heat stress using supplements such as zinc, in a heat stress induction model of GI permeability (Opgenorth et al., 2021; Rodriguez-Jimenez et al., 2025). Current studies have primarily focused on supplements that may enhance GI health and reduce inflammation during stressful events; however previous research has also helped us identify additional mitigation strategies based on the factors known to influence GI permeability and overall GI function. The three most reported mitigation approaches in the literature include nutritional management, probiotic supplementation, and stress reduction.

Nutritional Management

Nutritional management is the most important strategy for reducing the risk of increased GI permeability and maintaining GI health. Nutrition is the method that can be most controlled through human intervention; however, there are many considerations that must be made. As previously mentioned, forage inclusion rate and forage type can stabilize rumen pH, induce more ruminal contractions, and decrease permeability and inflammation associated with the GIT (Pereira et al., 2023b; Pereira et al., 2023a). Although, this may be beneficial there is a tradeoff between energy dense feeds such as concentrates and forage where including more forage could

mean less concentrates and decreased weight gain or milk production, which for many production systems is not ideal. Additionally, supplementation of yeasts has been hypothesized to improve GI function during periods of stress due to stabilization of the microbiome, although literature does not support that yeast supplementation either positively or negatively effects GI permeability (Pisoni and Relling, 2020; Coleman et al., 2023; Goetz et al., 2024). Antioxidant supplements, such as zinc, have also been studied and found to modulate inflammatory markers, improve intestinal morphology and physiology, and improve recovery from induced stress (Horst et al., 2020; Opgenorth et al., 2021; Rodriguez-Jimenez et al., 2025). Lastly, some other supplements that have been assessed are molasses, found to increase permeability (Silva et al., 2023), and L-glutamine, found to reduce physiological stress biomarkers and GI permeability (Ceja et al., 2023). Collectively, these findings suggest that nutrition plays a critical role in maintaining GI barrier integrity; however, additional research is needed to determine which dietary interventions are most effective in mitigating risk for increased GI permeability in cattle.

Stress reduction

As previously discussed, stressors such as transportation (González et al., 2012), heat stress (Baumgard and Jr, 2013), and weaning (Wood et al., 2015) can severely compromise GI health and function. Therefore, mitigating stressful events may help directly or indirectly reduce the development and consequences of decreased GI barrier integrity. Many dairy production systems implement heat stress mitigation strategies including evaporative cooling systems (Liao and Chiu, 2002) and optimal barn ventilation (Obando Vega et al., 2022). With regard to transportation, preventing overcrowding, minimizing travel distance, and proper handling practices may reduce stress imposed on cattle and potentially lessen the effects of GI hyperpermeability (Pisoni et al., 2022)Weaning, another challenge known to negatively impact gut health, is slightly more

difficult to mitigate. Strategies that may reduce stress associated with weaning include creep feeding, creep grazing, and maintaining fence-line contact between the dam and calf, although conclusions regarding the effectiveness of these practices remain inconsistent among studies (Enríquez et al., 2011). Continued research is needed to identify and refine management practices that effectively minimize weaning associated stress and support GI health during critical transition periods.

KNOWLEDGE GAPS AND FUTURE DIRECTIONS

Although multiple mitigation strategies have been proposed to minimize stress and support GI health in cattle, additional research is needed to better understand their effectiveness and direct impact on GI permeability. While nutritional and management interventions show promise for reducing the development and progression of increased permeability and compromised GI health, important questions remain regarding their mechanisms, long term efficacy, and practical implementation in commercial production systems. Consequently, several knowledge gaps exist, highlighting numerous opportunities for future research aimed at advancing the understanding of GI permeability in cattle.

Gastrointestinal permeability remains a relatively understudied area of research in cattle with many aspects of its pathogenesis, diagnosis, and long-term implications still poorly understood. Much of the current understanding is based on hypotheses and emerging evidence, leaving numerous knowledge gaps and opportunities for future investigation. Additionally, inconsistencies among methods used to evaluate GI permeability make it difficult to establish standardized protocols for both research and production settings.

There are substantial knowledge gaps in our understanding of GI permeability in cattle that continue to limit interpretation of existing findings. One important question is where permeability primarily occurs within the GIT. The rumen has been extensively studied and has been proposed as a major site of barrier dysfunction associated with conditions such as LA (Nagaraja and Lechtenberg, 2007a). However, the hindgut has recently gained attention, as acidosis can also occur in this region and may represent an additional consequence of ruminal disturbances (Abeyta et al., 2023c; Sanz-Fernandez et al., 2024). Another area requiring further investigation is the timing of barrier dysfunction throughout an animal's life cycle. Additional uncertainties include the precise mechanisms responsible for altered permeability, the time required for barrier dysfunction to develop, and the extent to which animals can recover from these alterations or experience long-term consequences. Although research in this area continues to expand, many questions remain unanswered.

Current research has largely focused on methods to induce and quantify GI permeability. Nevertheless, there is no universally accepted method or protocol for inducing barrier dysfunction and evaluating epithelial integrity *in vivo.* Commonly used induction methods include feed restriction (Zhang et al., 2013a) and NSAIDS, such as indomethacin and aspirin (Briggs et al., 2020; Cangiano et al., 2022). Other methods, including the use of gamma secretase inhibitors (Kvidera et al., 2017b), have been shown to successfully induce hyperpermeability of the gut. However, it remains unclear how accurately these models mimic naturally occurring epithelial barrier dysfunction in commercial production systems. Although these approaches reliably induced permeability, each method may result in varying degrees of barrier disruption, making comparisons among studies challenging. Similar inconsistencies exist among diagnostic approaches. As discussed previously, numerous biomarkers, tracers, and analytical methods have

been used to evaluate permeability, yet no standardized protocol currently exists. While quantification of Cr-EDTA in plasma or urine remains one of the most widely used approaches (Briggs et al., 2020; Horst et al., 2020; Wilms et al., 2024), further refinement and validation of diagnostic methods are needed. These inconsistencies likely reflect the evolving nature of the field and the substantial gaps that remain in our understanding of GI permeability.

Although significant progress has been made in understanding GI permeability in cattle, further research is needed to address existing limitations and improve the application of our knowledge to production settings. One promising area for future investigation is the impact of increased permeability during the pre-ruminant stage on long term GI health and animal performance later in life. Additional opportunities include evaluating permeability under commercial feedlot conditions, where barrier dysfunction may be particularly relevant. Feedlot cattle experience multiple dietary transitions as they adapt to high starch diets, making this population well suited for studying the relationships among dietary adaptation, GI permeability, LA, acidosis, and ruminal lesions. Advancing research in these areas may improve our understanding of disease development, facilitate the identification of practical mitigation strategies, and ultimately enhance cattle health, welfare, and productivity.

CONCLUSIONS

This narrative review summarized the current understanding of GI permeability in cattle including the normal physiology of the ruminal and intestinal barriers, factors associated with increased permeability and compromised GI health, available diagnosis approaches, potential consequences of barrier dysfunction, and current mitigation strategies. Collectively, the available literature suggests that GI barrier integrity plays an important role in maintaining animal health, immune function, and productivity. Emerging evidence suggests that both ruminal and intestinal

barrier dysfunction may contribute to systemic health challenges in cattle. Although considerable progress has been made in characterizing factors associated with GI permeability, substantial knowledge gaps remain regarding its diagnosis as there is a lack of standardized methods for inducing and measuring permeability, a major limitation in the field. Continued research is needed to improve our current understanding of GI barrier function, develop practical and standardized diagnostic methods, and identify strategies that support GI health under commercial conditions. Future studies should focus on validating diagnostic approaches and establishing causal relations between barrier dysfunction and disease outcomes as improved understanding in this area may provide opportunities to reduce economically significant conditions such as LA, inflammation, and production losses. Advancing knowledge in these areas has the potential to improve cattle health, welfare, and production efficiency while providing a stronger foundation for future research on GI permeability.

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Chapter 2 - Optimal sampling time for indigestible sugar markers of gastrointestinal permeability in yearling cattle

INTRODUCTION

Gastrointestinal (GI) permeability refers to the ability of an animal's intestinal epithelial barrier to regulate the passage of substances such as nutrients, toxins, water, and microbes from the gastrointestinal lumen into the bloodstream. Gastrointestinal permeability reflects how "leaky" the intestinal barrier is and can influence overall health through its role in the innate immune system. Gastrointestinal permeability can increase in response to disease syndromes, dietary changes, or stress, leading to altered movement of substances such as nutrients, water, and pathogens, between the lumen and the bloodstream. Additionally, GI permeability can be transcellular, where molecules are actively transported through epithelial cells, or paracellular, where molecules pass through tight junctions between cells, which is more common for macromolecules (Araujo et al., 2015). There are several methods to assess GI permeability in pre-ruminants typically involving oral administration of an indigestible biomarker followed by blood or urine sampling to confirm and quantify the amount of the biomarker that has crossed the intestinal epithelial barrier in a specified amount of time. The optimal sampling time corresponds to the time point when the biomarker reaches peak concentration in blood or urine.

Indigestible biomarkers are typically macromolecules and do not naturally cross the intestinal epithelial barrier in a healthy animal. Previous research (Branco Pardal et al., 1995; Klein et al., 2007; Minuti et al., 2013) to assess GI permeability with indigestible biomarkers has been performed in vivo using various sugar markers such as lactulose, mannitol, and sucralose. Little to no research has been completed using sugar molecules in fully functioning ruminants likely because of the uncertainty regarding the degradation and persistence of the sugar

markers in a fully functioning rumen environment. Sucralose, a novel synthetic sugar, has potential as a biomarker; however, there is a lack of research evaluating use in assessing GI permeability in a manner similar to lactulose (Klein et al., 2007; Minuti et al., 2013). In an in vitro study where the integrity and characteristics of some naturally occurring and synthetic sugars were tested, lactulose was found to be fermented at a low rate and sucralose was found to be almost completely non fermentable (Ahmed et al., 2013). Sucralose and lactulose may be effective biomarkers in functioning ruminants and sucralose may be a superior biomarker to assess GI permeability in functioning ruminants. In another in vitro study, the degradation of D-mannitol, lactulose, sucralose, and D-glucose were assessed for 48 hours in a continuous fermentation system with rumen fluid from a mature lactating cow (Ellett et al., 2022). Sucralose was detectable at consistently high concentrations throughout the 48-hour period compared to the other sugars that were only detectable up to 6 hours after being introduced to the in vitro system. These sugars have not been evaluated as in vivo markers of gastrointestinal permeability in ruminants, and information on optimal post-administration blood sampling time, as well as food safety and withdrawal considerations, are needed.

The objectives of this study were to determine usefulness of lactulose and sucralose for assessing GI permeability and to identify optimal post-administration sampling times in functioning ruminants with induced permeability. We hypothesized that across all sampling time points, sucralose would exhibit higher serum concentrations than lactulose, supporting its use as a more effective and quantifiable biomarker of GI permeability in animal with a functioning rumen.

MATERIALS AND METHODS

All animal care procedures used in this study were approved by the Kansas State University (KSU) Institution for Animal Care and Use Committee before the initiation of the study (IACUC-5032). This trial was conducted at the KSU College of Veterinary Medicine (CVM) in August 2024.

This pilot study used three yearling Holstein steers (290.3 ± 7.86 kg) with functioning rumens. Due to the nature of a pilot study and each animal serving as their own control, it was determined that three animals were sufficient for this study. No randomization or blinding occurred.

Prior to the study, steers were housed together and had free choice access to grass hay. At the initiation of the study, steers were housed individually in large animal pens (9m x 18m) with nose-to-nose contact. All steers were fed the same standard grass hay for the entirety of the study, fresh water was provided free choice, and daily health observations were conducted by trained personnel. Steers were fed standard grass hay ad libitum for 4 consecutive days to determine ad libitum hay intake (Table 1). On day 5, steers were restricted to 25% of their ad libitum intake for six consecutive days to induce GI permeability. The method used to induce GI permeability was determined as sufficient, according to a study conducted by Zhang (Zhang et al., 2013). During feed intake restriction, body condition was visually assessed for each animal using the beef cattle scale (1=emaciated, 5=optimal, 9=obese). Body weight (BW) was collected on day 5 prior to feed restriction and on day 9 prior to marker administration. The study ended on day 11 following completion of blood collection.

To assess GI permeability steers were orally administered a lactulose solution and a sucralose solution at the same time. Lactulose was purchased as a pre-mixed oral lactulose solution (Rising Pharmaceuticals, Inc., East Brunswick, NJ, USA) with a concentration of 0.67g lactulose/mL solution and stored according to label directions. Sucralose (Hard Eight Nutrition LLC, Henderson, NV, USA; bulksupplements.com) was purchased as a powder and stored according to label instructions. Prior to dosing, sucralose powder was mixed with deionized water at a concentration of 0.25g/mL and was stored at room temperature.

On day 9, steers were dosed with lactulose and sucralose solutions at a rate of 0.4 g sugar/kg of BW for each sugar at 0800 h, exact time was recorded to ensure blood collection at the proper timepoints. For oral sugar administration, steers were properly restrained in a squeeze chute, a stomach tube was passed orally into the reticulorumen, and each sugar solution was administered using an equine stomach pump (Neogen, Lansing, MI, USA). The stomach pump was flushed with water to ensure full dose of each sugar was administered. For a short period of time after sugar marker administration, steers were monitored for adverse events and regurgitation.

Blood samples were collected at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 hours following sugar marker administration via jugular venipuncture using 10 mL vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). and 18G X 1’’ needles (Air-Tite Products Co., Inc., Virginia Beach, VA, USA). Blood collection was performed by alternating sides of the neck (left then right) for sequential blood samples. Blood samples were allowed to clot at room temperature for 24 hours and centrifuged at 2000 x g at 4 °C for 20 minutes, se-

rum was extracted and stored at -20 °C until analysis. Serum was analyzed for lactulose and sucralose concentrations using gas chromatography-tandem mass spectrometry by Kansas State Veterinary Diagnostic Laboratory based on previous literature (Becker et al., 2013).

STATISTICAL ANALYSIS

All computations were performed in R (version 4.4.2; R Core Team, 2024). The average dry matter intake (DMI) was calculated for the ad libitum period and for the feed restriction periods respectively. For each sugar (lactulose and sucralose) and each sampling time point, arithmetic means and standard deviations (SD) of measured concentrations were calculated. To estimate the probability of a sugar being detected above the limit of quantitation (LOQ) at each time point, a Monte Carlo simulation was performed due to the small sample size and limited data obtained from sampling. Inference was conducted based on the Monte Carlo simulation rather than hypothesis testing, consequently, effects and interactions were not assessed statistically. For each sugar-time point combination, 1000 random values were generated from a normal distribution defined by the empirically calculated mean and SD. The probability of the simulated values exceeding the LOQ was calculated using the rnorm function. For sugar-time combinations with a SD of 0 (e.g., time 0 hr), the probability was defined as 1 if the observed mean exceeded the LOQ and 0 if mean was lower than the LOQ. No effects or interactions were tested, therefore, no statistical inference (i.e., P-values) was performed.

RESULTS

The calculated average DMI during the ad libitum period was 5.34 kg ± 0.566 kg. For the feed restriction period, the average DMI was 1.58 ± 0.169. The mean values, calculated from observations for sucralose from 3 to 36 hours were 4.05 ± 1.48 μg/mL and for lactulose 2.78 ±

1.40 μg/mL. The mean values for lactulose and sucralose at each sampling timepoint are presented in Figure 1. The Monte Carlo simulation revealed that the predicted probability of exceeding the LOQ for the 36-hr time period was greater for sucralose than lactulose (Lactulose = 0.857, Sucralose = 0.901). The probability for lactulose and sucralose at each sampling timepoint is presented in Figure 2. At 0 h, immediately before dosing, both sugars were not detectable (below LOQ) and were therefore defined as having zero probability of being above the LOQ. Furthermore, the probability of being detected above the LOQ was greater for sucralose than lactulose at every sampling time point from 3 to 36 hours.

DISCUSSSION

Three yearling Holsteins, with induced GI permeability, were dosed with lactulose and sucralose then blood was sampled at time 0 and every 3 hours after until 36 hours. In this study, lactulose and sucralose were detected at each sampling time following administration excluding 0 h. The probabilities of each sugar being detected above the LOQ were high from 3-33 h indicating the sugars were not completely degraded by rumen microbes and GI permeability was successfully induced. A primary concern for the use of sugars as biomarkers in any species, is the degradation of these sugars throughout the GI tract.

Previous studies have used lactulose, mannitol, and sucralose as well as other sugars such as xylose, galactose, and fructose in an in vitro fermentation system (Ahmed et al., 2013; Minuti et al., 2013; Ellett et al., 2022). In a study by Ahmed (Ahmed et al., 2013), fermentability of lactulose, sucralose, and mannitol were evaluated and they found mannitol and lactulose had decreased concentrations indicating that these sugars were degraded to some extent. Sucralose, on the contrary, was found to be almost completely unfermented. These results indicate the possibility of using lactulose, mannitol, and sucralose to assess GI permeability as a significant amount

may escape rumen fermentation. Similarly, in the current study, lactulose and sucralose were detected throughout the entire sampling period, excluding 0 h, with sucralose being detected at greater mean concentrations. In another in vitro fermentation study by Ellet (Ellett et al., 2022), lactulose, mannitol, D-glucose, and sucralose were incubated in rumen fluid for a 48-hour period and concentrations of the sugars were recorded. Lactulose was not quantifiable by high performance- liquid chromatography; therefore, there are no data on the concentration of lactulose. However, mannitol and D-glucose were detectable and their concentrations in rumen were found to decrease to nondetectable concentrations within 6 hours. In the same study, sucralose concentrations were found to remain consistently high throughout the entire 48 incubation period, indicating that it can escape rumen fermentation. This contrasts with the previous study mentioned by Ahmed (Ahmed et al., 2013) as mannitol was degraded quickly in this study, but was seen to have slower degradation rates in the previous study. However, sucralose was found to be unfermentable in both studies, indicating its potential use as a quantifiable biomarker in functioning ruminants. In addition to our findings, the data from these studies demonstrate that lactulose and sucralose can be used as biomarkers to assess GI permeability in functioning ruminants.

When assessing GI permeability in ruminants, the site of biomarker absorption remains unclear, particularly due to the uncertainty regarding stability of sugar biomarkers within the rumen. Sugars may be rapidly absorbed in the rumen, resulting in an early spike in serum concentration, or may escape ruminal fermentation and pass gradually to the lower gastrointestinal tract, where delayed absorption occurs. Given that the rumen functions as a reservoir with controlled outflow, this could lead to sustained or delayed increases in circulating sugars. Therefore, if absorption occurs across both ruminal and intestinal sites, serum concentration profiles

may exhibit a biphasic pattern, characterized by an initial increase followed by a plateau and subsequent decline. However, this is not the only factor impacting passage throughout the GI tract.

In addition, dietary factors such as feed intake level may influence the passage out of the rumen and absorption throughout the GI of biomarkers. Increased level of feed intake is associated with increased passage rates while decreased intake slows passage and increases rumen retention time (Colucci et al., 1982; Lechner-Doll et al., 1991). Under conditions of decreased feed intake or feed restriction, as applied in the present study, rumen fill and digesta flow may have been reduced, potentially lowering liquid passage rates. A reduction in passage rate could delay the transit of administered biomarkers through the gastrointestinal tract, thereby altering their absorption. Although this study fed grass hay, which typically promotes faster liquid passage via increased saliva production and rumination, the reduced feed intake likely limited these processes and slowed passage. Consequently, prolonged retention time and delayed absorption may explain the relatively higher concentrations of sugars observed, as well as their consistently high probability of detection across the entire 36-hour sampling period.

Although this study used sugars, other common biomarkers to assess GI permeability are liquid markers such as Cr-EDTA and Co- EDTA (Branco Pardal et al., 1995; Hall and Van Soest, 2019; Van Soest and Hall, 2020). These metal-based biomarkers are also used in dairy cattle to assess movement of ingesta (Van Soest and Hall, 2020) and in pasture cattle to asses urine excretion (Rodrigues et al., 2023a). Although, these studies are done in functioning ruminants, there is still uncertainty regarding how well these biomarkers can survive rumen conditions. An in vitro study done by Soest and Hall (Van Soest and Hall, 2020) found that while Cr-EDTA was slightly more stable than Co-EDTA under rumen-like reducing conditions, both showed signs of instability, warranting caution when used as biomarkers in ruminants; similarly,

their subsequent work in autoclaved rumen fluid demonstrated reduced absorbance consistent with partial dissociation of the metal-EDTA complex, suggesting these markers are not fully inert (Hall and Van Soest, 2019). Furthermore, there are potential hazards for both the environment and humans when using Cr-EDTA. Studies have shown that Cr (III), the ion of Cr used in Cr-EDTA, has been known to induce damage to DNA in cells, however there is uncertainty regarding its carcinogenicity, and should be used with caution for the health of the human workers (Wang et al., 2017). When considered alongside in vitro studies using sugars (Ahmed et al., 2013; Ellett et al., 2022), particularly sucralose, this compound may serve as an equally or more suitable biomarker for assessing GI permeability, given its lack of measurement challenges and potential hazards associated with metal-based biomarkers

Methods to assess GI permeability after administration of an indigestible biomarker involve obtaining a biological sample to quantify absorption. Previous studies have assessed GI permeability by analyzing urinary excretion of the biomarker (Klein et al., 2007; Rodrigues et al., 2023a), but urine collection is labor intensive and invasive, often requiring catheterization (Rodrigues et al., 2023b), urine bags (Klein et al., 2007), or in a funnel under slatted floors (Branco Pardal et al., 1995). Urinary biomarker concentrations are also highly variable, whereas blood or serum concentrations tend to be more consistent across species (Elia et al., 1987; Oriishi et al., 1995; Minuti et al., 2013). In a study by Rodrigues (Rodrigues et al., 2023b), it was found that chromium concentrations in blood were more stable but significant variation in urine following Cr-EDTA infusion in dairy cattle. Although that study constantly infused chromium, the variation in urinary samples can indicate inconsistency in using urine samples to assess concentrations of biomarkers. Similarly, Klein (Klein et al., 2007) observed high variability in urinary lactulose and mannitol in pre-ruminant calves. In contrast, a previous study

by Minuti (Minuti et al., 2013) reported that taking blood samples to evaluate GI permeability in adult sheep could be a non-invasive, sensitive, and more practical method than collecting urine from animals in a production setting. Consistent with these findings, the current study shows that although serum sugar concentrations fluctuate over time, the probability of detection remains high from 3-33 hours post dosing, supporting a robust sampling window in which a single timepoint can reliably indicate increased GI permeability.

Many pre-ruminant studies have used the lactulose mannitol test to assess GI permeability in young calves (Branco Pardal et al., 1995; Klein et al., 2007; Araujo et al., 2015) where blood is typically collected in a serial manner. However, this approach could potentially be reduced to a single sampling timepoint if the optimal time, corresponding to peak biomarker concentration in serum, is identified. A single collection point would be more practical for both the animals and researchers compared to repeated urine or blood sampling. However, there is a lack of studies identifying an optimal sampling timepoint for one blood collection. In a study by Araujo (Araujo et al., 2015), biomarkers were detected in serum as early as 60 minutes following administration in pre-ruminant animals, demonstrating rapid systemic appearance. Although this study used functioning ruminants, the blood sampling time ranges from 3-33 hours, which is much broader than the samples times used in pre-ruminant calves, but is consistent with the biomarker being detected in serum in a short amount of time after administration. In adult sheep, (2) biomarkers were also detected within a relatively short timeframe, with an optimal blood sampling window of 2-8 hours post administration, although lactulose remained detectable at 10 hours in lower concentrations. Collectively, these findings suggest that biomarkers appear in circulation rapidly across studies, irrespective of methodology, while the overall duration of detectability varies by physiological stage and species, with wider sampling

windows observed in ruminants compared to pre-ruminants. These studies provide data for determination of optimal timing for blood or urine samples in GI permeability studies, however the uncertainty and inconsistency in the results for functioning ruminants warrants further investigation.

LIMITATIONS

Limitations of this study include a short sampling duration and the method used to induce permeability. Samples were collected for 36 hr post-dosing, which was insufficient to observe serum sugar concentration falling below the LOQ. A longer sampling period may have better characterized the duration of sugar presence in serum and identified when concentration declined below the LOQ. As, mentioned previously, the method used to induce GI permeability could have decreased the passage rate of substances through the rumen, thereby prolonging biomarker absorption. Furthermore, the induction method may not accurately reflect more natural causes of hyperpermeability, such as those associated with disease states. Further research is warranted to refine dosing strategies and establish optimal sampling durations that more accurately capture biomarker kinetics under physiologically relevant conditions.

CONCLUSION

This study indicates that sucralose, a novel sugar, and lactulose can survive rumen degradation and can be used as biomarkers to assess gastrointestinal permeability in functioning ruminants. While the concentrations of sucralose and lactulose remained well above the LOQ from 3 to 36 hours, the mean concentrations and probabilities of being detected above the LOQ were greater for sucralose than lactulose. These findings highlight the potential of lactulose and sucralose being effective biomarkers for assessing gastrointestinal permeability. The present findings also help to identify an optimal blood sampling time period between 3 and 33 hours post

dosing. More research is warranted to develop a protocol for using indigestible sugars as biomarkers in functioning ruminants to assess gastrointestinal permeability in a research setting.

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FIGURES

Figure 2.1 Mean serum sugar concentrations for lactulose and sucralose at each of the sampling times.

Minimum standard deviation for lactulose and sucralose across time points were 0.590 and 0.806 μg/mL, respectively. Maximum standard deviation for lactulose and sucralose across time points were 2.28 and 2.14 μg/mL, respectively. Dotted line indicates the limit of quantitation of 1.0 μg/mL.

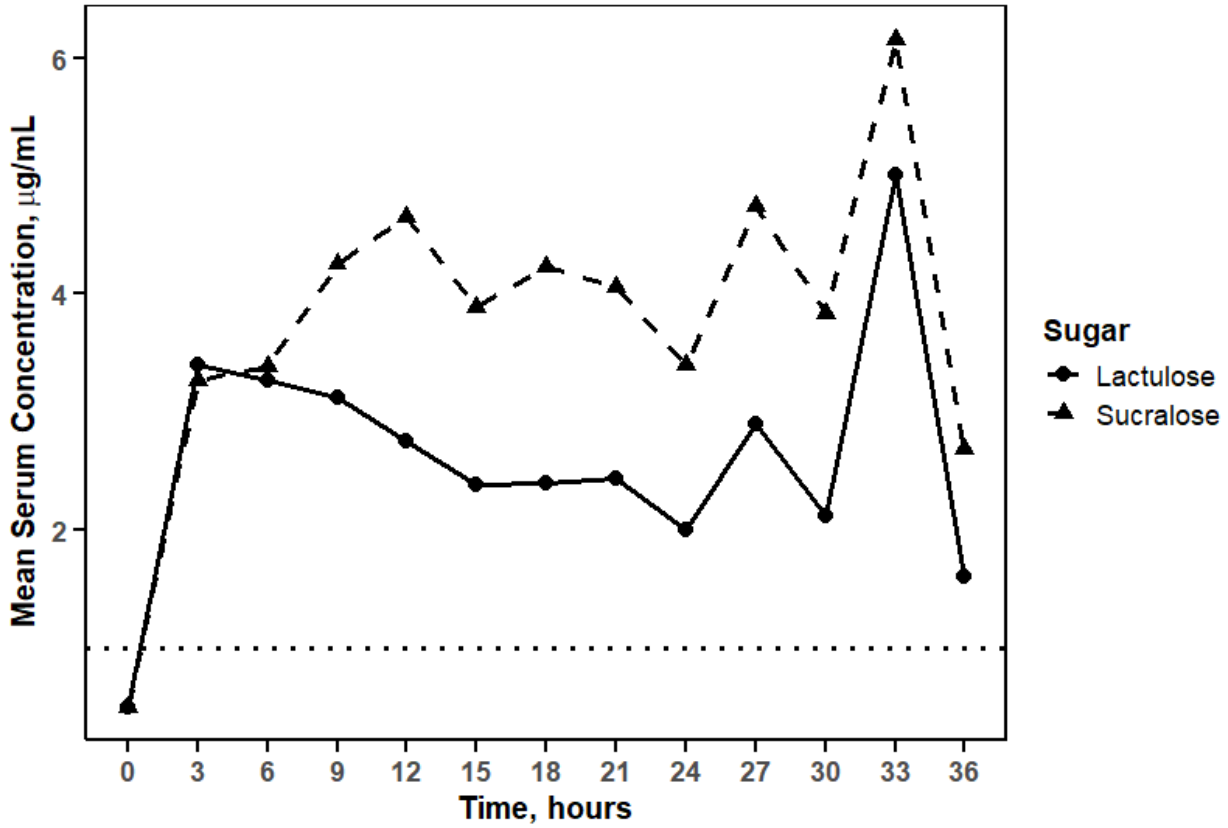
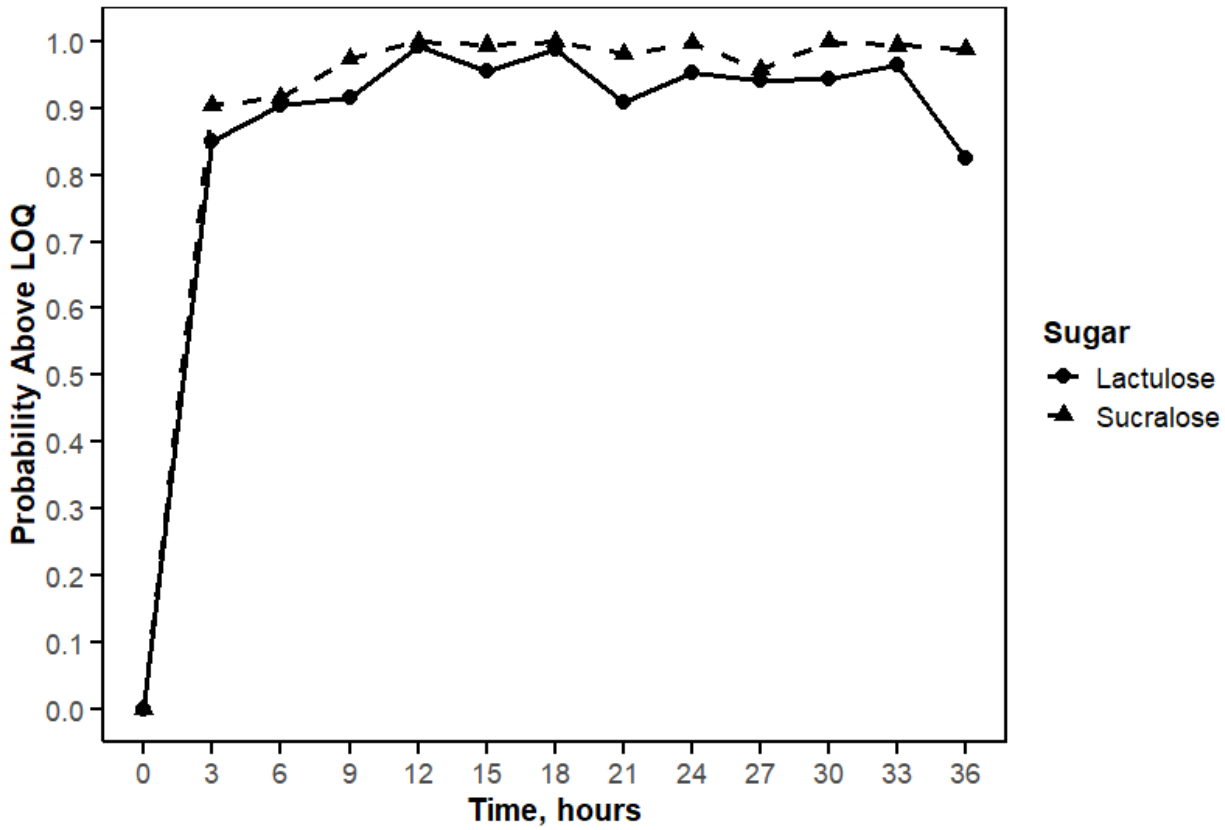


Figure 2.2 Probabilities of lactulose and sucralose being detected above the LOQ at each time point.



TABLES

Table 2.1 Nutrient composition of grass hay fed to Holstein steers

Nutrient	Dry Matter Basis	As Fed Basis
Moisture, %	–	8.65
Dry Matter (DM), %	–	91.35
Crude Protein (CP), %	6.39	5.84
Acid Detergent Fiber (ADF), %	40.91	37.37
Neutral Detergent Fiber (aNDF), %	62.81	57.38
Total Digestible Nutrients (TDN), %	50.63	46.25

Chapter 3 - The effect of starch inclusion level in calf starter on beef-dairy cross calf performance and gastrointestinal health at weaning and finishing

INTRODUCTION

Beef-dairy cross calves (BD) have become increasingly popular in recent years as producers breed dairy cows, such as Holstien and Jersey to beef sires, producing BD calves. Feedlots and packers have reported increased numbers of BD animals and recent research indicates that there are not only more beef-dairy calves being sold at auction markets, but they also have greater value than dairy calves, although slightly less than native beef calves (McCabe et al., 2022). As a result, there has been growing interest in the economic incentives and performance advantages of BD crosses. Beef-dairy cross calves have more economic value than 1-day old Holstein bull calves contributing to the improved economic viability the dairy industry (Dal Zotto et al., 2009; Mc Hugh et al., 2010; Wilson et al., 2020). Cross breeding dairy cattle with beef sires has been shown to improve growth performance and carcass characteristics compared with straightbred dairy cattle (Foraker et al., 2022). In addition, BD crosses have demonstrated greater dressing percentages relative to straightbred dairy cattle (Güngör et al., 2003). However, BD crosses exhibit a liver abscess (LA) prevalence of 40 to 60% (Foraker et al., 2022) compared to conventional beef breeds (15 to 30%) and dairy breeds (50 to 80%) (Nagaraja and Chengappa, 1998). These differences highlight an important area of ongoing research focused on understanding the underlying causes, mechanisms, and potential prevention strategies for LA development.

Liver abscesses are a common health disorder in cattle characterized by localized bacterial infections and suppurative lesions within hepatic tissue, often resulting from bacterial translocation across a compromised rumen epithelium. This condition is rarely detected in live animals and is typically diagnosed only at harvest. Consequently, LA continue to impose substantial economic costs on the cattle industry through reduced performance, decreased carcass value, and loss of liver salvageability. Although first documented in cattle in the 1940s, the economic losses of liver abscesses have become increasingly significant with the intensification and growing value of beef cattle (Smith, 1940). Beyond this, LA negatively impact growth performance and carcass yield by reducing feed intake, weight gain, and feed efficiency depending on the severity of LA (Nagaraja and Chengappa, 1998). Incidence is highly variable ranging from 0-95% (Nagaraja and Chengappa, 1998) but average between 12 to 32% in most feedlots (Brink et al., 1990), however BD calves experience higher prevalence. Furthermore, BD cross calves can exhibit higher proportions of open and adhered LA resulting in the greatest loss of the entire visceral organ mass estimated at $189.00 per animal, as well as additional losses associated with processing delays (Lawrence, 2022; Grote et al., 2025). Although, LA are multifactorial in origin, this variability in incidence across production systems suggest that multiple interacting factors contribute to disease development: however, the primary mechanism is thought to originate within the rumen environment.

The primary mechanism underlying LA is the “rumenitis-liver abscess complex,” which is characterized by an association between rumen lesions and LA occurrence in cattle at slaughter (Amachawadi and Nagaraja, 2022). The accepted pathogenesis theorizes that dietary factors particularly high starch rations, commonly fed in feedlots to provide energy to cattle, can cause

increased organic acid production, which includes volatile fatty acids (VFAs) and lactic acid. Increased starch fermentation can lead to acid accumulation and prolonged periods of low ruminal pH, which damages epithelial integrity and promotes inflammation in the rumen wall. Inflammatory responses associated with rumenitis may further compromise tight junction and epithelial repair mechanisms, increasing susceptibility to bacterial translocation (Amachawadi and Nagaraja, 2022). Opportunistic pathogens such as *Fusobacterium necrophorum* can then enter the portal blood stream and colonize hepatic tissue, resulting in abscess formation (Nagaraja and Lechtenberg, 2007b). In addition, physical damage to the rumen barrier can occur from hair ingestion during grooming, ingested foreign objects, or sharp feed particles (Nagaraja and Lechtenberg, 2007b). Overall, GI integrity is a key factor in understanding and mitigating LA development in feedlot cattle. Consequently, understanding how BD crosses differ from traditional beef cattle may be important when evaluating factors associated with GI health and integrity.

There are notable early life management differences between native beef calves and dairy beef calves (BD crosses and Holstein calves) that may influence GI health and subsequent LA development. Dairy calves are typically separated from the dam shortly after birth, transported to a calf ranch and are raised there from a few days of age to 5-6 months of development, depending on the operation. Calf ranches specialize in raising calves for future meat production as well as dairy replacement heifers. In contrast, native beef calves typically raised in cow calf systems, remain on pasture with their dam until weaning at 7 months of age. Dairy calves are commonly introduced to grain-based diets, high in rapidly fermentable starch, upon arrival at the calf-ranch or within the first few days of life. Although, management strategies vary, all operations feed some form of starter feed differing in ingredients, levels of starch, texture, and feeding protocol (Bigelow et al., 2025). Furthermore, dairy calves are weaned fairly early from 40 days of age to

slightly later at 70 days of age (Bigelow et al., 2025). Conversely, native beef animals have limited access to grain and higher starch feed stuffs until weaning. These differences in feeding management, may contribute to the increased incidence of LA observed in BD cross cattle, as these animals are exposed to higher starch diets from an early age through harvest. Additionally, Holsteins and Holstein-influenced cattle exhibit greater feed intake than traditional beef breeds, potentially increasing total starch consumption and further elevating LA risk. Because starch inclusion is a major component of dairy calf starter diets and has been implicated as a primary driver of GI health and LA development, understanding its effects on GI development is critical.

Starch inclusion level in the diet can both positively and negatively impact rumen development, pH regulation, and absorptive capacity. Proper rumen papillae development depends on both physical and chemical stimulation. Forages, provide physical stimulation for rumination, saliva production, and abrasion of the rumen wall, all of which contribute to normal rumen development (McBurney et al., 1983; Pazoki et al., 2017). In contrast, concentrate diets provide chemical stimulation through increased production of VFAs, particularly butyrate, which promotes papillae growth and microbial activity within the rumen (Govil et al., 2017). Increased ruminal fermentation and energy availability can improve animal performance. High concentrate diets can result in abnormal papillae morphology, including clumping, branching and keratinization, which may reduce absorptive capacity and impair GI health and performance (McGavin and Morrill, 1976). As previously mentioned, excessive starch fermentation can lower ruminal pH, damaging the rumen epithelium and potentially contributing to LA development. Collectively these findings demonstrate that starch is a major driver of GI adaptation in dairy calves, emphasizing the need to better define starch inclusion strategies that optimize rumen development and

performance while minimizing negative impacts on GI health. The balance between rumen development and negative effects on GI health is important in BD crosses, as their unique combination of dairy and beef genetics, along with early-life management practices, may influence rumen development, GI adaptation, and responses to dietary starch differently than traditionally raised beef cattle.

The objectives of this study were to evaluate the effect of starch level in a calf starter diet on performance, GI morphology, rumen pH, and GI permeability during weaning and finishing transition phases. We hypothesized that calves fed increased starch in the calf starter diet during the hutch phase will have decreased growth performance, decreased rumen pH, and decreased morphological measurements when compared to calves fed the less starch in the calf starter in both weaning and finishing phases.

MATERIALS AND METHODS

All animal care procedures used in this study were approved by the Kansas State University (KSU) Institution for Animal Care and Use Committee (IACUC) before the start of the study (IACUC-5037). The hutch phase was conducted at the KSU Sheep and Meat Goat Center from September to December 2024. The grower phase of this trial was conducted at the KSU Beef Stocker Unit from December 2024 to April 2025. The finishing phase of this trial was conducted at the KSU Beef Cattle Research Center from April to May 2025. All facilities were located in Manhattan, Kansas.

Animals and management

Hutch Phase

Twenty pre-weaned castrated, male Angus-Holstein cross calves (<1 week of age) weighing between 34 and 45 kg were used in this study. All animals were sourced from a single dairy

farm in Northeast Kansas and were not castrated or vaccinated until arrival at KSU facilities, however they received colostrum according to farm protocol. Upon arrival calves were individually weighed on a calibrated livestock scale adjacent to the pens. A blinded, randomized control design was used to randomize calves to treatment groups, which were high starch starter diet (HS, n=10) or low starch starter diet (LS, n=10). Starter diets were formulated to be similar in crude protein and metabolizable energy concentrations, but differ in the starch and fiber concentrations (Table 1). Calves were randomized to treatment A or B then two calves were systematically switched to even out the mean and standard deviation of arrival body weight between the treatments. After an adjustment period of three days, blinded veterinarians assessed health of the animals, surgically castrated all calves, and tagged each animal with a unique study identification number. All calves were vaccinated with a viral respiratory vaccine (IBR, BVD, PI3, BRSV) and given lidocaine and meloxicam (1mg/kg) for pain management following castration. An unblinded collaborator, who was not involved in data collection and analysis, unloaded the starter diets into grain totes labeled A or B to maintain the blinding of daily caretakers.

Calves were individually housed inside the climate-controlled facility on raised pens (1.5 x 2.4 m), with separators between each pen allowing calves visual and nose to nose contact with immediate neighboring calves. Each pen was equipped with a water bucket, dry feed bucket, and a milk bottle holder. During the experiment, pens and all other calf supplies were cleaned twice daily. Milk replacer was reconstituted at a concentration of 150 g/L throughout the study. A step-up, step-down milk replacer feeding protocol was followed where from study day 0 to 9 calves were offered 1.89 L of milk replacer (Land o Lakes Cow's Match Cold Front, Arden Hills, MN, USA; 26% crude protein, 20% crude fat) twice daily at 0630 and 1830 h. From days 10 to 39 calves were stepped-up to 2.84 L of milk replacer twice daily. From days 40 to 47, calves were

stepped down to 1.89 L of milk replacer twice daily, then from days 48 to 53 calves were stepped down again to 1.89 L milk replacer once daily, where the afternoon feeding was withdrawn. On day 54, milk replacer was removed completely and calves were weaned.

Starting on day 10, calves were offered one of the two starter grain diets at 0.5 lb per calf. Once daily, starter grain intake was recorded by subtracting the unconsumed amount of feed from the amount offered the previous day and calves were stepped-up in feed by 0.5-lb increments if the refusal weighed less than 0.5 lb for three consecutive days. Feed refusals were collected only if dry matter of feed was altered. Samples of starter grain diets were collected directly from the grain totes once per week. Wet and dry weight were recorded to calculate dry matter percentage and adjust starter grain intake to dry matter basis. Fresh water was available *ad libitum*. Twice daily trained personnel conducted daily health checks, recorded milk replacer intake, and recorded fecal scores. Antimicrobials and electrolytes were given as needed. The same animal caretaker did daily health observations and fecal scores each day to remain consistent.

On days 62 and 63 half of the animals (n=10, LS=5, HS=5) were harvested at the KSU College of Veterinary Medicine Veterinary Diagnostic Laboratory for gastrointestinal tissue sample collection. The remaining calves (n=10, LS=5, HS=5) continued receiving their allocated starter diet with the same feeding regimen as stated before until the end of the hutch phase. On day 72, calves were vaccinated with a seven-way clostridial vaccine and a viral/ bacterial respiratory vaccine (IBR, BVD, PI3, BRSV, *Mannheimia haemolytica*). On day 80, calves were transported to the KSU Beef Stocker Unit and this concluded the hutch phase.

Grower Phase

Upon arrival at the KSU Beef Stocker Unit, calves were weighed in the calibrated, stationary squeeze chute and housed in outdoor group pens (1pen / treatment) with fresh water *ad*

libitum and the starter grain offered that day in the feed bunk. Calves were monitored daily and treated with antimicrobials as needed. The grower ration (70:30 concentrate to forage; Table 3.2) was offered the next morning. After four weeks, due to a change at the KSU Beef Stocker Unit, sweet bran was no longer offered and dried distillers' grains (DDGS) was offered in place of the sweet bran for the remainder the grower phase. Every morning bunks were assessed by trained, blinded personnel and the feed call was adjusted according to previous day refusals following slick bunk management. Refusals were collected and weighed when there was an adverse weather event that affected the dry matter of the feed. On the last day of the grower phase (D180), calves were weighed and transported to the KSU Beef Cattle Research Center.

Finishing Phase

The ten beef-dairy steers that continued from the hutch and grower phases (n=10, LS=5, HS=5) were used in the finishing phase. Upon transportation to the Beef Cattle Research Center, each animal was placed in an individual pen on slotted floors allowing for visual and nose-to-nose contact with immediate neighboring calves, received the finishing phase starter ration where initial intake was based on a percentage of their body weight, and given access to fresh water *ad libitum.* Ingredient and nutrient composition of the starter, intermediate step-up, and finishing diets are presented in Table 3.3. Calves received the starter ration for ten days, then received the first step-up diet for seven days. After seven days, the calves received the second step-up diet for another seven days then received the full finishing ration for four days before calves were harvested for tissue sample collection. Feed ingredients were weighed out each morning and the ration was mixed in a stationary mixer. Calves were fed twice daily at 0800 and 1700. Once daily, feed refusals were obtained from every animal and feed intake was calculated by subtracting the weight of the refusal from the total weight of feed offered the previous day. The

amount of feed offered was adjusted following slick bunk management. Twice daily, trained personnel conducted health checks, cleaned pens, and provided calves with clean water. On day 28, calves were weighed in the morning and transported on a trailer for processing in Junction City, Kansas. Calves were removed from the trailer and harvested individually by captive bolt. This concluded the live animal trial.

Data collection and sample analysis

Slaughter

Calves were randomly allocated to slaughter groups and slaughter groups were assigned to either day 62 or 63. On days 62 and 63 of the hutch phase, calves were weighed and transported to the Kansas State University College of Veterinary Medicine Diagnostic Laboratory. Calves were euthanized via injection of pentobarbital and were separated from all other animals during the process. Abnormalities such as gross lesions and liver abscesses were assessed and a full systemic necropsy was performed (Schmidt et al., 2023).

On day 28 of the finishing phase, calves were weighed in the morning and transported on a trailer for processing in Junction City, Kansas. Calves were removed from the trailer and euthanized one by one using a captive bolt, performed by trained personnel at the processing plant.

Feed refusals

Feed refusals and feed ingredients were prepared for analysis using similar methods. All feed refusals and orts were dried in a forced air oven at 55C until the weights of the samples no longer changed, which was approximately 72 hours. Feed intake for each day was adjusted based on the DM percentage from the dried sample. Wet feed ingredients including corn silage, sweet bran, and high moisture corn were dried using the same process. The DM percentage was calculated using the following equation: (dry weight with pan – pan weight) / (wet weight with pan –

pan weight) X 100. To prepare for analysis, dry feed samples from the hutch phase were composited into a 100g sample for each treatment. Furthermore, a 100g composite of each feed ingredient from each week from the grower and finishing phases were created. Feed sample composites and feed ingredient composites were ground through a 1mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). All samples were sent to Cumberland Valley Analytical Services for analysis.

Performance

Body weight was measured upon arrival and on days 10, 40, 47, 51, 54, 57, 61, at harvest and on day 80 before the morning feeding. On day 80 before transportation, calves were weighed at the KSU Sheep and Meat Goat Center and then transported to the Kansas State University Beef Stocker Unit. Thereafter, calves were weighed individually each month before the morning feeding.. During the finishing phase, BW was measured upon arrival to the Beef Cattle Research Center and on days 12, 19, 26, and 28before evening feeding in a squeeze chute placed on calibrated load cells. No withdrawal from feed or water was imposed and all body weight measurements were considered full body weight.

Body weight and pen dry matter intake (DMI) were recorded to calculate interim average daily gain (ADG) and gain-to-feed ratio (G:F). Interim ADG was calculated for each calf as the change in individual body weight between consecutive weigh dates divided by the number of days between measurements. Individual DMI was estimated by allocating pen DMI to each calf based on its proportion of the total pen body weight during the corresponding period. Gain-to-feed ratio was then calculated by dividing each calf's interim ADG by its estimated DMI.

Fecal scores

During the hutch phase, fecal scores were assigned twice daily Monday through Friday by a single evaluator for consistency. Fecal consistency was assessed and scored using a 4-point scoring system (Table 3.4) ranging from 0 to 3. Higher fecal scores indicated greater severity of fecal looseness and digestive disturbance, while lower fecal scores indicated normal, well-formed feces.

GI contents

During harvest at weaning and finishing, GI contents were collected directly from the rumen, duodenum, jejunum, ileum, and spiral colon in 250 mL plastic jars and frozen at -20 °C until nutrient concentration analysis. The pH of the content samples was obtained using a calibrated benchtop pH probe (Milwaukee Instruments Inc, Rocky Mount, NC) immediately after the sample was collected. Gastrointestinal content samples from the hutch phase were dried in a forced air oven at 55 °C until weights of the samples didn't change. This resulted in loss of sample as the sample became baked on to the aluminum pans and could not be removed. Finishing phase samples were dried in a freeze drier at -50 °C until a powdery consistency and decreased odor was observed, which was approximately 1-2 weeks depending on type and amount of sample. Dry matter was calculated using the same equation used for DM of feed. A coffee grinder was used to grind rumen and colon samples, whereas, a mortar and pestle were used to grind sample from the duodenum, jejunum, and ileum. All GI content samples were sent to Cumberland Valley Analytical Services for analysis of starch, NDF, and CP.

GI Permeability

Gastrointestinal (GI) permeability in both the hutch and finishing phases was assessed by orally drenching calves with a sucralose solution followed by collecting a blood sample a few hours later. The gastrointestinal permeability assessment was conducted on days 51, 57, and 61

of the hutch phase with the first time point being before animals were weaned, and the two later timepoints being after animals were completely weaned. During the finishing phase, GI permeability was assessed on days 12, 19, and 26, each time point being evaluated 2 days after animals were stepped up on the first intermediate, second intermediate, and final finishing diets. Sucralose powder was purchased from an online vendor (Hard Eight Nutrition LLC, Henderson, NV, USA; bulksupplements.com) and was stored according to label directions. Prior to dosing a solution with a concentration of 0.25g/mL was made by mixing the sucralose powder with deionized water and was stored at room temperature. Sucralose was dosed at a rate of 0.4g/kg of BW where time of dosing was recorded to ensure blood samples were collected at correct time points. With proper restraint, a speculum was placed and a stomach tube was passed from the mouth to the reticulo-rumen and the sugar solution were administered using an equine stomach pump (Neogen, Lansing, MI, USA). In both the hutch and finishing phases water was pumped into the rumen following administration of the sugar solution to ensure the complete delivery of the biomarker, with approximately 200 mL of water used in the hutch phase and 1000 mL of water used in the finishing phase. For a short period of time following the oral drench process, each calf was monitored for adverse effects and regurgitation.

Blood was obtained using 10 mL vacutainer tubes and an 18G x 1’ needle via jugular venipuncture approximately 3 hours following dosing in the hutch phase. and 15 hours following dosing in the finishing phase. Blood was allowed to clot at room temperature and spun at 2000g at 4 °C for 20 minutes, serum was extracted and stored in a -20 °C freezer until analysis. Serum sucralose concentrations were obtained by gas chromatography-mass spectrometry at the Kanas State University Veterinary Diagnostic Lab (KSVDL).

pH Boluses

Small ruminant pH boluses (Small ruminant sensors; 20.6-mm diameter, 138-mm length, 245-g mass; Dascor, Escondido, CA) were used to asses changes in rumen pH during step down weaning. Boluses were randomized to a subset of calves from each treatment (n=4, LS=2, HS=2) and pH was measured every 2 minutes as described in a previous study (Penner et al., 2009). A pH sensor was placed into the bolus and a pre-trial calibration was performed using pH 4.0 and 7.0 solution. Boluses were inserted on d 47 and collected data until harvest on d 62 or 63 when the boluses were recovered at harvest, a post-trial calibration was performed, and data were downloaded to a laptop computer.

Tissue samples

At slaughter in the hutch phase, the GIT was completely emptied and each section of the GIT (forestomaches, small intestine, and large intestine) was weighed. A 1 x 1 cm square tissue sample from the ventral sac of the rumen, duodenum-, jejunum, ileum, and spiral colon were fixed in 10% neutral buffered formalin solution. Upon conclusion of the finishing phase, the GIT was removedand tissue samples were obtained and stored in a similar matter to the hutch phase. Tissue samples were stored at room temperature until histopathological analysis at the KSUVDL.

Slide preparation and analysis followed procedures comparable to those previously described by (Champagne et al., 2025). Briefly, all GI tissues were stained using hematoxylin and eosin (H&E). In addition, Alcian Blue staining (pH 2.5 brazilliant blue counterstain) was applied to small intestinal segments (duodenum, jejunum, ileum), as well as the spiral colon. All slides were assessed by a single evaluator, with up to 5 replicate measurements collected for each histological parameter per tissue (Table 3.5). For both morphologic assessment and goblet cell analysis, slides were digitalized at 400x magnification using a brightfield Leica Aperio AT2 scanner.

Quantitative measurements from H&E-stained sections were obtained using the measurement tools within Aperio eSlide Manager software (Version 12.4.3.5008; Leic Biosystems, Deer Park, IL). Goblet cell area in small intestine and spiral colon samples was determined by capturing five representative digital images per slide and analyzing them with the Colour Deconvolution2 plugin in the Fiji image analysis platform, following established methodologies (Ruifrok and Johnston, 2001; Schindelin et al., 2012; Landini et al., 2021). Goblet cell area was initially quantified as the percentage of tissue section occupied by goblet cells. The normalized values were then subjected to an arcsine square-root transformation prior to statistical analysis to improve normality and stabilize variance. Measurement criteria are summarized accordingly (Table 3.5). During microscopic evaluation of gastrointestinal tissues, it was not always possible to obtain the targeted five replicate measurements per sample. This limitation arose from several factors including mild autolysis at the microscopic level, mechanical damage that occurred during slide preparation, and suboptimal staining quality. When feasible, affected tissue samples were recut and stained in an effort to improve measurement yield. Despite these efforts, some samples still did not reach the intended number of replicates (Appendix 1).

STATISTICAL ANALYSIS

All data were analyzed using R statistical software (R v.4.5.1). Significance was set at $P \leq 0.05$ with tendencies described at $0.05 > P \leq 0.10$ for all variables.

Intake and performance

Body weight milk replacer intake, and starter feed intake (calculated on a dry matter basis) were used to calculate ADG and a gain to feed ratio (G:F) to determine feed conversion. Body weight and DMI were analyzed using linear mixed effects (*lme4* package in R) models with fixed effects of treatment, day and treatment x day interaction with calf included as random

effect in the Hutch Phase, using a compound symmetry covariance structure. Average daily gain was estimated as the slope of the BW regression line from the BW linear mixed effects model. Gain to feed ratio was analyzed using a simple linear model (*lm* function in R) with fixed effect of treatment. In the hutch phase DMI and G:F were analyzed using MR DMI values, starter grain only DMI values, and total (MR and starter grain combined) during the preweaning hutch phase. In the finishing phase, BW, ADG, and G:F were analyzed similarly to the method described in the hutch phase. Dry matter intake was analyzed with a similar model used in the hutch phase with fixed effects of treatment, stage, and treatment by stage interaction, where stage represented the different rations fed during the finishing phase. Calf was included as a random effect.

Fecal scores

Fecal scores were converted to a binary response variable representing the absence (score 0-1) or presence (score 2-3) of a fecal event. The effect of treatment on the number of fecal events was evaluated separately for the pre-weaning and post weaning periods using a linear model (*lm* function in R). Treatment was included as a fixed effect and the number of fecal events was the response variable. Analysis of variance (ANOVA) was used to determine the overall effect of treatment. When appropriate, treatment means were estimated using *emmeans* function and pairwise comparisons were performed using Tukey's adjustment for multiple comparisons.

Hutch phase pH and GI weights at slaughter

Gastrointestinal tract pH measurements were converted to hydrogen ion concentration prior to analysis to account for the logarithmic nature of pH. The equations used to transform pH values to hydrogen ion concentration and back transform to pH values were ([H^+] = 10^{-pH})

and (pH = -\log{10}[H^+]). Data were analyzed using linear mixed effects models (*nlme* package) in R with treatment, GI section, and their interaction included as fixed effects with calf included as a random effect. A compound symmetry correlation structure was used to model within calf correlations among sections and heterogenous residual variances were allowed across sections using a variance identity structure. Competing covariance structures were evaluated using AIC values and the model with the lowest AIC was selected. Least squares means were generated using the *emmeans* package. Results were back transformed and are presented as pH values.

Gastrointestinal tract weights were also calculated as a percentage of the animal's body weight. As a percentage of BW, these GI weights were analyzed using linear mixed effects models (*nlme* package in R). Fixed effects included treatment, GI section, and their interaction with calf included as random effect. A compound symmetry covariance structure was used to model correlations among sections with a heterogenous variance identity structure. Other covariance structures were modeled and the best fit was selected as the model with the lowest AIC value. Least squares means were generated and pairwise comparisons were conducted when appropriate.

GI contents

Starch, CP, and NDF concentrations for the hutch phase were analyzed using a linear mixed effects model implemented in R. Treatment, GI section, and their interaction were included as fixed effects. Calf ID was included as random effect to account for repeated measures. A compound symmetry covariance structure was specified to model within subject correlations. Observations with missing values were excluded from the analysis.

During the finishing phase, starch, CP, and NDF content were not normally distributed and were evaluated using untransformed, log-, square root-, and cube root- transformed models. Model fit was assessed using AIC and BIC, with final selection based on the lowest AIC and inspection of residual Q-Q plots. Cube root transformation provided the best fit for starch (whereas a log transformation was most appropriate for NDF. Final analyses were conducted using linear mixed effect models (*nlme* package) with treatment, GI section, and their interaction as fixed effects, calf ID as a random effect, and a compound symmetry correlation structure.

GI permeability

Serum sucralose concentrations were obtained from analysis at the KSVDL. Serum sucralose concentrations were analyzed using a linear mixed effect model (*lme4* package). Separate models were fit for the hutch and finishing phases using restricted maximum likelihood (REML), including treatment, day, and their interaction as fixed effects and calf and experimental batch as random intercepts. Model estimated marginal means were generated to compare the effects of treatment, sampling day, and their interaction.

Histopathology

To evaluate differences in gastrointestinal histologic features between HS and LS treatments, a dataset was constructed using measurements obtained from formalin-fixed tissues, along with calf ID and treatment group. Because multiple measurements were collected per animal, all numeric variables were averaged within calf to generate a single representative value per individual prior to analysis. Because the data for goblet cell counts were bounded and not normally distributed, values were first normalized by dividing each observation by the maximum value observed withing the respective tissue. Statistical analyses were performed using transformed values.

Multivariate structure within each tissue was first assessed using principal component analysis (PCA) implemented with the *prcomp* function in R statistical software. Analyses were conducted separately by tissue (rumen, duodenum, jejunum, ileum, and colon) and by phase (hutch or finishing). Principal component analysis was used to reduced correlated histologic measurements into a smaller set of orthogonal principal components (PCs) that capture the primary sources of variation. Scree plots were generated for each tissue to guide selection of the most informative PCs. Selected PCs were subsequently analyzed using multivariate analysis of variance (MANOVA), using the *manova* function in R, to test for overall differences between treatment groups. In addition, individual PCs were evaluated using linear models, with treatment included as a fixed effect to determine whether HS and LS groups differed along specific axes of histologic variation.

To complement the multivariate approach and provide interpretable estimates of effect size and direction, univariate analyses were also performed for each histologic measurement. For each tissue and phase, individual histologic measurements were analyzed using linear models with the *lm* function in R, with treatment specified as a fixed effect.

pH boluses

Rumen pH was continuously monitored using indwelling ruminal boluses that recorded pH every 2 minutes throughout the monitoring period. Raw pH data were summarized descriptively using R. Mean ruminal pH was calculated by calf and by day. The duration of ruminal acidosis was quantified as the cumulative time spent below pH 5.6 by counting observations below each threshold and converting counts to minutes and hours.

Daily ruminal pH was also summarized alongside daily starch and body weight to facilitate graphical assessment of relationship between daily starch intake and ruminal pH. Graphs of

GI permeability measures and average histology measurements per calf were also assessed. Owing to the limited sample size, analyses were descriptive, and no formal hypothesis testing or inferential statistical comparisons were performed.

RESULTS

Hutch Phase

Growth and Feed Intake

Preweaning BW revealed a treatment by day interaction where BW increased over time from initial BW (d0) to weaning (d54), and the LS group gained more per day than the HS group (P=0.034; Table 3.6). The slope of the BW regression line represented ADG, where calves in the LS had a greater ADG of 0.78 kg/d compared to 0.70 kg/d for the HS calves (Figure 3.1). There was no effect of treatment on gain to feed ratio in the preweaning phase when based on starter grain only G:F (P=0.24) and milk replacer and grain G:F (P=0.51).

Pre-weaning total (milk replacer + grain) DMI was not affected by treatment, time, or treatment by time interactions (P=0.44). Milk replacer DMI was not affected by treatment (P=0.76) or treatment by day interactions (P=0.44), however, a day effect was observed due to the step-down methods used to wean calves (P<0.01). Starter grain DMI had an effect of day (P<0.01) and a treatment by day interaction (P=0.006). On d 40, there were no treatment differences observed in grain intake (P=0.20). On d 47, during the second MR step-down, there was a treatment effect in starter grain DMI, where LS and HS calves consumed 1.5 and 1.3 ± 0.063 kg/d, respectively. On d 54, when calves were weaned completely, there was a treatment effect identified (P <=0.014), where the LS calves consumed 2.1 ± 0.068 kg/d and the HS group consumed 1.8 ± 0.068 kg/d.

Post weaning initial BW was not different among treatments (P=0.15). Post weaning final BW also was not different among treatments (P=0.55). Therefore, the slope of the BW regression line, ADG, was not different between treatment groups (P=0.95). Similarly, there was no evidence of significant treatment, day or treatment by day interaction observed for post weaning DMI (P=0.23). Post weaning G:F was not different among treatments (P=0.23).

Fecal scores

Treatment did not affect the number of days on which calves experienced at least one fecal event during either the pre-weaning or post-weaning periods (Table 3.7). During the pre-weaning period, calves assigned to the HS diet experienced at least one fecal event on an average of 1.7 ± 0.57 days, whereas calves assigned to the LS diet experienced at least one fecal event on an average of 0.7 ± 0.57 days; however, this difference was not significant (P = 0.23). Likewise, during the post-weaning period, no treatment effect was observed (P = 0.88), with calves in the HS and LS groups experiencing at least one fecal event on an average of 2.0 ± 0.88 and 1.8 ± 0.88 days, respectively.

Slaughter

At slaughter during the hutch phase, no lesions or abnormalities were detected in any section of the GI system. During the finishing phase, at harvest, there were no lesions or abnormalities detected throughout the GI system and no liver abscesses were detected.

Gastrointestinal Tract Weights and pH

pH differed significantly among GI sections (P<0.0001), while no effect of treatment (P=0.690) and no interaction (P=0.44) were detected (Table 3.8). The ileum had significantly higher pH than the rumen (P=0.0016) and the jejunum (P=0.055); however, the pH in the ileum

did not differ from the duodenum or colon. No other section contrasts were statistically significant ($P>0.18$).

Gastrointestinal tract section weights differed among anatomical regions ($P<0.0001$; Table 3.9), while no treatment effect ($P=0.43$) or section by treatment interactions ($P=0.44$) were detected. All GI sections differed significantly from one another ($P<0.05$), where our reticulorumen was the heaviest, followed by our small intestine, large intestine, abomasum, and omasum.

Body weight adjusted GI section weights (g/kg BW) differed among regions ($P<0.0001$; Table 3.9); however, there were no significant differences among treatments ($P= 0.29$) and no significant interaction ($P=0.75$). Similar to GI weights in kg, all sections differed from one another ($P<0.05$), where our reticulorumen compromised the largest percentage of calf body weight, followed by our small and large intestines, abomasum, and omasum.

Gastrointestinal morphology

Principle component analysis (PCA) of rumen variables identified 8 principal components (PCs). Based on inspection of the Scree plot, the first 5 PCs were retained for further interpretation, accounting for 95% of the total variance (Table3.10). Papillary width, papillary area, and stratum basale were strongly correlated with PC1. Papillary width and keratin thickness were most influential in PC2, whereas, PC3 was largely driven by stratum corneum and stratum spinosum. Variation in PC4 was primarily associated with stratum granulosum and stratum corneum, while stratum basale was the dominant contributor to PC5. Multivariate analysis of variance (MANOVA) of rumen PCs indicated no significant difference between treatments ($P=0.54$: Table 3.13). No significant differences were detected in any rumen measurements in the linear models ($P>0.05$: Table 3.14).

In the duodenum, the PCA generated 6 PCs, of which 4 were retained based on the Scree plot, explaining 95% of the variance (Table 3.11). Principle component 1 was characterized by villus height, crypt death, and total mucosal thickness. Principle component 2 was driven by villus width, goblet cell area, and crypt width. Goblet cell area and villus width were strongly correlated with PC3, while PC4 was similarly influenced by villus width and goblet cell area. Multivariate analysis of variance of the 4 PCs results showed no differences between treatments (P=0.27; Table 3.13). In the duodenum, however, total mucosal thickness was greater in the HS groups (P=0.003) with the average total mucosa thickness measurements being 740 and 940 ± 56.1 µm for the LS and HS groups, respectively (Table 3.15)

For the jejunum, 6 PCs were identified, with four selected for further evaluation, representing 95% of the variance (Table 3.11). Principle component 1 was primarily associated with crypt depth, total mucosal thickness, and crypt width. Villus height and villus width were strongly correlated with PC2. In PC3, goblet cell area and crypt width were the dominant variables, whereas, PC4 was mainly influenced by crypt width and villus width. Multivariate analysis of variance of the 4 PCs results indicated no significant treatment effect (P=0.088; Table 3.13). No evidence of treatment effects on individual histologic measurements were observed (P>0.05; Table 3.15).

Similarly, PCA of ileal measurements produced 6 PCs, with four retained, accounting for 95% of the variation (Table 3.11). Principle component 1 was largely defined by crypt depth, total mucosal thickness, and crypt width. Principle component 2 was influenced by villus width and crypt width, while goblet cell area was the primary contributor to PC3. Principle component 4 was driven by villus height and crypt depth. No significant treatment differences were detected

by the multivariate analysis of variance of the 4 PCs (P=0.87; Table 3.13). There were no significant effects of treatment on histologic measurements (P>0.05; Table 3.15).

In the colon, 4 PCs were identified, with 3 retained based on the Scree plot, accounting for 97% of the variation (Table 3.12). Gland depth, total mucosal thickness, and gland width were strongly correlated with PC1. Principle component 2 and 3 were influenced by goblet cell area and gland width. Multivariate analysis of variance of the 3 PCs results indicated no significant differences between treatments (P=0.61; Table 3.13). No significant effects of treatment on spiral colon individual histological measurements were observed (P>0.05; Table 3.16).

GI permeability

Serum sucralose concentration did not differ between dietary treatments at any sampling day (days 51, 57, and 61), and no effects of treatment (P = 0.606), day (P = 0.199), or the treatment × day interaction (P = 0.849) were detected (Table 3.17).

Gastrointestinal nutrient concentrations

There were no effects of treatment (P = 0.75), GI section (P = 0.27), or their interaction (P= 0.44) on starch concentration (Table 3.8). Due to lack of duodenal samples, duodenal NDF and CP concentration was not analyzed. In the rumen, jejunum, ileum, and colon NDF concentration was found to be influenced by treatment (P=0.003), GI section (P<0.001), and treatment x section interaction (P=0.050; Figure 3.2). Pairwise comparisons were conducted and treatment differences only occurred in the ileum, where LS animals had significantly greater NDF concentration in the ileum than the HS calves (LS=33.69 vs HS=4.36 ± 8.17 %DM). No treatment differences in NDF concentration were detected in other GI sections. Crude protein concentration differed significantly among GI sections (P<0.0001) and there was a significant treatment x section interaction (P<0.0001; Figure 3.2) but no significant effect of treatment (P=0.36). Pairwise

comparisons revealed that HS animals had substantially greater CP concentration in the ileum than the LS animals (HS= 42 vs LS=20 ± 2.93 %DM). No other differences in CP concentration were detected between treatments in another other section of the GI tract.

Rumen pH

The mean ruminal pH for calves 7, 8, 9, and 12 was 6.14, 6.17, 6.20, and 5.44, respectively (Table 3.18) Calves in the LS treatment had pH values ranging from 5.21 to 6.81 (calf 7) and 5.03 to 6.92 (calf 8). In the HS treatment, calf 9 had a pH ranging from 5.24 to 6.96, whereas calf 12 exhibited the widest range, with pH values from 4.54 to 6.59.

Mean hourly ruminal pH for each calf is presented in Figure 3.3. Distinct diurnal pH patterns were observed among calves, with substantial individual variation throughout the monitoring period. Average daily ruminal pH and daily starch intake are presented in Figure 3.4. In the LS treatment, daily mean ruminal pH generally followed changes in daily starch intake, whereas calves in the HS treatment exhibited greater day-to-day variability in both starch intake and ruminal pH.

The total time spent below a ruminal pH of 5.6 is shown in Figure 3.5. Within the LS treatment, calves 7 and 8 spent 3.0 and 7.4 h below pH 5.6 over the entire monitoring period, respectively. In the HS treatment, calf 9 spent 2.8 h below pH 5.6, whereas calf 12 spent 145.2 h below this threshold over the entire monitoring period. This translates to around 13 hours per day that calf 12 spent below a pH of 5.6 indicating substantially greater exposure to low ruminal pH than the other calves.

Individual ruminal morphology measurements, including papilla length, papilla width, papilla area, and keratin thickness are presented in Figure 3.6 where calf 12 exhibits greater papillae width than the other calves, while calves 7, 8, and 9 experience papilla length than calf 12,

indicating more normally developed papillae. Each animal's rumen histology slide is presented in Figure 3.7. The rumen morphology slide for calf 12 reveals very abnormal, papillae with branching when compared to calves 7, 8, and 9 rumen morphology slides that show longer, more developed papillae. Gastrointestinal permeability measurements for each calf at all three sampling time points are presented in Figure 3.8. Permeability measurements vary by calf and sampling day, however calves 7 and 12 experience higher serum sucralose concentrations on the third sampling day compared to the previous two sampling days, while calves 8 and 9 experience relative low serum sucralose concentrations throughout all three sampling days.

Growing Phase

Growth and Intake

Grower phase growth and performance measures are reported as arithmetic means as there was only one pen per treatment and intake was measured at the pen level. All values are pen level averages (Table 3.19). Average BW was 436 kg and 378 kg for LS and HS groups, respectively. Average daily gain for the LS calves was 3.06kg/d and for the HS calves 3.02kg/d. Average DMI for the LS pen was 12.7kg/d and for the HS pen was 13.1kg/d. Gain to feed ratio for the LS group was calculated at 0.238 kg/ kg and for the Hs group it was 0.233 kg/ kg.

Finishing Phase

Growth and Intake

Finish phase BW showed only a significant effect of time ($P<0.001$) where BW increased from d 0 to d 28 of the finishing phase (Table 3.20). On d 0, calves weighed 280 ± 12 kg on average, whereas on D 28 of the finishing phase calves had an average weight of 330 ± 12 kg.

There was no evidence of statistical difference in ADG between the treatments (P=0.13), but numerically the LS group had increased ADG compared to the HS group (1.9 vs 1.7 ± 0.11 kg/d; Table 3.20). Dry matter intake during the finishing phase revealed only a significant step-up diet effect (P<0.001) where on average, calves consumed less feed when offered the second step-up diet compared to when they were offered the starter, the first step-up diet, and the finishing diet (Figure 3.9). While on the step-up 2 diet, calves consumed 5.6 kg/day compared to 7.3, 7.4, and 7.4 ± 0.30 kg/dd on the starter, step-up 1, and finishing diets, respectively. Gain to feed revealed no significant effects of treatment (P=0.17), although numerically, the LS group on average, had increased G:F when compared to the HS group (0.29 vs 0.25 +/- SE kg/kg; Table 3.20).

Gastrointestinal pH at slaughter

Gastrointestinal pH of rumen, duodenal, jejunal, ileal, and colon contents at slaughter revealed no significant treatment (P=0.713) or treatment by section interaction (0.831); however, there was a significant effect of GI section (P<0.001; Table 3.21). However, pH did not differ between the colon and rumen (P = 0.9995), the duodenum and jejunum (P = 0.2313), or the ileum and rumen (P = 0.7014). All other pairwise comparisons among GI sections were significant (P < 0.05).

Gastrointestinal morphology

The principal component analysis of rumen measurements identified 8 PCs, of which five were retained based on the Scree plot, explaining 94% of the total variance (Table 3.22). Principle component 1 was primarily driven by papillae length, keratin thickness, and stratum granulosum thickness. In PC2, papillae width, papillary area, and stratum spinosum thickness were the dominant contributors. Principle component 3 was largely influenced by stratum basale thickness and keratin thickness, while PC4 reflected variation in stratum spinosum thickness.

Multivariate analysis indicated a significant overall effect for rumen PCs (P=0.025), with PC2 contributing significantly to differences between treatments (P=0.0073; Table 3.25). Subsequent linear modeling of individual rumen variables revealed a significant treatment effect for papillae width (P=0.049; Table 3.26). Additionally, papillae area (P=0.13), stratum spinosum thickness (P=0.14), and stratum basale thickness (P=0.084) tended to be greater in HS compared to LS. No differences were observed for papillae length, keratin thickness, stratum corneum thickness, or stratum granulosum thickness (P>0.05).

In the duodenum, 6 PCs were generated and 4 were retained for further evaluation, accounting for 93% of the variance (Table 3.23). Principle component 1 was characterized by crypt depth, total mucosal thickness, and crypt width. Villus height and goblet cell area were most strongly correlated with PC2. Principle component 3 was influenced by goblet cell area and crypt width, whereas, PC4 was driven by total mucosal thickness and villus width. No significant difference between treatments was detected by the multivariate analysis of variance of the 4 PCs (P=0.61; Table 3.25). In duodenum, no treatment effects were detected for any variables (P>0.05; Table 3.27).

For the jejunum, 6 PCs were identified, with 4 explaining 97% of the total variance (Table 3.23). Principle component 1 was strongly correlated with crypt depth, total mucosal thickness, and crypt width. Goblet cell area and villus width contributed most to PC2. Principle component 3 was associated with villus height and crypt width, while PC4 again reflected goblet cell area and villus width. Although the overall MANOVA did not detect a significant multivariate effect among treatments (P = 0.26), scores on the second principal component (PC2) differed significantly among treatments (P = 0.038, Table 3.25). This indicates that while the combined multivariate response was not statistically different, variation captured by PC2 was associated with

treatment differences. Jejunal analyses indicated tendencies for increased goblet cell area in the HS group (P=0.099) and reduced villus width (P=0.19) compared to LS (Table 3.27).

Similarly, ileal PCA produced 6 PCs with 4 retained, explaining 98% of the variance Table 3.23). Principle component 1 was strongly correlated with villus height, crypt depth, and total mucosal thickness. Goblet cell area and crypt width were the primary contributors to both PC2 and PC3, while villus width was most correlated with PC4. No significant treatment differences were observed in the multivariate analysis of variance of the 4 PCs (P=0.96; Table 3.25). Ileal morphologic measurements showed no significant differences or trends between treatments (P>0.05; Table 3.27).

In the colon, 4 PCs were identified, and 3 were retained following Scree plot evaluation explaining 99% of the variance (Table 3.24). Principle component 1 was strongly correlated with gland depth, total mucosal thickness, and gland width, whereas, PC2 and PC3 were primarily correlated with gland width and goblet cell area. Multivariate analysis of variance results indicated no significant difference between treatments (P=0.52; Table 3.25). In the colon, total mucosal thickness tended to be greater (P=0.097) in the HS than LS group (Table 3.28).

Gastrointestinal permeability

Serum sucralose concentration in the finishing phase differed by sampling day (P = 0.053), whereas no effects of treatment (P = 0.820) or the treatment × day interaction (P = 0.870) were detected (Table 3.29). Pairwise comparisons indicated that serum sucralose concentration was greater on day 19 than on day 26 (P = 0.045). Serum sucralose concentrations did not differ between days 12 and 19 (P = 0.606) or between days 12 and 26 (P = 0.243).

Gastrointestinal nutrient concentrations

During the finishing phase, starch concentration was affected by GI section (P <0.01), but not treatment (P = 0.71) or treatment by section interaction (P = 0.52; Table 3.21). Pairwise comparisons indicated that starch concentration was greater in the colon (6.43 %DM) than the duodenum (5.41 %DM: P=0.0190), jejunum (5.75 %DM: P=0.032)), and the rumen (6.37%DM; P=0.019). Starch concentration in the colon did not differ from the ileum (7.04 %DM; P=0.84), and no other differences among GI sections were detected (P>0.05).

There was a significant effect of treatment (P=0.043), GI section (P<0.0001), and a treatment x section interaction (P<0.001) for NDF concentration in the finishing phase (Figure 3.10). Calves receiving the HS diet experienced greater NDF concentration in the duodenum than calves receiving the LS treatment (HS=3.06 vs LS=1.22 ± 0.35 %DM; P=0.0045). No treatment differences were detected in the colon, ileum, jejunum, or rumen.

In the finishing phase, CP concentration was affected by treatment (P<0.0001), GI section (P<0.0001), and treatment x section interaction (P=0.018; Figure 3.10). Calves receiving the LS diet had greater CP concentration in the duodenum than calves receiving the HS diet (LS=3.82 vs HS=2.93 ± 0.16 %DM; P=0.0047). No further treatment differences were detected in other GI sections.

DISCUSSION

Growth performance and intake

One of the primary objectives of this study was to determine whether increasing starch inclusion in calf starter diets would improve growth performance during the hutch phase and have any carryover growth effects during the finishing phase. We hypothesized that growth performance and intake would be decreased in animals given the HS diet as there would be less inflammation of the GI system and therefore, less metabolic stress, allowing animals to consume

more, therefore, gaining more weight. In contrast to our hypothesis, calves receiving the LS starter feed exhibited greater BW at weaning and greater ADG during the pre-weaning period compared to calves fed the HS starter. These differences coincided with greater DMI by LS calves suggesting that the greater DMI overserved likely contributed to the increased BW and ADG during the pre-weaning period (Khan et al., 2007). However, following weaning, treatment differences in BW, ADG, and DMI were no longer evident even in the finishing phase, indicating that the early advantage in growth performance did not persist once calves were transitioned to common post hutch management and diets. The disappearance of treatment differences following weaning reflects the transition of both treatment groups to similar grower and finisher diets, which may have reduced any nutritional programming effects associated with the pre-weaning starter diets. Once nutrient intake and dietary composition became comparable between treatments, opportunities for compensatory growth and similar rates of tissue accretion may have minimized the initial performance differences (Drouillard et al., 1991; Hornick et al., 2000).

Previous studies evaluating starch concentration in calf starter diets have reported inconsistent effects on growth performance, feed efficiency and intake. Some studies have demonstrated improved ADG and feed efficiency when calves were offered highly fermentable, starch rich starters due to greater ruminal production of VFAs (Pazoki et al., 2017; Dennis et al., 2018; Quigley et al., 2018; Satoh et al., 2023). In contrast, the present findings do not align with what has previously been found, and that could be due to differences in starch source, starch fermentability, forage inclusion, milk allowance, and weaning strategy. Furthermore, many studies conducted have used Holstein bull calves (Dennis et al., 2017; Quigley et al., 2018; Satoh et al., 2023). Therefore, differences between the present study and previous reports may partially reflect physiological and growth differences associated with BD crosses. These findings suggest

that increasing starch concentration in calf starter may not be as influential as previously thought on carryover growth performance later in the production cycle. Under the conditions of the present study, increasing starch concentration in calf starter did not confer a sustained growth advantage beyond the hutch phase, suggesting that early dietary starch concentration alone maybe have limited influence on subsequent performance.

Fecal scores

During the hutch phase, encompassing both the preweaning and postweaning periods, dietary starch concentration did not influence the number of days calves experienced a fecal event (fecal score ≥ 2). These findings are consistent with those of Satoh et al. (2023), who reported no effect of calf starter starch concentration on fecal scores despite evaluating three dietary starch inclusion levels (41.8%, 31.9%, and 22.0%). Similarly, Dennis et al. (2018) observed no differences in fecal scores among calves fed textured starter diets that varied in starch concentration while evaluating growth performance, nutrient digestibility, and ruminal fermentation. In contrast, other studies have reported increased fecal scores or a greater incidence of abnormal feces in calves consuming high-starch starter diets (Osorio et al., 2012; Dennis et al., 2017). These conflicting findings may, in part, reflect differences in fecal scoring systems, including the frequency of scoring, scoring scale, and criteria used to classify feces as abnormal. Although fecal score is commonly used as a practical indicator of GI health and digestive disturbances in young calves, studies differ in fecal scoring systems. Fecal scoring systems differ in numerical scales, thresholds used to classify abnormal feces, and the frequency at which fecal scores are recorded, all of which may influence the reported incidence of fecal abnormalities. Under the management conditions of the present study, dietary starch concentration alone did not appear to have adversely affected fecal consistency or GI health during the hutch phase.

pH of contents

Gastrointestinal content pH at harvest was not influenced by calf starter starch concentration during either the hutch or finishing phases, suggesting that the dietary starch concentrations evaluated in the present study did not substantially alter the luminal environment of the GI system. This finding is consistent with the absence of treatment effects observed for GI weights, permeability, and most histological measurements, indicating that early life starch intake had minimal effects on overall GI development and function. In contrast, significant differences in pH among GI sections were expected and reflect the differences in physiological functions of each section. The rumen maintains a relatively acidic environment due to microbial fermentation, production of VFAs, whereas the pH of the small intestine increases as there is an increase in pancreatic and biliary secretions that neutralize the acidic digesta from the abomasum (Diao et al., 2019) . Furthermore, hindgut acidosis is typically characterized by decreased fecal pH due to increased starch flow into the hindgut and subsequent fermentation (Wheeler and Noller, 1977; Gressley et al., 2011). Although the present study evaluated colonic pH rather than fecal pH, the pH measurements obtained in this study, suggest that increasing starch concentration in calf starter did not result in GI conditions consistent with hindgut acidosis.

There is currently a lack of studies assessing pH in the GI system of pre-weaned calves. To our knowledge, the only other study (Zhang et al., 2017) that has conducted a similar protocol to what was done is the present study. The pattern of digesta pH observed in the present study is generally consistent with that reported by Zhang who found lower pH values in the rumen and proximal small intestine and greater pH values in the distal small intestine. In both studies, the ileum exhibited one of the highest pH values among GI sections. However, unlike Zhang, dietary treatment did not affect digesta pH in the present study, suggesting that the starch concentrations

evaluated were insufficient to alter GI pH despite normal regional variation. Most studies look at abomasal, ruminal, or fecal pH in calves or fully functioning ruminants (Gressley et al., 2011), whereas this study looked at pH of almost all compartments of the GIT. It should also be recognized that GI pH measured at harvest represents a single timepoint that may be influenced by recent feed intake, digesta passage rate, and the stage of digestion at harvest. Therefore, although there were section specific differences in pH, the lack of treatment effects suggests that early dietary starch concentration did not produce any sustained alterations in GI luminal concentrations and did not greatly affect GI function to produce carryover affects during the finishing phase.

GI weights

Gastrointestinal tract weights at hutch phase harvest were not influenced by calf starter starch concentration, regardless of whether weights were expressed on an absolute basis or as a percentage of BW. Differences among the sections of the GIT reflect the distinct capacities and physiological functions of each different compartment. In pre-weaning calves, the abomasum comprises a greater proportion of the GIT because it functions primarily in the digestion of milk (Meale et al., 2017). During this period, solid feed intake is relatively limited and the gradual increase in dry matter intake promotes ruminal growth and maturation as calves transition toward a functional ruminant digestive system. Previous research evaluating ruminal and GI development has reported similar differences in the weights of GI sections with the abomasum representing a larger proportion of the digestive system in pre-weaning calves (Greenwood et al., 1997; Steele et al., 2017). However, those studies also assessed the effects of forage inclusion on GI development, which likely contributed to treatment differences that were not observed in the present study. Although dietary starch concentration has been proposed to influence growth through changes in nutrient availability and fermentation end products, the range of starch concentrations

evaluated in the present study were insufficient to alter organ mass. These findings are consistent with the absence of treatment effects on overall growth performance following weaning and indicate that early life management had limited influence on the structural development of the GI tract at the organ level.

GI morphology

Overall, calf starter concentration had minimal effects in GI morphology during the hutch phase. Although greater total mucosal thickness was observed in the duodenum of calves receiving the HS diet, no treatment differences were detected in any other GI section. Various other studies have demonstrated enhanced ruminal or intestinal development in calves fed more fermentable diets as starch fermentation can increase butyrate production, the main VFA responsible for papilla development (Anderson et al., 1982; Lesmeister and Heinrichs, 2004; Moeini et al., 2017; Govil et al.). In contrast, in a study done by Beharka, it was found that starch rich diets can induce papillae changes that cause more branched, and clumped papilla as compared to more tongue shaped, uniform papilla (Beharka et al., 1998). The present study did not align with either of these hypotheses suggesting that the dietary treatments produced only limited structural adaptations during early GI development. These inconsistencies among studies, however, are due to differences in starch source, starch concentration, forage inclusion, feed strategy, and duration of dietary exposure, as a majority of studies have evaluated the effect of forage inclusion or particle size on rumen development. Furthermore, because pre-weaning calves derive a large portion of their nutrients from milk replacer, the contribution of starter intake to GI development may have been insufficient to elicit widespread histological changes before weaning.

Although few treatment differences were detected during the hutch phase, calves that received the HS starter exhibited subtle alterations in ruminal morphology during the finishing

phase. Specifically, papilla width was greater in HS calves, while papilla area, and stratum spinosum and basale thickness tended to be greater when compared to calves fed the LS diet. Increased dietary starch generally promotes microbial fermentation and the production of VFAs, particularly butyrate, which aids in papilla ruminal epithelial proliferation and papilla development (Meale et al., 2017). Although increased papillary development is generally associated with greater absorptive capacity, these structural adaptations did not translate into improvements in growth performance or feed efficiency during the finishing phase, which could likely be due to the short finishing period as prolonged exposure to high starch is the primary driver of increased papilla size and area in finishing animals (Estevam et al., 2020; Jennings et al., 2021). Interestingly, these morphological differences were not evident immediately following the hutch phase. Both treatment groups were managed similarly for approximately 159 days before the finishing-phase evaluation. This may suggest that early life starch exposure induced subtle developmental adaptations or programming of the ruminal epithelium that persisted throughout the production cycle, although the biological significance of these changes remains uncertain given the absence of differences in growth performance and GI permeability.

Although these findings generally agree with the proposed mechanisms of ruminal epithelial adaptation described in dairy calves, caution should be exercised when making direct comparisons because the present study evaluated BD cross calves. Differences in growth potential, nutrient partitioning, and GI development between crossbred and Holstein calves may influence their physiological response to early life nutrition, highlighting the need for additional research in crossbred production systems.

GI Permeability

One of the primary objectives of this study was to determine whether starch inclusion level in calf starter influenced GI permeability during early life and whether these effects persisted into the finishing phase. To our knowledge, no previous studies have directly evaluated the effects of starch concentration on GI permeability in calves using orally administered sugar biomarkers as a measure of permeability. In the present study, dietary starch concentrations did not influence GI permeability as evident by the lack of treatment effects on serum sucralose concentrations during weaning and finishing transition. These findings are consistent with the absence of treatment effects observed for histopathology, and luminal pH, collectively indicating that early life starch concentration had minimal influence on GI development and function under the conditions of the present study.

Previous studies have investigated nutritional strategies to mitigate GI permeability early in life primarily through alterations in milk replacer composition with multiple studies showing that milk replacer composition can negatively impact GI permeability (Amado et al., 2019; Wilms et al., 2019). There has also been studies looking at yeast (Pisoni and Relling, 2020), glutamine (Ceja et al., 2023), and butyrate (Gao et al., 2025) supplementation on GI permeability particularly when calves are exposed to physiological or environmental stressors. Serum sucralose was detected in calves throughout the study (Table 3.29, Table 3.17, Figure 3.8), indicating baseline gastrointestinal absorption of the marker. Although some individual calves exhibited greater serum sucralose concentrations than others, treatment had minimal effect on serum sucralose concentrations ($P > 0.05$), and it is unclear whether these differences reflected increased gastrointestinal permeability or normal biological variation

During the finishing transition, DMI for step up diet 2 was significantly less than the rest of the finishing rations (Figure 3.9, Table 3.20) and serum sucralose concentrations were significantly greater during the period where animals were on the step-up 2 ration, (Table 3.29). It is unclear if feed intake decreased due to possible increase in permeability, or if permeability was increased due to a decrease in feed intake, as feed restriction has been proven to decrease epithelial barrier function (Zhang et al., 2013b; Pisoni et al., 2022); however, the decrease in epithelial barrier function during this study was not as severe as seen in previous studies. The lack of treatment differences indicates that both dietary treatments provided sufficient nutritional support to maintain normal GI integrity and exhibit exceptional growth performance throughout development. Furthermore, all calves transitioned to common grower and finishing diets, which may have minimized any subtle programming of early life nutrition, explaining the lack of effects during the finishing transition. Overall, the present findings demonstrate that altering starch concentration in calf starter had minimal effects on GI development and function, indicating the dietary starch concentration alone may not be a major determinant of GI health in healthy BD cross calves.

Nutrient concentrations

Nutrient concentrations differed among GI section, reflecting the normal processes of microbial fermentation, enzymatic digestion, nutrient absorption, and digesta passage. As digesta progresses through the GIT, starch, protein, and fiber undergo varying degrees of microbial degradation and enzymatic digestion, resulting in expected differences in nutrient composition among GI compartments (Harmon and Swanson, 2020). Despite isolated treatment differences in duodenal CP and NDF concentrations, no treatment effects were observed for starch concentration, and differences in CP and NDF were not detected in the remaining GI sections. Because

crude protein represents total nitrogen, including both undigested dietary protein and microbial protein, the observed differences in duodenal CP and NDF may reflect treatment-related alterations in ruminal fermentation or microbial populations established during the hutch phase. Early-life dietary starch concentration has the potential to influence rumen microbial colonization and development, which may subsequently affect fiber degradation, microbial protein synthesis, and the flow of nutrients into the small intestine. Although the rumen microbiome was not evaluated in the present study, differences in microbial development provide a plausible explanation for the treatment effects observed in the duodenum.

Rumen pH

One of the primary objectives of this study was to characterize ruminal pH patterns in calves receiving starter diets differing in starch concentration during the weaning transition. Although statistical analyses were not performed because only four calves were monitored, continuous ruminal pH measurements revealed notable differences among individual animals. Mean ruminal pH was generally similar among three of the four calves; however, calf 12, which received the HS diet, exhibited a decreased mean ruminal pH, a wider pH range, and substantially more time below the commonly accepted threshold for subacute acidosis of pH 5.6 than the remaining calves (Figure 3.6). Greater day-to-day variability in ruminal pH and starch intake (Figure 3.5) was also observed in HS calves, 9 and 12, suggesting that individual responses to increased dietary starch may vary considerably.

As, dietary starch increases, rapidly fermentable carbohydrates promote greater ruminal fermentation and production of VFAs, particularly lactic acid , resulting in the reduction in ruminal pH (Owens et al., 1998). Although ruminal pH fluctuates throughout the day with periods of intake and fermentation, prolonged periods of decreased ruminal pH can be detrimental to the

rumen environment and to the animal, developing a condition known as acidosis, which can lead to altered epithelial integrity (Owens et al., 1998; Amachawadi and Nagaraja, 2022). Continuous ruminal pH monitoring is a helpful method in assessing the true pH patterns in the rumen environment rather than a single timepoint measurement, as seen in previous calf studies where a small ruminant pH bolus is used (Penner et al., 2009; Laarman et al., 2012). Continuous pH measurements more accurately collect the fluctuations in frequency, duration, and severity of low pH bouts.

The descriptive trends observed in the present study are consistent with the established effects of dietary starch on ruminal fermentation. In the present study, calves receiving the HS starter, generally exhibited more variability in ruminal pH and calf 12 exhibited substantially more time below a pH of 5.6. However, calf 9 was able to maintain a more stable pH throughout the sampling time period than calf 12. While starch inclusion in the diet has been proven to promote a more acidic rumen environment in some animals, the considerable individual variation observed indicates that factors such as feeding pattern, fermentation dynamics, and animal specific responses are likely to contribute to the variation in ruminal pH (Schwartzkopf-Genswein et al., 2003).

Despite descriptive evidence that one HS calf experienced prolonged exposure to low ruminal pH, these observations were not accompanied by corresponding differences in overall treatment effects on GI permeability, GI histology, GI nutrient concentrations, pH at harvest, or growth performance. However, evaluation of the individual histologic measurements of the calves equipped with a rumen boluses revealed interesting descriptive relationships between ruminal pH dynamics and ruminal morphology. Calf 12, which exhibited the lowest pH values, the greatest variability and the longest duration below pH 5.6, also demonstrated more pronounced

alterations in ruminal papilla morphology than the other calves (Figure 3.8 and Figure 3.7). In contrast, calf 9, the other HS calf, maintained ruminal pH patterns comparable to those of the LS calves (calf 7 and 8) and exhibited ruminal papilla development similar to calves 7 and 8. Although these observations are based on individual animals and should not be interpreted as treatment effects, they suggest that prolonged exposure to lower ruminal pH may influence ruminal epithelial adaptation in susceptible animals.

Similarly in individual permeability responses did not consistently correspond with treatment assignment. Evaluation of serum sugar concentrations for each bolus monitored calf (Figure 3.9) indicated that calves 7 (LS) and 12 (HS) exhibited greater permeability at the final sampling time point, whereas calves 8 (LS), and 9 (HS) demonstrated comparatively smaller changes over time. These observations further indicate that factors beyond dietary starch concentration, including individual animal variation, likely contribute to GI barrier function.

Research evaluating continuous ruminal pH in pre-weaned calves remains limited, particularly using indwelling ruminal pH boluses, making direct comparisons with previous studies difficult. Furthermore, most published studies have been conducted in Holstein calves (Laarman et al., 2012; Kim et al., 2016; Gelsinger et al., 2020), whereas relatively little information is available for BD cross calves. Because BD cross calves may differ in growth patterns, feed intake behavior, and nutrient utilization, additional research is needed to determine whether these factors influence ruminal pH regulation and adaptation to dietary starch. Collectively, the present findings suggest that while dietary starch concentration may contribute to ruminal pH dynamics, individual animal characteristics, including feeding behavior, ruminal buffering capacity, and physiological variation, may also play important roles in determining ruminal pH responses.

LIMITATIONS

A limitation of the present study includes the relatively small sample size, particularly for the continuous ruminal pH monitoring, in which only four calves received ruminal pH boluses. Consequently, ruminal pH observations were evaluated descriptively and should be interpreted with caution. Nevertheless, this study provides novel information regarding the effects of starch inclusion level in calf starter on growth performance, GI permeability, GI development and ruminal pH in beef-on-dairy animals and establishes a foundation for future research. Collaboration with commercial calf ranches and following a larger population of calves throughout the production cycle would improve the external validity and provide greater statistical power to evaluate growth performance, ruminal development, and LA incidence under commercial conditions. Future research should also investigate ruminal VFA production, continuous ruminal pH, and GI permeability in BD cross calves compared with native beef and Holstein calves to better understand the differences in nutrient utilization and the mechanisms contributing to the greater incidence of LA reported in BD animals.

CONCLUSION

Results from the current study indicate that starch inclusion in calf starter had limited effects on growth performance, GI permeability, ruminal pH, and GI morphological development in BD cross calves. Although calves receiving the low starch starter exhibited greater body weight at weaning and average daily gain during the hutch phase, these differences were not maintained following transition to common grower and finishing diets. Likewise, early life starch concentration had minimal effects on GI permeability, GI tract weights, luminal pH, and nutrient concentrations throughout the GI tract. Subtle differences in ruminal morphology observed dur-

ing the finishing phase suggest that early dietary starch exposure may influence ruminal epithelial development; however, these structural differences were not accompanied by corresponding differences in GI function or animal performance. Although formal statistical analyses were not performed for the continuous ruminal pH data, dynamic, descriptive observations indicated substantial individual variation in ruminal dynamics that corresponded to differences in rumen histologic measurements and highlighted the value of continuous monitoring for evaluating responses to dietary changes. Collectively, these findings suggest that calf starter starch concentration alone is unlikely to be a primary driver of LA in BD cattle. Rather, LA pathogenesis is likely multifactorial, and additional research is warranted to further define the roles of early life nutrition, ruminal fermentation, GI barrier function, and animal specific factors in the development of liver abscesses.

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Tables

Table 3.1 Nutrient and ingredient composition of calf starter treatment diets fed to beef x dairy steers during the hutch phase

	Treatment	
Ingredient, % DM	**High starch**	**Low starch**
Oat grain fine ground	17.83	17.83
Corn grain cracked	19.7	19.7
Sugarcane molasses	4.62	4.62
Soybean meal solv.	19.4	14.29
Soy best with gums	0.67	3.38
Blood meal	3.17	3.17
Corn grain fine	31.56	1.51
Beet pulp shreds	0	10.98
Soybean hulls ground	0	11.02
Fiber plus	0	1.68
Soft wheat middlings	0	8.76
Youngstock VTM	0.17	0.17
Vit/ Min Mix[1]	2.86	2.86
Decoxx[2]	0.02	0.02
Nutrient	**High starch**	**Low starch**
DM, % as-fed	93.1	93.3
Starch, % DM	45.3	19.5
Crude protein, % DM	22.9	23.6
aNDF, % DM	10.7	25.5
ME, Mcal/kg DM[3]	3.04	2.76
NEm, Mcal/kg DM	2.09	1.09
NEg, Mcal/kg DM	1.43	1.26

[1] Youngstock VTM mineral concentrations: Mn - 36,520 ppm, Cu - 9,360 ppm, Fe - 5,200 ppm, Zn - 52,000 ppm, I - 936 ppm, Co - 312 ppm, Se - 312 ppm, Vit. A - 2,500,000 IU/lb, Vit. D3 - 600,000 IU/lb, Vit. E50 - 500,000 IU/kg
[2]Active ingredient is Decoquinate at 27.2 g/lb
[3]ME, NEm, NEg calculated from diet formulated values

Table 3.2 Nutrient and ingredient composition for grower rations

	Grower ration	
Ingredient, % DM	**Grower 1**	**Grower 2**
Sweet bran	39.5	0
Dry rolled corn	40.6	39.6
Prairie hay	12.6	13.1
KSU supplement[1, 2]	7.1	7.1
Water	0	15.0
Dried distiller grains	0	40.2
Nutrient		
% as-fed		
CP, % DM	14.7	17.6
Starch, % DM	31.1	30.9
aNDF, % DM	28.6	28.3
ME, Mcal/kg DM[2]	2.84	2.83
NEm, Mcal/kg DM[2]	1.92	1.91
NEg, Mcal/kg DM[2]	1.30	1.30

[1] KSU supplement ingredient formulation (%DM): Wheat Middlings - 58.6, Limestone - 34.9, Salt - 5.1, Soy oil - 0.8, Rumensin 90 - 0.2, Zinc sulfate - 0.1, Vit A 30,000 IU/g - 0.09, Manganese sulfate - 0.08, Vit E 50% - 0.06, Copper sulfate 25% - 0.05, Selenium 0.4% - 0.02, EDDI 9.2% - 0.008

[2]KSU supplement mineral concentrations: Co - 0.328 ppm, Cu -133.5 ppm, I - 6.12 ppm, Fe - 1306 ppm, Mn - 316 ppm, Se - 1.06 ppm, Zn - 442 ppm, Vit. A - 29.4 IU/g, Vit. D - 0, Vit. E - 16.5 IU/g

[3] ME, NEm, NEg, calculated from NRC values

Table 3.3 Nutrient and ingredient formulations for finishing rations

		Finishing Transition Ration		
Ingredient, % as-fed	**Starter**	**Step-up 1**	**Step-up 2**	**Finishing**
HMC	39.2	51.4	55.6	58.9
DDGS	27.7	19.5	11.1	54.7
Corn silage	21.1	21.3	27.5	31.4
Alfalfa hay	6.92	5.02	3.09	1.63
Urea	0	0.179	0.339	0.457
Limestone	0	0.642	0.589	0.529
Mineral[1]	0	0.756	0.692	0.622
KSU supplement[2]	5.06	0	0	0
Purina Supermix	0	1.19	1.09	0.977
Nutrient				
DM, % as fed	72.1	69.3	70.9	69.6
CP, % DM	18.8	16.9	15.8	14.2
Starch, % DM	33.4	46.7	48.4	56.4
aNDF, % DM	24.0	20.2	17.5	14.4
ME, Mcal/kg DM	3.02	2.95	2.95	2.95
NEm, Mcal/kg DM	2.02	2.01	2.01	2.00
Neg, Mcal/kg DM	1.37	1.37	1.37	1.37

[1] MKC optimal beef balancer 4% concentrations: Ca - 18-20%, P - 4%, Salt - 20-22%, K - 0.3%, Mg - 2%, S - 5%, Zn - 7,000 ppm, Cu - 2,000 ppm, Mn - 2,800 ppm, I - 70 ppm, Se - 25 ppm, Vit. A - 200,000 IU/lb, Vit. D - 50,000 IU/lb, Vit. E - 250 IU/lb, Co - 95 ppm
[2] See table footnotes in table 3.2 for information on KSU supplement

Table 3.4 Fecal scoring system used during the hutch phase to score feces twice daily

Description of the scoring system used to score feces twice a day (Monday-Friday) for each animal during the hutch phase. Images represent example feces in each category.

Fecal score	Description	Example
0	Normal, firm but not hard, piles easily	
1	Semi formed, pasty, does not hold form, piles but easily spreads	
2	Runny, spreads easily, loose but stays on top of bedding/ grates	
3	Watery, foul smelling, completely soaks into bedding, translucent or blood present	

Table 3.5 Description and definitions of histologic measurements collected from the rumen, small intestine, and spiral colon of beef × dairy calves

Description of histologic measurement collected from the rumen, duodenum, jejunum, ileum and spiral colon using Aperio eSlide Manager software.

Measurement Name (units)	Measurement Definition
Rumen [1]	
Papilla Length (µm)	Papilla tip to papilla base
Papilla Width (µm)	Measured at mid-papilla height
Papilla Area (µm^2)	Papilla length x papilla width
Papilla Keratin Thickness (µm)	Measured from mucosal surface of papilla to where stratum granulosa began or where nucleated stratum corneum cells ended
Stratum Corneum (µm)	Measured from epithelial surface to stratum granulosum
Stratum Granulosum (µm)	Measured from stratum granulosum to stratum spinosum
Stratum Spinosum (µm)	Measured from stratum spinosum to stratum basale
Stratum Basale (µm)	Measured from stratum spinosum to muscularis mucosa
Small Intestine (Duodenum, Jejunum, Ileum)	
Villus Length (µm)	Villus tip to villus base
Villus Width (µm)	Measured at mid-villus height
Crypt Depth (µm)	Measured from villus base to base of deepest crypt
Crypt Width (µm)	Measured across crypt at most even plane of section
Total Propria Thickness (µm)	Measured from villus base interface to muscularis mucosa
Spiral Colon	
Gland Depth (µm)	Measured from epithelium to base of deepest gland present
Gland Width (µm)	Measured across gland as most even plane of section
Total Propria Thickness (µm)	Measured from epithelium surface to muscularis mucosa

[1] All rumen epithelial measurements made on rumen epithelium not on papilla

Table 3.6 Table 3.6. Hutch phase growth performance of beef × dairy calves fed low- or high-starch starter diets during the pre-weaning and post-weaning periods. Hutch phase performance estimated marginal means (emmeans) for both pre-weaning and post weaning phases and model results

Variable	Low starch	High starch	SEM	P-value
Preweaning Phase				
Initial BW, kg	41	39	2.4	0.31
Weaning BW, kg	83	77	2.5	0.15
ADG, kg/day	0.779	0.704	0.025	0.03
DMI, kg				
MR[1] DMI	0.69	0.68	0.00032	0.50
Grain DMI D40[2]	0.95	0.84	0.062	0.20
Grain DMI D47[2]	1.5	1.3	0.063	0.021
Grain DMI D54[3]	2.1	1.8	0.068	0.0014
Total DMI [4]	1.2	1.2	0.062	0.44
G:F, kg/kg[5]				
Grain G:F	1.4	1.6	0.084	0.24
Total G:F	0.61	0.59	0.221	0.51
Postweaning Phase				
Initial BW, kg	83	77	2.5	0.15
Final BW, kg	117	111	6.1	0.55
ADG, kg/day	1.3	1.3	0.14	0.95
DMI, kg	3.3	2.9	0.39	0.47
G:F, kg/kg	0.35	0.42	0.035	0.23

[1]MR is milk replacer

[2]Day 40 and 47 are days when milk replacer was decreased

[3]Day 54 is when calves were completely weaned.

[4] Total DMI includes milk DMI calculated with milk replacer and grain averaged over the pre-weaning period

[5]Values used for G:F and ADG are fitted to a regression line, not calculated from raw data

Table 3.7 The effect of diet on the average number of fecal events a calf experienced in the pre-weaning and post-weaning period of the hutch phase.

Period	Low Starch	High Starch	SEM	P-value
Pre-weaning[1]	0.7	1.7	0.57	0.23
Post-weaning	1.8	2.0	0.88	0.88

[1]Only for a portion of the pre-weaning period

Table 3.8 Effects of dietary starch level and gastrointestinal tract section and their interaction on digesta pH and nutrient composition in the hutch phase

	Treatment				Section[1,2]							Trt X Section
Variable	**LS**	**HS**	**SEM[3]**	**P-value**	**Ru**	**Duo**	**Jej**	**Ile**	**Col**	**SEM**	**P-value**	**P-value**
pH[4]	5.49	5.16	3.77e0-6	0.69	5.75[b]	4.69[ab]	6.11[ab]	6.86[a]	5.67[ab]	1.33e-05	<0.01	0.44
Contents												
Starch %DM	9.9	11.5	3.9	0.75	13.8	7.9	6.1	11.6	13.9	4.9	0.25	0.44
NDF %DM	30.7	21.1	2.3	<0.01	43.5[a]	-	8.95[c]	19.0[bc]	32.2[ab]	4.8	<0.01	0.05
CP %DM	28.8	31.1	1.8	0.36	23.6[a]	-	43.9[c]	31.1[b]	21.2[a]	1.8	<0.01	<0.01

[1] LS= Low starch, HS= High starch, Ru= Rumen, Duo= Duodenum, Jej= Jejunum, Ile= Ileum, Col= Colon, SEM= Standard Error of the Mean

[2] The number of samples analyzed for each nutrient by gastrointestinal tract section was as follows: starch—Ru (n = 10), Duo (n = 8), Jej (n = 10), Ile (n = 10), and Col (n = 10); NDF—Ru (n = 9), Duo (n = 0), Jej (n = 4), Ile (n = 4), and Col (n = 7); and CP—Ru (n = 9), Duo (n = 0), Jej (n = 4), Ile (n = 4), and Col (n = 8).

[3] SEM for pH is expressed as hydrogen concentration as SEM was not able to be back transformed as pH is not linear.

[4] pH values were log transformed, statistical analysis was done on transformed values, and then those values were back transformed for presentation

Table 3.9 Effects of dietary starch level in calf starter and gastrointestinal tract section on gastrointestinal tract weight during the hutch phase.

Treatment					Section[1]							Trt X Section
Variable	**LS**	**HS**	**SEM**	**P-value**	**RR**	**Om**	**Ab**	**SI**	**LI**	**SEM**	**P-value**	**P-value**
Weight (kg)	1.10	1.31	0.091	0.43	1.68^{b}	0.376^{d}	0.565^{c}	2.46^{a}	0.954^{e}	0.186	<0.01	0.443
g/kg BW	131	140	6.48	0.290	190^{b}	41.0^{e}	6.18^{d}	274^{a}	113^{c}	11.5	<0.01	0.746

[1] RR= Reticulorumen, Om= Omasum, Ab= Abomasum, SI= Small Intestine, LI= Large Intestine, SEM= Standard Error of the Mean

Table 3.10 Pearson correlation coefficients of histologic measurements in the rumen with each of the top 5 principal components in hutch phase

Item	PC1[1]	PC2	PC3	PC4	PC5
Rumen					
Papilla Length	-0.83	0.37	-0.09	0.20	-0.32
Papilla Width	-0.29	-0.88	0.18	0.28	-0.04
Papilla Area	-0.93	0.13	-0.12	0.25	0.19
Papilla Keratin Thickness	-0.19	0.86	0.36	-0.07	-0.25
Stratum Corneum	-0.62	0.19	-0.48	-0.45	-0.25
Stratum Granulosum	-0.58	-0.20	0.29	-0.64	0.23
Stratum Spinosum	-0.36	-0.17	0.85	0.03	-0.11
Stratum Basale	0.85	0.04	0.14	-0.20	0.39

[1] PC is principal component

Table 3.11 Pearson correlation coefficients of histologic measurements in the small intestine with each of the top 4 principal components in hutch phase

Item	PC1[1.]	PC2	PC3	PC4
Duodenum				
Villus Length	-0.79	0.47	0.14	0.22
Villus Width	0.16	0.74	-0.31	-0.55
Crypt Depth	-0.83	-0.32	-0.19	-0.34
Crypt Width	-0.06	-0.65	-0.72	0.14
Total Propria Thickness	-0.98	0.14	-0.01	0.04
Goblet Cell Area	-0.10	-0.76	0.47	-0.36
Jejunum				
Villus Length	0.53	-0.77	0.00	0.01
Villus Width	-0.40	-0.74	-0.28	0.37
Crypt Depth	0.95	0.10	-0.07	-0.18
Crypt Width	0.66	0.11	0.54	0.50
Total Propria Thickness	0.89	-0.35	-0.12	-0.21
Goblet Cell Area	0.50	0.50	-0.60	0.34
Ileum				
Villus Length	0.69	-0.25	0.11	0.65
Villus Width	-0.15	-0.92	-0.15	-0.28
Crypt Depth	0.91	0.16	-0.05	-0.33
Crypt Width	0.84	-0.38	-0.05	0.02
Total Propria Thickness	0.93	0.22	-0.01	-0.22
Goblet Cell Area	-0.01	-0.12	0.98	-0.13

[1] PC is principal component

Table 3.12 Pearson correlation coefficients of histologic measurements in the spiral colon with each of the top 3 principal components in hutch phase

Item	PC1[1.]	PC2	PC3
Spiral Colon			
Gland Depth	0.97	-0.03	-0.07
Gland Width	0.67	0.63	0.39
Total Propria Thickness	0.90	-0.27	-0.27
Goblet Cell Area	-0.16	0.93	-0.35

[1] PC is principal component

Table 3.13 Multivariate analysis of variance of principal components of histologic measurements to evaluate overall effect of a high starch or low starch diet on gastrointestinal morphology

Tissue	Wilks lambda	F-value	P-value
Rumen	0.66	0.66	0.65
Duodenum	0.42	1.80	0.27
Jejunum	0.82	0.27	0.088
Ileum	0.78	0.29	0.87
Colon	0.76	0.65	0.61

Table 3.14 The effect of dietary starch level in calf starter on histologic measurements in the rumen in the hutch phase

Measurement (units)	Low Starch	High Starch	SEM	P-value
Rumen				
Papilla Length (µm)	1370	1400	271	0.94
Papilla Width (µm)	327	378	229	0.16
Papilla Area (mm^2)	0.499	0.521	0.115	0.89
Papilla Keratin Thickness (µm)	12.6	8.4	1.58	0.13
Stratum Corneum (µm)	13.7	12.6	9.04	0.41
Stratum Granulosum (µm)	14.7	14.6	8.35	0.97
Stratum Spinosum (µm)	25.2	25	2.76	0.95
Stratum Basale (µm)	33.1	33.6	3.22	0.92

Table 3.15 The effect of dietary starch level in calf starter on histologic measurement in the small intestine in the hutch phase

Measurement (units)	Low Starch	High Starch	SEM	P-value
Duodenum				
Villus Length (µm)	264	358	15.9	0.0031
Villus Width (µm)	111	115	10.8	0.81
Crypt Depth (µm)	558	596	56.9	0.65
Crypt Width (µm)	67.4	63.5	3.56	0.47
Total Propria Thickness (µm)	735	937	56.1	0.035
Goblet cell area (%)	3.90	3.77	0.85	0.918
Jejunum				
Villus Length (µm)	310	346	47.5	0.608
Villus Width (µm)	131	109	10.9	0.201
Crypt Depth (µm)	615	621	89.0	0.966
Crypt Width (µm)	64.5	61.9	3.86	0.655
Goblet cell area (%)	2.05	2.15	0.43	0.163
Total Propria Thickness (µm)	908	926	109	0.906
Ileum				
Villus Length (µm)	311	287	20.1	0.400
Villus Width (µm)	126	144	12.1	0.366
Crypt Depth (µm)	518	487	76.4	0.797
Crypt Width (µm)	69.5	63.5	6.03	0.482
Goblet cell area (%)	10.8	4.24	4.64	0.350
Total Propria Thickness (µm)	807	721	94.6	0.521

Table 3.16 The effects of dietary starch level in calf starter on histologic measurements in the spiral colon in the hutch phase

Measurement (units)	Low starch	High Starch	SEM	P-value
Spiral Colon				
Gland Depth (µm)	416	467	33.4	0.31
Gland Width (µm)	66.1	68.1	5.37	0.804
Total Propria Thickness (µm)	510	554	30.4	0.337
Goblet Cell Area %	23.9	17.9	3.62	0.275

Table 3.17 The effect of dietary starch level in calf starter on serum-sugar concentration in the hutch phase

	Treatment				Day					Trt X D
Variable	**LS**	**HS**	**SEM**	**P-value**	**51**	**57**	**61**	**SEM**	**P-value**	**P-value**
Serum-sucralose conc µg/ml	7.90	6.78	4.93	0.61	5.36	6.94	9.73	5.98	0.20	0.85

Table 3.18 Descriptives of rumen pH for calves with pH boluses in the hutch phase, where pH was monitored every 2 minutes for 11 days

	Low Starch		High starch	
Variable	ID:7	ID:8	ID:9	ID:12
Mean	6.14	6.17	6.20	5.44
Median	6.14	6.19	6.20	5.41
Min	5.21	5.03	5.34	4.54
Max	6.81	6.92	6.96	6.59
SD	0.243	0.303	0.270	0.377

Table 3.19 Arithmetic means for growth performance of calves fed low or high-starch calf starters during the growing phase

Variable	Low Starch	High Starch
BW, kg	436	378
ADG, kg/day	3.06	3.02
DMI, kg/day	12.7	13.1
G:F kg/kg	0.238	0.233

Table 3.20 The effect of dietary starch level in calf starter on growth performance and feed intake in the finishing phase

Variable	Low Starch	High Starch	SEM	P-value
BW, kg				
Starter diet	278	281	16.3	0.88
Step-up 1	310	318	16.3	0.73
Step-up 2	321	331	16.3	0.681
Finishing	325	335	16.3	0.666
ADG, kg/d	1.92	1.68	0.11	0.131
DMI, kg/d				
Starter Diet	7.27	7.28	0.403	0.975
Step-up 1	7.56	7.33	0.403	0.690
Step-up 2	5.25	5.86	0.403	0.296
Finishing	7.44	7.36	0.403	0.891
G:F, kg/kg	0.286	0.247	0.0183	0.171

Table 3.21 Main effects of gastrointestinal section and treatment on digesta pH and nutrient composition at harvest during the finishing phase

Treatment					Section[1, 2]							Trt x Section
Variable	**LS**	**HS**	**SEM[3]**	**P-value**	**Ru**	**Duo**	**Jej**	**Ile**	**Col**	**SEM**	**P-value**	**P-value**
pH[4]	5.90	5.87	1.35e-06	0.57	6.37^{ab}	5.41^{c}	5.75^{bc}	7.04^{a}	6.43^{b}	9.22e-07	<0.01	0.83
Contents												
Starch, %DM	7.73	7.11	1.21	0.71	4.84^{b}	5.04^{b}	5.38^{b}	10.4^{ab}	14.2^{a}	2.66	<0.01	0.52
NDF, %DM	17.3	22.2	2.15	0.04	47.6^{ab}	8.40^{c}	6.43^{bc}	32.9^{a}	33.5^{a}	10.0	<0.01	0.01
CP, %DM	23.9	27.3	0.59	<0.01	19.4^{ab}	38.3^{c}	45.5^{bc}	16.6^{a}	16.7^{a}	4.10	<0.01	0.02

[1] LS= Low starch, HS= High starch, Ru= Rumen, Duo= Duodenum, Jej= Jejunum, Ile= Ileum, Col= Colon, SEM= Standard Error of the Mean

[2] The number of samples analyzed for each nutrient by gastrointestinal tract section was as follows: starch—Ru (n = 9), Duo (n = 8), Jej (n = 10), Ile (n = 10), and Col (n = 10); NDF—Ru (n = 9), Duo (n = 17), Jej (n = 9), Ile (n = 7), and Col (n = 10); and CP—Ru (n = 9), Duo (n = 7), Jej (n = 9), Ile (n = 7), and Col (n = 10).

[3] SEM for pH is expressed as hydrogen concentration as SEM was not able to be back transformed

[4] pH values were log transformed, statistical analysis was done on transformed values, and then those values were back transformed for presentation

Table 3.22 Pearson correlation coefficients of histological measurements in the rumen with each of the top 5 principal components in the finishing phase

Variable	PC1[1]	PC2	PC3	PC4	PC5
Rumen					
Papilla Length	0.76	-0.36	-0.25	0.44	-0.12
Papilla Width	-0.53	0.76	0.05	-0.09	0.19
Papilla Area	0.49	-0.75	-0.29	0.31	0.02
Papilla Keratin Thickness	0.84	-0.02	0.40	-0.14	-0.18
Stratum Corneum	-0.74	0.10	0.09	0.43	-0.48
Stratum Granulosum	-0.80	-0.34	0.01	0.26	0.09
Stratum Spinosum	-0.16	-0.60	0.20	-0.67	-0.33
Stratum Basale	0.08	-0.46	0.86	0.09	0.19

[1] PC is principal component

Table 3.23 Pearson correlation coefficients of histological measurements in the duodenum, jejunum, and ileum with each of the top 4 principal components in the finishing phase

Variable	PC1[1]	PC2	PC3	PC4
Duodenum				
Villus Length	-0.79	0.47	0.14	0.22
Villus Width	0.16	0.74	-0.31	-0.55
Crypt Depth	-0.83	-0.32	-0.19	-0.34
Crypt Width	-0.06	-0.65	-0.72	0.14
Total Propria Thickness	-0.98	0.14	-0.01	0.04
Goblet cell area	-0.10	-0.76	0.47	-0.36
Jejunum				
Villus Length	0.53	-0.77	0.00	0.01
Villus Width	-0.40	-0.74	-0.28	0.37
Crypt Depth	0.95	0.10	-0.07	-0.18
Crypt Width	0.66	0.11	0.54	0.50
Total Propria Thickness	0.89	-0.35	-0.12	-0.21
Goblet cell area	0.50	0.50	-0.60	0.34
Ileum				
Villus Length	0.69	-0.25	0.11	0.65
Villus Width	-0.15	-0.92	-0.15	-0.28
Crypt Depth	0.91	0.16	-0.05	-0.33
Crypt Width	0.84	-0.38	-0.05	0.02
Total Propria Thickness	0.93	0.22	-0.01	-0.22
Goblet cell area	-0.01	-0.12	0.98	-0.13

[1]PC is principal component

Table 3.24 Pearson correlation coefficients of histologic measurements in the spiral colon with each of the top 3 principal components in the finishing phase

Variable	PC1[1]	PC2	PC3
Spiral Colon			
Gland Depth	0.97	-0.03	-0.07
Gland Width	0.67	0.63	0.39
Total Propria Thickness	0.90	-0.27	-0.27
Goblet Cell Area	-0.16	0.93	-0.35

[1]PC is principal component

Table 3.25 Multivariate analysis results of principal component of histologic measurements to evaluate overall effect of a high starch or a low starch diet on gastrointestinal morphology at the end of finishing transition

Tissue	Wilks lambda	F-value	P-value
Rumen	0.656	0.656	0.03
Duodenum	0.415	1.76	0.274
Jejunum	0.820	0.274	0.883
Ileum	0.775	0.289	0.871
Colon	0.755	0.648	0.613

Table 3.26 The effect of dietary starch level in calf starter on histologic measurements in the rumen in the finishing phase

Measurement (units)	Low Starch	High Starch	SEM	P-value
Rumen				
Papilla Length (µm)	3340	3720	1090	0.732
Papilla Width (µm)	359	468	47.1	0.0488
Papilla Area (mm^2)	1.050	1.720	0.394	0.129
Papilla Keratin Thickness (µm)	7.68	9.7	2.00	0.330
Stratum Corneum (µm)	13.7	12.2	1.14	0.209
Stratum Granulosum (µm)	13.6	13.8	1.50	0.897
Stratum Spinosum (µm)	23.1	27.2	2.50	0.144
Stratum Basale (µm)	39.4	45.1	2.90	0.0843

Table 3.27 The effect of dietary starch level in calf starter on histologic measurements in the small intestine in the finishing phase

Measurement (units)	Low Starch	High Starch	SEM	P-value
Duodenum				
Villus Length (µm)	425	418	52.6	0.90
Villus Width (µm)	184	181	13.5	0.817
Crypt Depth (µm)	629	611	57.4	0.755
Crypt Width (µm)	70.1	64.3	5.24	0.300
Total Propria Thickness (µm)	1110	1130	66.7	0.846
Goblet cell area (%)	3.88	4.71	0.764	0.306
Jejunum				
Villus Length (µm)	485	456	28.3	0.480
Villus Width (µm)	171	196	12.4	0.188
Crypt Depth (µm)	587	583	32.2	0.930
Crypt Width (µm)	69.7	69.4	1.61	0.951
Goblet cell area (%)	1.78	3.33	0.59	0.0999
Total Propria Thickness (µm)	1080	1060	51.9	0.807
Ileum				
Villus Length (µm)	390	400	44.9	0.161
Villus Width (µm)	164	163	9.73	0.976
Crypt Depth (µm)	522	521	43.9	0.991
Crypt Width (µm)	67.0	66.4	2.76	0.888
Goblet cell area (%)	6.59	7.64	0.99	0.477
Total Propria Thickness (µm)	914	922	84.6	0.952

Table 3.28 The effect of dietary starch level in calf starter on histologic measurements in the spiral colon in the finishing phase

Measurement (units)	Low Starch	High Starch	SEM	P-value
Spiral Colon				
Gland Depth (µm)	490	548	30.9	0.219
Gland Width (µm)	59.3	58.7	3.68	0.916
Total Propria Thickness (µm)	584	666	30.9	0.0971
Goblet Cell Area %	14.0	15.1	1.78	0.665

Table 3.29 The effect of dietary starch level in calf starter on serum sugar concentration in the finishing phase

	Treatment				Day					Trt X D
Variable	**LS**	**HS**	**SEM**	**P-value**	**12**	**19**	**26**	**SEM**	**P-value**	**P-value**
Serum- sucralose conc µg/ml	4.60	4.52	0.27	0.82	4.64	4.96	4.08	0.28	0.05	0.87

Figures

Figure 3.1 The effect of the dietary starch x day interaction on body weight gain in the hutch phase.

The effect of a low starch (LS) or high starch (HS) diet on average daily gain in the pre-weaning hutch phase, where the high low starch calves had significantly higher average daily gain (P=0.03). Average daily gain is represented by the slope of the BW regression model for each treatment

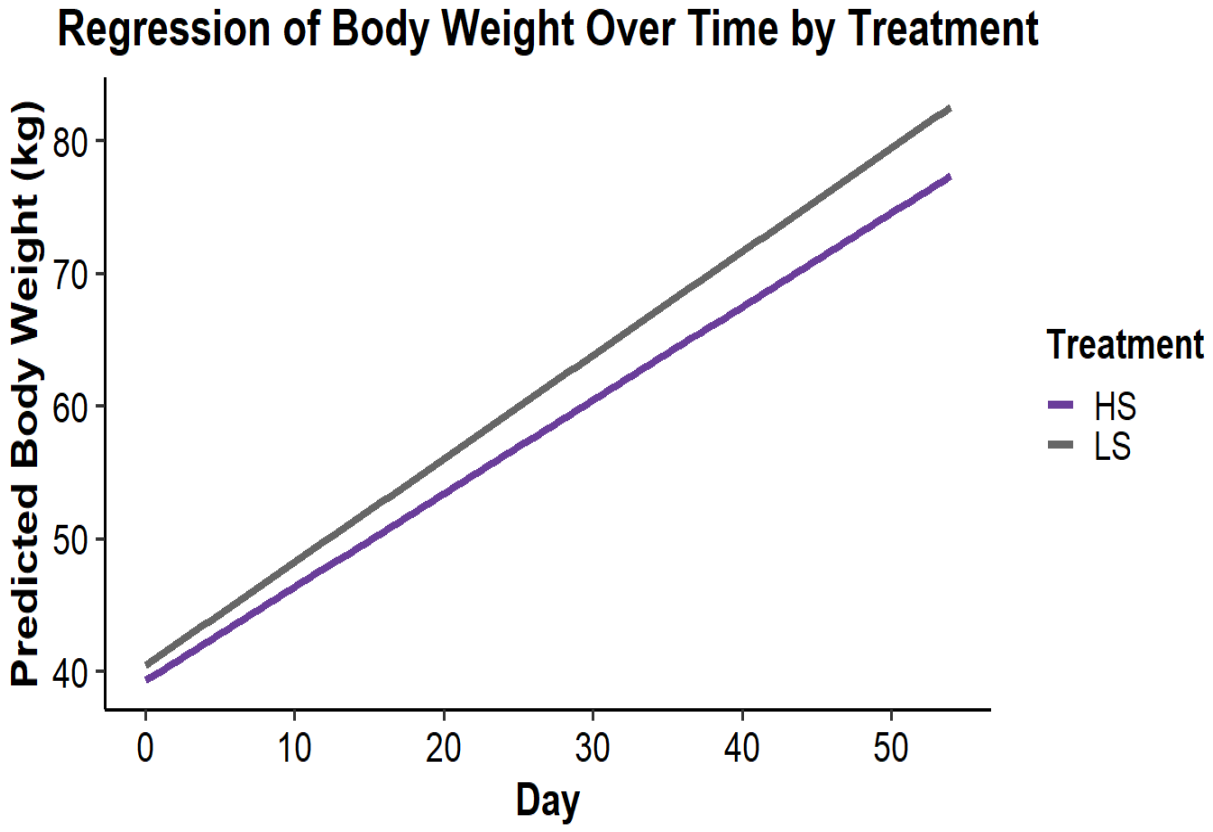


Figure 3.2 The effect of dietary starch x gastrointestinal section interaction on NDF and CP nutrient concentration of digesta in the hutch phase

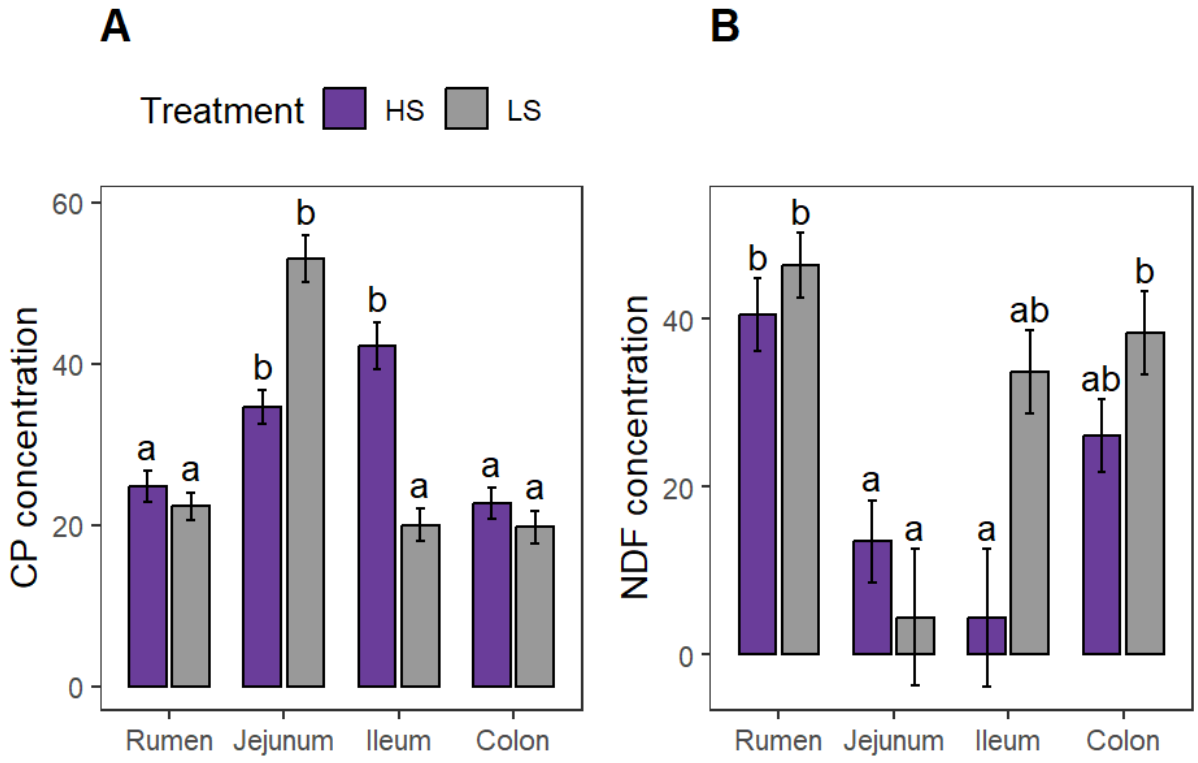


Figure 3.3 Average hourly rumen pH for each of the calves with a ruminal pH bolus fed low or high starch calf starter during the weaning transition.

Hourly pH averages during the time calves were monitored by rumen pH boluses for each calf with a bolus.

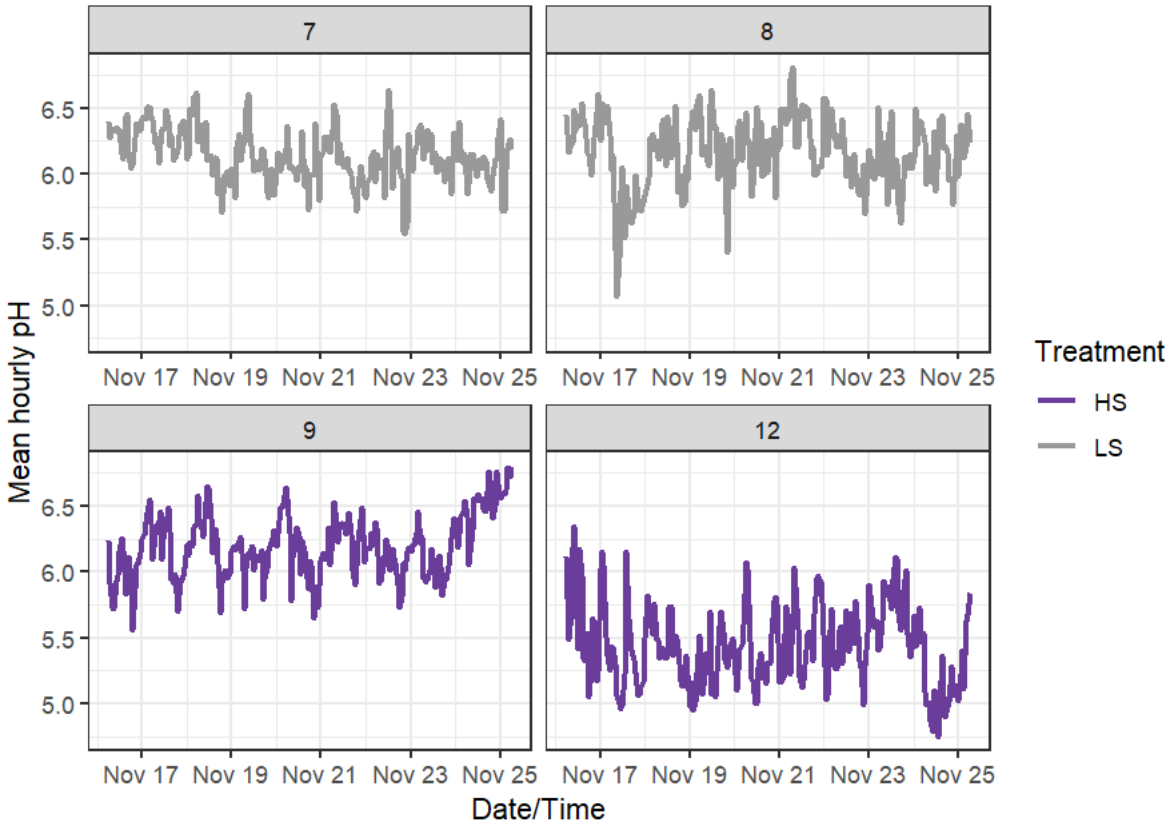


Figure 3.4 The average starch intake per day and average rumen pH per day for each calf with a rumen pH bolus fed low (7 and 8) or high (9 and 12) starch calf starter during weaning transition.

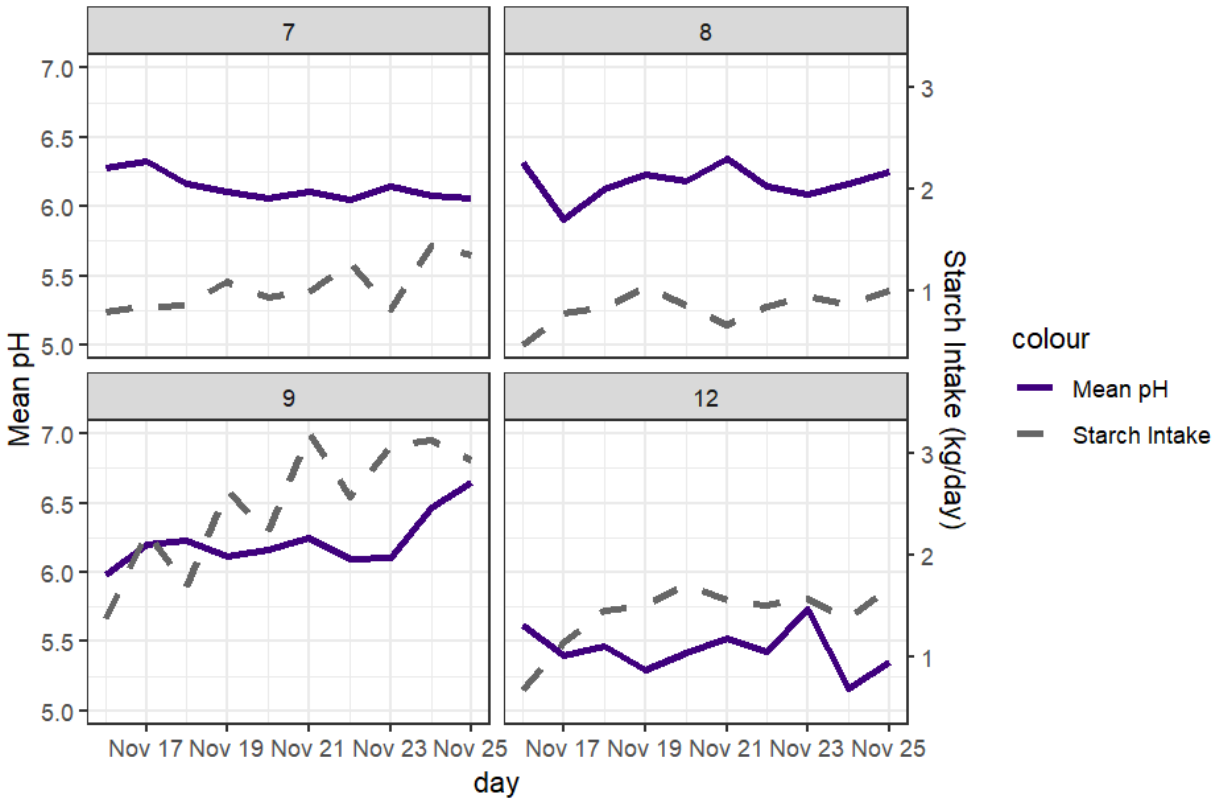


Figure 3.5 The total minutes per day rumen pH was below the threshold for subacute rumen acidosis (pH≤5.6) each day for each calf with a rumen pH bolus fed low (7 and 8) or high (9 and 12) starch calf starter.

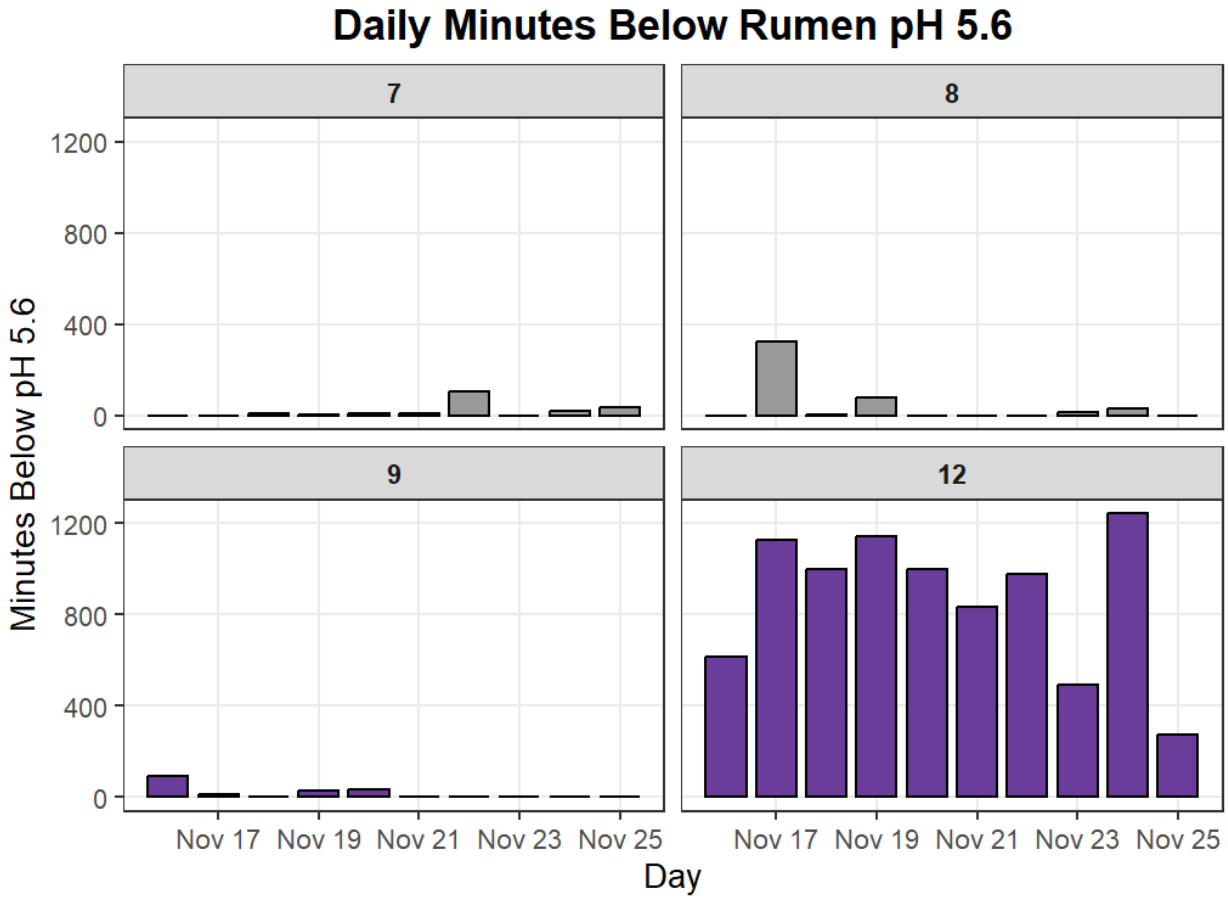


Figure 3.6 Average rumen papilla histological measurements at weaning for each calf with a rumen pH bolus fed the low and high starch calf starter

Histological measurements for rumen papilla length, papilla width, papilla area, and keratin thickness for each calf that were monitored by rumen pH boluses during the hutch phase.

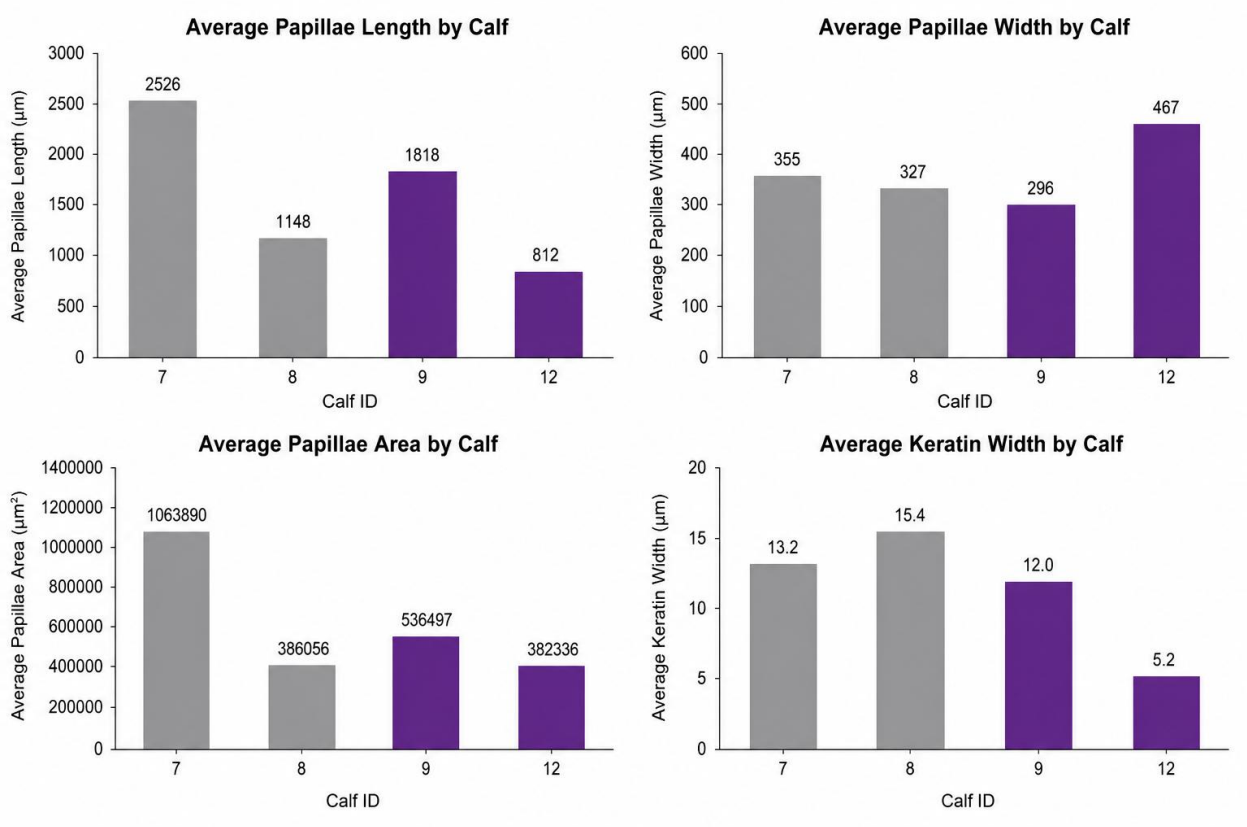


Figure 3.7 Snapshots of rumen histology slides showing papilla structure for each of the four calves with rumen boluses.

Calves 7 and 8 are received the low starch diet and calves 9 and 12 received the high starch diet.

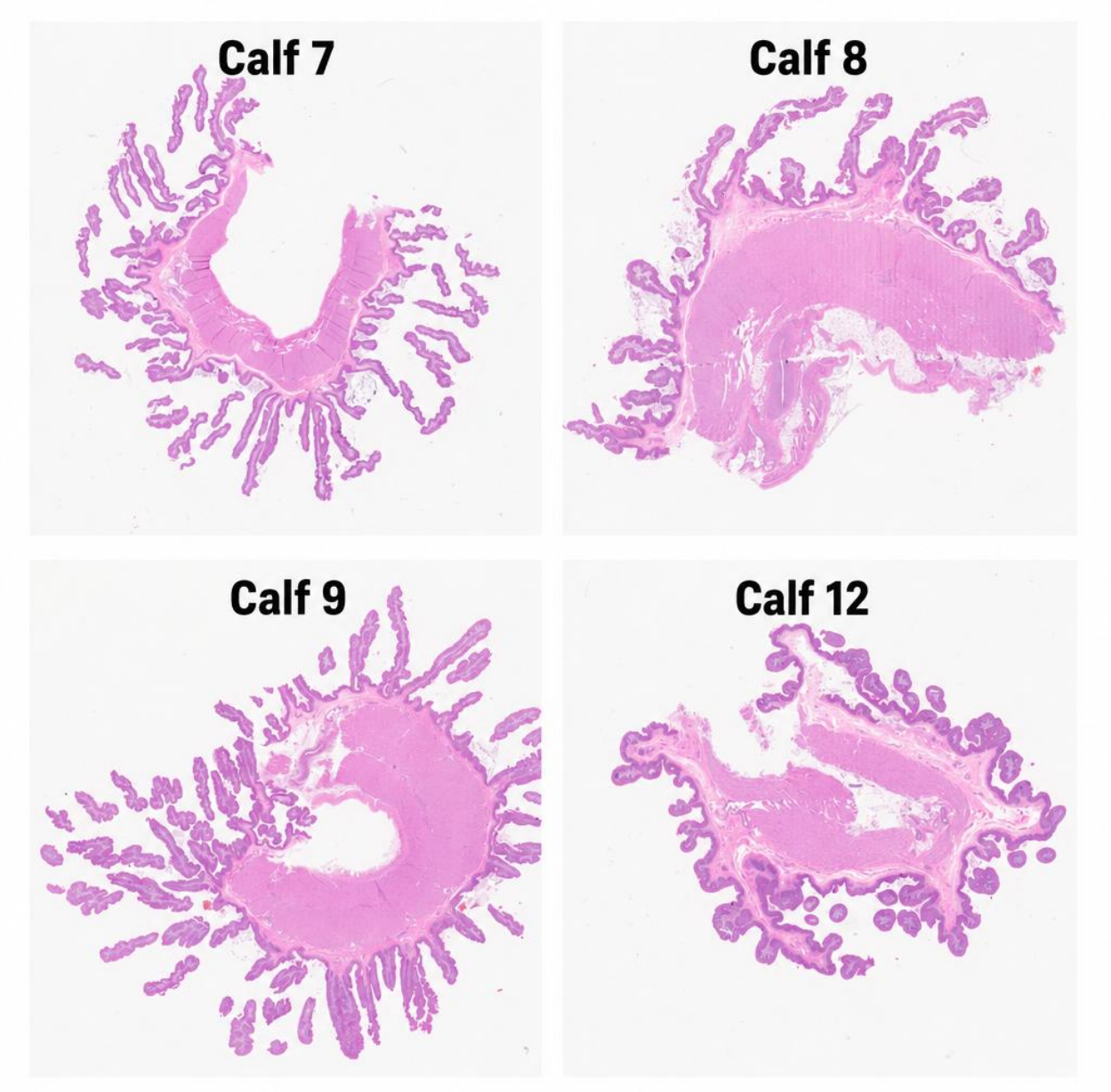


Figure 3.8 Serum sucralose concentration on each sampling day during the hutch phase for each calf with a rumen pH bolus fed a low or high starch calf starter during weaning transition.

Weaning occurred on day 54 of the trial.

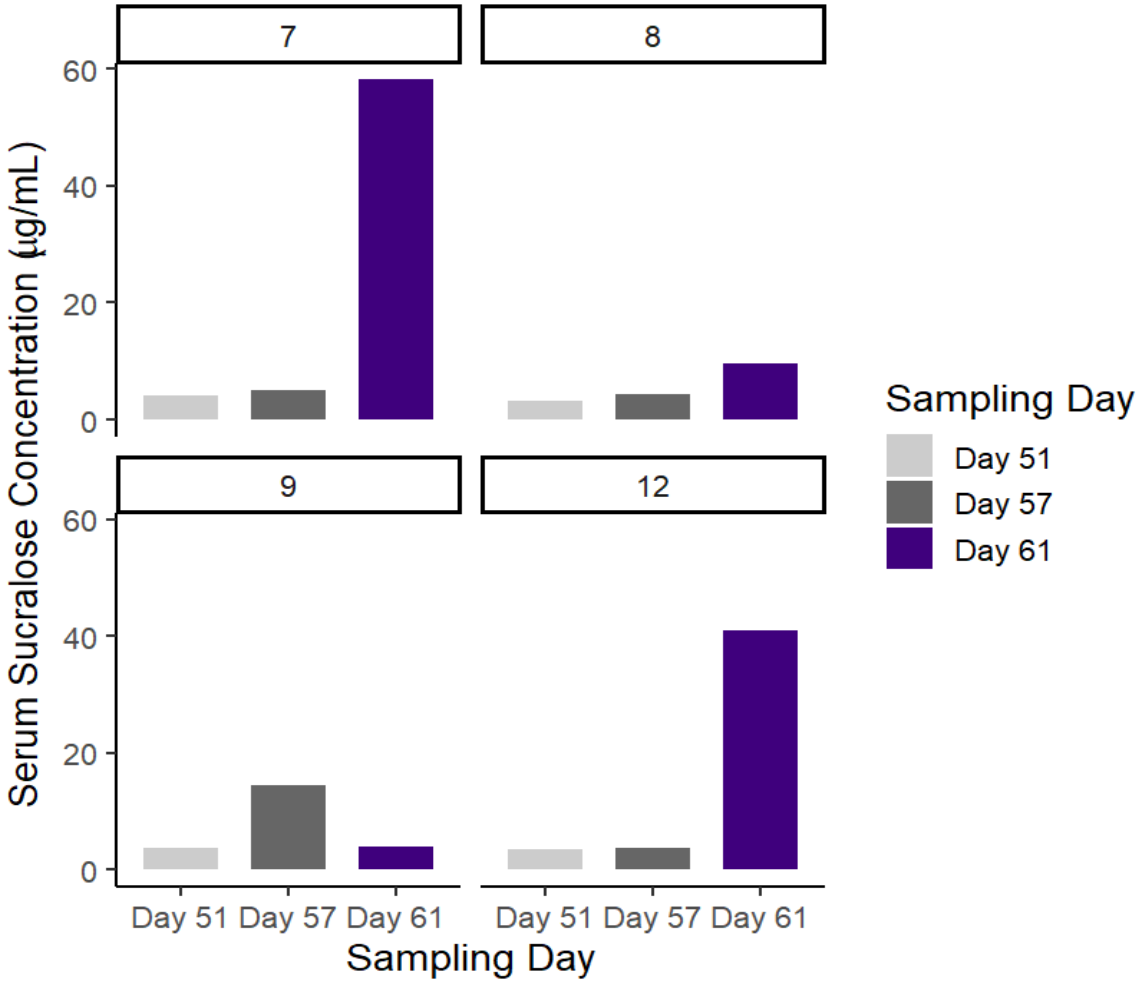


Figure 3.9 The differences in dry matter intake in the finishing phase for each of the finishing rations.

The effect of finishing ration on dry matter intake during the finishing transition. Step-up 2 had lesser DMI (P<0.001). S = Starter diet; SU1 = step-up 1 transition diet; SU2 = step-up 2 transition diet; F = final finishing diet.

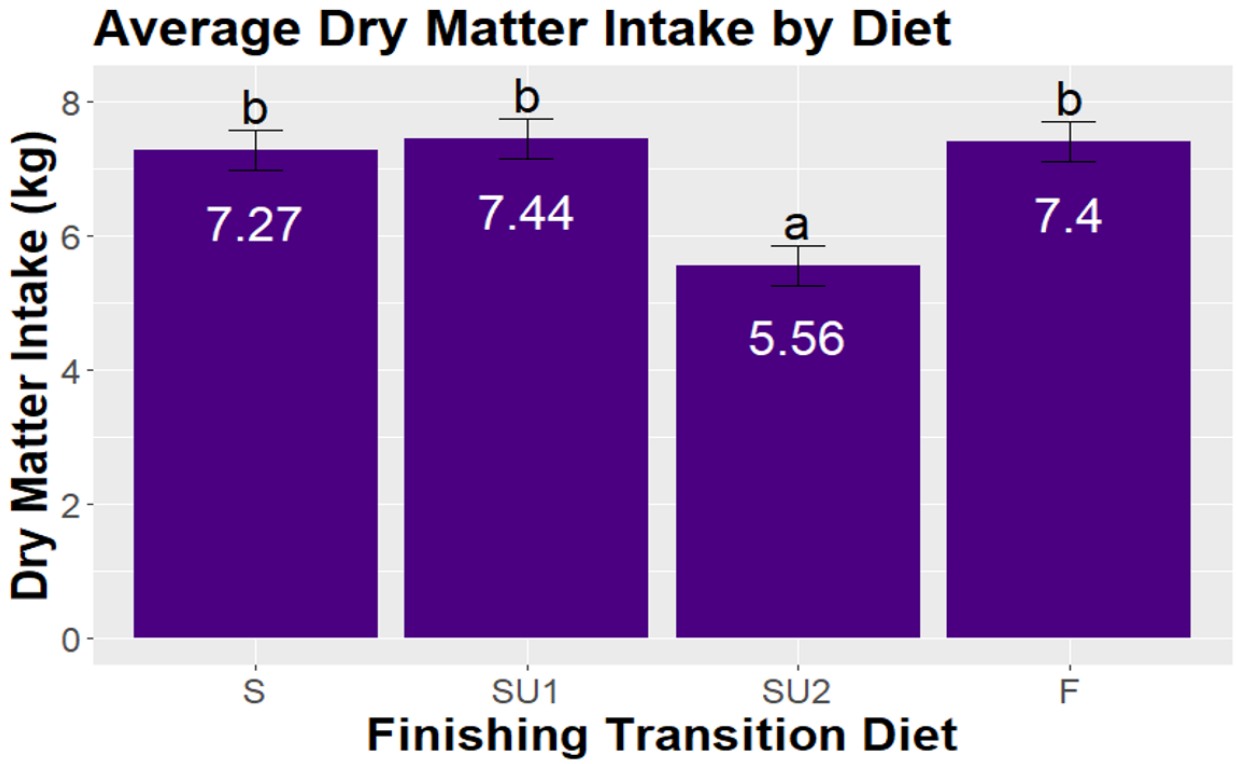
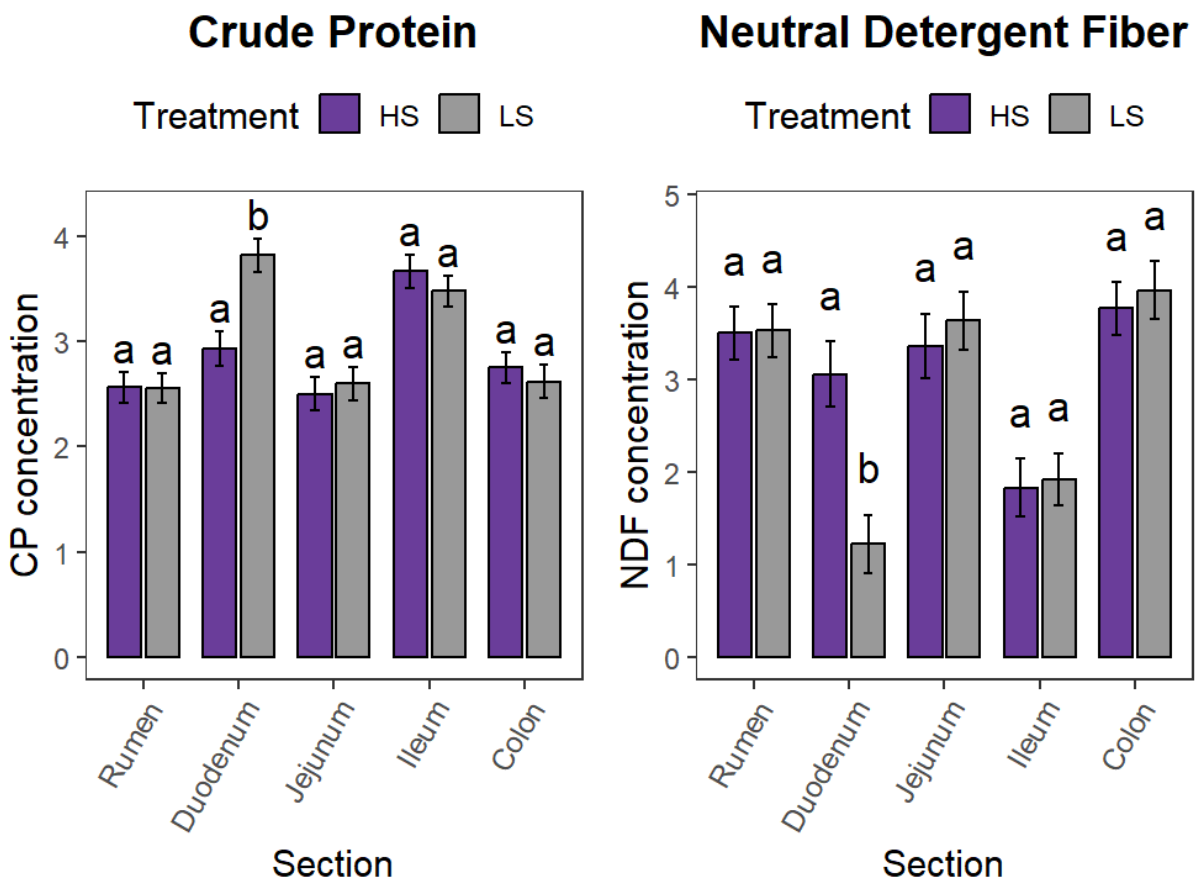


Figure 3.10 The effect of dietary starch level in calf starter x gastrointestinal section on NDF and CP nutrient concentrations of digesta at harvest in the finishing phase.



Appendix A - Counts of replicates for each histological measurement in the hutch and finishing phases

Appendix Table A.1 Counts and percentages of rumen histological measurements obtained in the hutch and finishing phases

Measurement	Weaning (n=10 animals; 50 measurements	Finishing (n=10 animals; 50 measurements
Papilla Length	50/50 (100%)	48/50 (96%)
Papilla Width	50/50 (100%)	48/50 (96%)
Papilla Area	50/50 (100%)	48/50 (96%)
Keratin Thickness	50/50 (100%)	50/50 (100%)
Stratum Corneum	50/50 (100%)	50/50 (100%)
Stratum Granulosum	50/50 (100%)	50/50 (100%)
Stratum Spinosum	50/50 (100%)	50/50 (100%)
Stratum Basale	50/50 (100%)	50/50 (100%)

Appendix Table A.2 Counts and percentages of duodenal, jejunal, and ileal histological measurements obtained in the hutch and finishing phases

Measurement	Weaning (n=10 animals; 50 measurements	Finishing (n=10 animals; 50 measurements
Duodenum		
Villus Length	50/50 (100%)	50/50 (100%)
Villus Width	50/50 (100%)	50/50 (100%)
Crypt Depth	50/50 (100%)	50/50 (100%)
Crypt Width	50/50 (100%)	50/50 (100%)
Propria Thickness	50/50 (100%)	50/50 (100%)
Goblet Cell Area	50/50 (100%)	50/50 (100%)
Jejunum		
Villus Length	48/50 (96%)	48/50 (96%)
Villus Width	48/50 (96%)	48/50 (96%)
Crypt Depth	48/50 (96%)	48/50 (96%)
Crypt Width	48/50 (96%)	48/50 (96%)
Propria Thickness	48/50 (96%)	48/50 (96%)
Goblet Cell Area	50/50 (100%)	50/50 (100%)
Ileum		
Villus Length	40/50 (80%)	49/50 (98%)
Villus Width	40/50 (80%)	49/50 (98%)
Crypt Depth	40/50 (80%)	49/50 (98%)
Crypt Width	40/50 (80%)	49/50 (98%)
Propria Thickness	40/50 (80%)	49/50 (98%)
Goblet Cell Area	50/50 (100%)	48/50 (96%)

Appendix Table A.3 Counts and percentages of spiral colon histological measurements obtained in the hutch and finishing phases

Measurement	Weaning (n=10 animals; 50 measurements	Finishing (n=10 animals; 50 measurements
Gland Width	46/50 (96%)	42/50 (84%)
Gland Depth	46/50 (96%)	42/50 (84%)
Propria Thickness	46/50 (96%)	42/50 (84%)
Goblet Cell Area	50/50 (100%)	50/50 (100%)