Investigating inflammation management of dairy cows during the calving transition period

by

Lauren Elizabeth Engelking

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

The calving transition is a challenging period for dairy cows characterized by negative energy balance, metabolic dysfunction, and inflammation, each of which may compromise milk production. Despite extensive research in this area, much is still unknown on how to best manage this period. The overall objective of this thesis research was to investigate inflammation management in dairy cows during the calving transition including 1) evaluation of nutritional (Chapter 2, 3, 5) and anti-inflammatory treatment (Chapter 2 and 3), 2) assessment of variation in transition cow physiology and metabolism, and the relationship with inflammation (Chapter 4 and 5), and 3) validation of a marker specific to gastrointestinal inflammation (Chapter 6).

Chapter 2 and 3 assessed the effects of dietary butyrate supplementation and oral non-steroidal anti-inflammatory drug (NSAID) administration, in a 2 × 2 factorial arrangement of treatments, on serum inflammatory markers, productivity, uterine inflammation, and interval to first ovulation. Cows were randomly assigned to dietary butyrate supplementation (n = 42), or a control diet (n = 41) from 28 d before calving, until 21 days in milk (DIM). In the same study, cows were also assigned to receive oral NSAID (n = 42), or an oral placebo (n = 41), at 12-24 h after calving. Dietary butyrate supplementation increased plasma fatty acid concentration, and tended to increase serum inflammatory marker concentration, haptoglobin (Hp), 4 d before calving. Cows receiving NSAID tended to have delayed ovulation, compared to cows that did not receive NSAID. Overall, dietary butyrate supplementation and oral NSAID administration were ineffective in improving milk yield or reproductive performance, or reducing systemic or uterine inflammation in dairy cows.

Chapter 5 assessed the effects of offering free choice hay for the first 5 DIM on productivity, serum inflammatory markers, gut permeability, and colon gene expression in fresh dairy cows. Cows were randomly assigned to receive free choice hay, separate from total mixed ration (n = 20), or only total mixed ration (n = 12). Cows offered free choice hay tended to have lower concentration of serum Hp at 3 DIM. Amongst cows offered free choice hay, cows that consumed more hay had a smaller increase in serum amyloid A, and tended to have a smaller increase in Hp concentration from calving to 3 DIM. However, milk yield was not increased in cows offered free choice hay, perhaps due to reduced total dry matter intake (DMI).

Chapter 4 and 5 assessed factors associated with inter-cow variation in intake, metabolism, and physiology. Cows assigned to free choice hay, described in Chapter 5, were examined in Chapter 4 to assess factors associated with variable free choice hay intake; cows with lower pre-calving DMI, and higher concentration of plasma β-hydroxybutyrate and serum Hp at calving consumed more free choice hay. Chapter 5 evaluated factors associated with variation in gut permeability; gut permeability tended to be negatively correlated with pre-calving DMI ($P = 0.07$, r = -0.36), and tended to be positively correlated with serum Hp concentration at 3 DIM ($P = 0.07$, r = 0.35), indicating postpartum greater gut permeability is related to lower pre-calving DMI and higher serum Hp concentration after calving.

Chapter 6 investigated fecal calprotectin as a marker of gastrointestinal tract inflammation. Preliminary data obtained using a commercial calprotectin analysis kit showed little variation amongst samples, regardless of the cow the sample was collected from, or the time-point at which the sample was collected (prepartum vs. postpartum). Four additional commercial calprotectin analysis kits were then assessed; results obtained were infeasible or

highly variable amongst kits, thus it is unknown which kit, if any, provides accurate calprotectin concentrations.

In conclusion, dietary butyrate supplementation and oral non-steroidal anti-inflammatory drug administration are not recommended for transition dairy cows. However, offering free choice hay may be an effective strategy to accommodate inter-cow variation in periparturient factors; cows with lower intake before calving, and cows with greater fat mobilization and serum inflammatory marker concentration at calving may voluntarily increase free choice hay intake, and free choice hay intake may reduce serum inflammatory marker concentration in cows. Finally, this research highlights the remaining need for validation of a marker specific to gastrointestinal inflammation in dairy cows which would vastly improve our ability to manage such inflammation.

Preface

All experimental protocols received research ethics approval from the University of Alberta's Animal Care and Use Committee (AUP00003364 for Chapter 2 and 3; AUP00003716 for Chapter 4, 5, and 6; AUP00003193 for Chapter 6). All procedures, sampling, and animal care was conducted according to the Canadian Council on Animal Care.

Chapter 2 of this thesis has been published as Engelking, L. E., D. J. Ambrose, and M. Oba. "Effects of dietary butyrate supplementation and oral nonsteroidal anti-inflammatory drug administration on serum inflammatory markers and productivity of dairy cows during the calving transition" in the Journal of Dairy Science in 2022; 105:4144-4155. https://doi.org/10.3168/jds.2021-21553. I was responsible for experimental design, sample and data collection and analysis, and preparation of the manuscript. D. J. Ambrose was involved in experimental design and manuscript preparation. M. Oba was the corresponding author and was involved in experimental design and manuscript preparation.

Chapter 3 of this thesis has been published as Engelking, L. E., M. Gobikrushanth, M. Oba and D. J. Ambrose. "Effects of dietary butyrate supplementation and oral non-steroidal anti-inflammatory drug administration on uterine inflammation and interval to first ovulation in postpartum dairy cows" in the Journal of Dairy Science Communications in 2022; 3:362-367. https://doi.org/10.3168/jdsc.2022-0207. I was responsible for experimental design, sample and data collection and analysis, and preparation of the manuscript. M. Gobikrushanth assisted with statistical analysis. M. Oba was involved in experimental design and manuscript preparation. D. J. Ambrose was the corresponding author and was involved in experimental design, sample collection and analysis, and manuscript preparation.

Chapter 4 of this thesis has been accepted as Engelking, L. E., and M. Oba. “Peripartum factors associated with variation in voluntary postpartum hay intake in dairy cows” for publication in the Journal of Dairy Science Communications. I was responsible for experimental design, sample and data collection and analysis, and preparation of the manuscript. M. Oba was the corresponding author and was involved in experimental design and manuscript preparation.

Chapter 5 of this thesis has been accepted as Engelking, L. E., and M. Oba. “The effects of offering free choice hay for the first five days postpartum on productivity, rumination, plasma metabolites, serum inflammatory markers, gut permeability, and colon gene expression in fresh dairy cows” to the Journal of Dairy Science. I was responsible for experimental design, sample and data collection and analysis, and preparation of the manuscript. M. Oba was the corresponding author and was involved in experimental design and manuscript preparation.

Chapter 6 of this thesis is in preparation as Engelking, L. E., and M. Oba. “Evaluation of commercially-available Enzyme-Linked Immunosorbent Assay kits for calprotectin quantification in dairy cows” to BMC Research Notes. I was responsible for experimental design, sample and data collection and analysis, laboratory troubleshooting approaches and execution, and preparation of the manuscript. M. Oba was involved in experimental design, troubleshooting approaches, and manuscript preparation. I will be the corresponding author.

Dedication

This thesis is dedicated to my parents, Henry and Lesley Engelking.

Acknowledgments

Firstly, I would like to extend my most heartfelt gratitude to Dr. Masahito Oba for accepting me as a graduate student four and a half years ago. The growth I have experienced in the last few years, both academically and personally, has been largely attributed to Dr. Oba, and I am incredibly thankful for this time in my life. Dr. Oba has pushed me to evaluate research critically and contextually, allowing me to investigate problems with a refined perspective and deeper overall understanding. Dr. Oba has ignited my adoration for research and cows, and my love for each has grown exponentially in the time I have spent as Dr. Oba's student. I am continuously inspired by Dr. Oba's passion and knowledge of dairy research, and I will strive to exemplify these characteristics throughout my career.

I thank each of my committee members, Drs. Leluo Guan, Divakar Ambrose, and Anne Laarman, for their extensive contribution to my thesis research and career growth. Dr. Guan has opened my eyes to a whole new world of genetic research which has vastly improved my understanding of cow physiology. Dr. Ambrose has greatly enhanced my understanding of dairy cow reproduction, and allowed me to tie my immunology interests into another discipline of dairy cattle research. Dr. Anne Laarman has been instrumental in my graduate student career through challenging my previous beliefs, facilitating fruitful scientific discussions amongst graduate students, and most importantly, acting as a mentor through many of my grad school woes. Thank you to Dr. Gleise da Silva for serving as my arms-length examiner, and to Dr. Lance Baumgard for serving as my external examiner. In addition, I want to thank Dr. Toshihisa Sugino for providing me with the adventure of conducting research in Japan and allowing me to considerably improve my lab skills. As well, thank you to Drs. Frank Robinson, Leanna Grenwich, Clover Bench, and Adina Bujold for their kindness and support in my program.

I am incredibly grateful for the friendships I have made along the way in grad school; to Tianna Larson, Satoko Hisadomi, Matthew Burdick, Jen Haisan, Reillee Duperron, Courtnee Hainstock, Stephanie Chang, Hannah Lantz, Maddi Degenshein, Maddie Bennett, Becs Hiltz, Ali Wolfe, Alison Mark, Diego Martinez, Kayla Johnson, Marina Tortades i Valero, Hector Perez Marquez, Jen Carrelli, Nicole Zukiwsky, Nella Batres, Iswarya Rajesh, Yajing Ban, Jo Ann Chew, Taresa Chieng, and Matt Oryschak, I am so thankful to have encountered each of you throughout my program. You have been incredibly valuable to my success in graduate school through not only helping with studies, but also through our shared laughter and tears, reminding me I am not alone in this crazy grad school experience. To all my colleagues and friends at the Dairy Research and Technology Center, thank you for your consistent support and help throughout all my studies. Your contributions are instrumental to this research.

Finally, I must thank my family for their continued unwavering support of me throughout my education. I thank my grandparents, aunts, uncles, cousins, and brother, Bradley, for their ongoing encouragement through every chapter of my university career. I would especially like to thank my Newton side of the family for paving the way for me in agriculture research at U of A 100 years ago. Finally, and most importantly, I thank my parents for always being my number one supporters in every aspect of my life, without fail. Their belief in me and my capabilities allowed me to persist through the hardest chapter of my life, even when I believed I could not. I certainly would not be pursuing a Ph.D. today if it were not for their endless encouragement at every stage of my schooling. I have come a long way since my first year as an undergraduate when I swore to my parents I would drop out because I was “simply not cut out for university”. It was through their unyielding encouragement and love that I am where I am today, and because of that, I want to give my most sincere, whole-hearted, **thank you to my parents.**

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Acetyl CoA	Acetyl-coenzyme A
ACTB	β-actin
ADF	Acid detergent fiber
APP	Acute phase protein
BCS	Body condition score
BHB	β-hydroxybutyrate
BW	Body weight
cDNA	Copy deoxynucleic acid
CLDN1	Claudin 1
CLDN4	Claudin 1
COX	Cyclooxygenase
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
DPP	Days postpartum
ELISA	Enzyme-linked immunosorbent assay
FA	Fatty acid
FCM	Fat-corrected milk
GIT	Gastrointestinal tract
HAY	Free choice hay treatment group
Hp	Haptoglobin
ICFO	Interval from calving to first ovulation
IgA	Immunoglobulin A
IL10	Interleukin 10
Li·Cr-EDTA	Lithium chromium ethylenediaminetetraacetic acid
LPS	Lipopolysaccharide
ME	Metabolizable energy
MRP8	Migration inhibitory factor related protein 8

MRP14	Migration inhibitory factor related protein 14
MP	Metabolizable protein
MUN	Milk urea nitrogen
NDF	Neutral detergent fiber
NE_L	Net energy for lactation
NFC	Non-fiber carbohydrate
NH	No free choice hay treatment group
NSAID	Non-steroidal anti-inflammatory drug
OCLN	Occludin
OM	Organic matter
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PMN	Polymorphonuclear
Proportional forage NDF intake	Forage neutral detergent intake as a % of total dry matter intake
Proportional starch intake	Starch intake as a % of total dry matter intake
PVDF	Polyvinylidene difluoride
qPCR	Quantitative polymerase chain reaction
SAA	Serum amyloid A
SARA	Subacute ruminal acidosis
SCC	Somatic cell count
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Starch:forage NDF ratio	Starch intake (kg/d)/forage neutral detergent fiber intake (kg/d)
TBST	Tris-buffered saline (7.4 pH) + 1% tween
TCA	Tricarboxylic acid cycle
TMB	Tetramethylbenzidine
TG	Triglyceride
TLR	Toll-like receptor
TLR4	Toll-like receptor 4
TMR	Total mixed ration
VFA	Volatile fatty acid
HRP	Horseradish peroxidase

ZO1	Zonula occludens 1

Chapter 1. Literature Review

Adult dairy cows face considerable challenges throughout their lifetime, particularly during the calving transition period, defined as three weeks before and three weeks after calving. Challenges during the calving transition period include parturition itself as well as increased energy requirements to support milk production and reduced feed intake around calving, typically leading to a state of negative energy balance (Trevisi and Minuti, 2018). Negative energy balance is commonly associated with inflammation, gut barrier dysfunction, and metabolic disorders, although the precise relationship of each is not fully elucidated (Martins et al., 2022).

Nutritional management is a popular theme in dairy cow research, and has been widely explored in improving animal health and production, and reducing metabolic dysfunction and inflammation. However, despite abundant research conducted in this area, much is still unknown regarding optimal nutritional management of dairy cows. The unique anatomy and physiology of ruminants adds an additional layer of complexity to dairy cow management. The gastrointestinal tract (GIT) is of interest due to its dual-purpose role in nutrient digestion and absorption, as well as maintaining gut barrier function, each of which are crucial in managing energy requirements, health, and inflammation in dairy cows. Although it is established that GIT function, inflammation, and nutrition each play a role in cow performance and health, the relationship between each of these parameters is not well understood. As such, improved understanding of each of these areas and their relationship with one another will vastly improve our ability to manage dairy cows.

1.1 Gastrointestinal Tract

1.1.1 Anatomy and Structure

This review defines the foregut as the reticulum, rumen, omasum, and abomasum. The first three digestive compartments of the foregut, the reticulum, rumen, and omasum, each contain a stratified squamous epithelium, meaning their apical walls are multilayered, with the outermost epithelial cell layer, the corneum, containing keratinized cells (Graham and Simmons, 2005; Stumpff et al., 2011, 2018). The last section of the foregut, the abomasum, is a glandular compartment with a single layered columnar epithelium made up of many epithelial folds to increase surface area and contact with digesta (Umphrey and Staples, 1992).

The small intestine follows the abomasum and is made up of the duodenum, jejunum, and ileum. The columnar polarized epithelial cells of the small intestine are covered by a layer of mucus, and include enteroendocrine cells, enterocytes, goblet cells, tuft cells, Paneth, and stem cells (Peterson and Artis, 2014; Steele et al., 2016). Together these cells contribute to hormone secretion, digestion (Peterson and Artis, 2014), and absorption (Meneses et al., 2016), mucus production, (Kim and Ho, 2010), immune function (Hendel et al., 2022), and antimicrobial action (Gallo and Hooper, 2012). The various cell types form the small intestine epithelium, arranged together to form villi and crypts within the small intestine (Tivey and Smith, 1989).

The cecum and large intestine follow the ileum and are often considered together as one unit, “the hindgut”, due to a lack of distinct anatomical division and similar fermentation profiles (Elsden et al., 1946). The hindgut also contains a monolayer of cells including goblet, enteroendocrine, and enterocyte cells covered in a layer of mucus, but the hindgut differs from the small intestines as it does not contain Paneth cells, and only contains crypts, but no villi (van

Kruningen, 1988). Finally, after the hindgut is the rectum which also contains a mucus-covered monolayer of cells, composed of enterocytes, goblet cells, and Paneth cells (Wu et al., 2022).

1.1.2 Carbohydrate Digestion and Absorption

Carbohydrates are primarily digested in the rumen, where a microbial community made up of bacteria, protozoa, and fungi, ferment feedstuffs into volatile fatty acids (VFA), butyrate, propionate, and acetate, which account for up to 70% of total dietary energy in ruminants (Flint and Bayer, 2008). Following carbohydrate fermentation, papillae on the epithelial surface of the reticulorumen absorb VFA which are then transported to the portal vein and enter circulation where they may be utilized by the cow (Ma et al., 2017).

Carbohydrates are broadly categorized as either plant cell contents or plant cell wall components (Hall and Eastridge, 2014). Cell contents include starches and sugars which can be digested by both microbes in the GIT (Owens and Basalan, 2016) or by endogenous enzymes (Huntington et al., 2006). However, cell wall components are made up of fibrous plant material which are minimally digested by endogenous enzymes, but this is compensated for by microbial fermentation of plant material in the rumen and hindgut (Nozière et al., 2010; Taschuk and Griebel, 2012). Starches that escape ruminal digestion continue to the small intestine where they are digested by endogenous amylase and may be absorbed (Harmon, 1992).

Fiber not degraded in the rumen, and starches not degraded in the rumen or small intestine, flow to the hindgut where additional microbial fermentation occurs. Next to the rumen, the hindgut is the largest fermentation chamber in ruminants, providing 5-10% of dietary energy through fermentation products which can influence animal health and production (Gressley et al., 2011). Through microbial fermentation, the hindgut digests carbohydrates that were not

previously hydrolyzed by previous digestive processes, producing VFA (McNeil, 1988; Hume, 1997; Váradyová et al., 2000). Carbohydrates arriving in the hindgut are typically slowly digested polymers, such as crystalline starches and lignin, that have not been digested by the foregut (Van Soest, 1994). However, hindgut fermentation is influenced by an array of factors including rumen mat function, feed intake and passage rate, starch source, and overall starch intake, with greater starch intakes typically resulting in increased arrival of starch in the hindgut leading to increased fermentation (McCarthy et al., 1989; Gressley et al., 2011; Plaizier et al., 2018).

Following carbohydrate fermentation, VFA can be absorbed through the hindgut epithelium, primarily through passive absorption (Argenzio et al., 1975), however, the extent of absorption is somewhat limited compared to the rumen (Gressley et al., 2011). This is partially due to the mucus layer covering the hindgut mucosa with a tightly regulated pH of 7 that appears to be unaffected by digesta pH (Hume et al., 1997), which reduces pH-mediated VFA absorption (Gressley et al., 2011). As well, some degree of VFA absorption is bicarbonate-dependent (Gäbel et al., 1991; Sehested et al., 1999; Aschenbach et al., 2009), and there is less bicarbonate available in the hindgut compared to the foregut due to the absence of salivary bicarbonate (Gressley et al., 2011).

1.1.3 Passage of Feed through the Gastrointestinal Tract

Nutrient digestion and absorption are largely impacted by passage rate of digesta through the GIT (Krizsan et al., 2010; Moyo and Nsahlai, 2017). Rate of passage is affected by intrinsic animal factors, dietary and environmental factors, and particle size (Ellis et al., 1994; Huhtanen et al., 2006). Passage rate determines the length of time feedstuffs spend in each digestive compartment, and slower passage rate generally increases digestion as feed has increased

duration of exposure to digestive enzymes and microbes (Merchen et al., 1997; Wang and McAllister, 2002). With increased intake, passage rate increases to facilitate ruminal emptying and create space for additional feed intake (Colucci et al., 1982), thus decreasing digestion in the rumen. Forages typically have a slower passage rate than grains in the rumen (Hartnell and Satter et al., 1979; Colucci et al., 1982, NASEM, 2021), and beyond the rumen (Colucci et al., 1982). Dietary NDF content is described as the single best predictor of passage rate (Krizsan et al., 2010), thus the majority of research evaluating passage rate involves assessment of various forage characteristics and their effect on GIT flow. It should be noted that the majority of research investigating passage rate focuses on ruminal passage rate, and more attention is required for post-ruminal factors.

Within forages, those with smaller particle size, whether due to smaller chop lengths or greater fragility, may have a faster rate of passage as less ruminal digestion and rumination is required to further reduce particle size and allow passage through the reticulo-omasal orifice (Allen, 2000), which requires particles to be less than 2-3 mm (Kammes and Allen, 2012). In addition to particle size, characteristics such as forage maturity (Krizsan et al., 2010), functional specific gravity, and stem: leaf ratio can influence passage rate (Huhtanen et al., 2006). Increases in forage maturity and stem: leaf ratio typically decrease passage rate as they are inversely related to digestibility, thus increases in either of these parameters increases retention time in the rumen, slowing passage rate (Huhtanen et al., 2006). Forages lower in specific gravity are more buoyant in the rumen, thus such forages often have a reduced passage rate as they join the rumen mat away from the reticulo-omasal orifice (Kammes and Allen, 2012; Oba and Kammes-Main, 2023). Rumen pH is also thought to play a role in passage rate, with increased rumen pH resulting in an increased passage rate (Stokes, 1983), perhaps due to increased penetration of

feed particles with cations and water (Parrott and Thrall, 1978), or due to alterations in microbial populations (Hooper and Welch, 1985), however, the mechanisms behind this are not well described.

1.1.4 Barrier Function

The GIT is semipermeable, allowing absorption of nutrients, electrolytes, and water (Ferraris and Diamond, 1997; Kunzelmann and Mall, 2002), but prevents passage of toxic molecules from the gut lumen to portal circulation (Podolsky et al., 1999; Blikslager et al., 2007). This review will focus on the barrier function of reticulorumen, small intestine, and hindgut due to the paucity of research exploring barrier function of other segments.

Absorption in the GIT may occur transcellularly or paracellularly (Meneses et al., 2016). Paracellular transport occurs in the space between epithelial cells, modulated by tight junctions, adherend junctions, and desmosomes (Ballard et al., 1995; Arrieta et al., 2006). Tight junctions allow selective absorption (Bruewer and Nusrat, 2000), but also provide mechanical strength and barrier function, preventing toxins within luminal contents from entering portal circulation (Graham and Simmons, 2005; Green and Simpson, 2007). Transcellular transport involves endocytosis or exocytosis, sometimes mediated by selective transporters on the apical membrane of the GIT (Ménard et al., 2010; Eckel and Ametaj, 2016). The rumen, small intestine, and hindgut have toll-like receptors (TLR) on the apical and basal side of epithelial cells that contribute to barrier function through detection (Taschuk and Griebel, 2012), and elimination of pathogens by initiating local inflammatory responses (Iwasaki and Medzhitov, 2004; Rakoff-Nahoum et al., 2004; Sansonetti, 2006).

The barrier function of the rumen is largely attributed to the stratified squamous epithelium (Zebeli and Metzlier-Zebeli, 2012), with the outermost keratinized corneum layer providing physical protection from pathogen entry (Baldwin et al., 1998; Graham and Simmons, 2005; Steele et al., 2016). The apical epithelial layer of the rumen has been suggested to contain mucus-producing cells, resulting in some mucus coverage and additional protection of the rumen epithelium (Habel, 1963; Lavker, 1969a,b). However, mucus in the rumen has not received attention in recent research, thus the presence of mucus and the contribution of mucus to barrier function in the rumen is unknown. In addition to host cells present in the corneum, a symbiotic microbial population adheres to host cells in the corneum, but do not penetrate it (Steele et al., 2009; Steele et al., 2011a). Beneath the corneum is the stratum granulosum which contains epithelial cells with tight junctions, adherend junctions, and desmosomes (Graham and Simmons, 2005), followed by metabolically active layers, the stratum spinosum and stratum basale (Baldwin et al., 2004). With shifts in the rumen environment, such as during increased grain intake, the rumen epithelium can adapt, to a certain extent, through increased epithelial cell proliferation (Goodlad, 1981, Shen et al., 2004), morphogenesis (Steele et al., 2009), and altered gene expression (Steele et al., 2011b). If the rumen mucosa layer of the GIT is compromised and microbes begin to penetrate the epithelium, an immune response is initiated and immune cells migrate from the basal epithelial cells to the upper layers of the epithelium to provide further barrier from entry (Steele et al., 2009).

In the small intestine and hindgut, barrier function is composed of mucus, intestinal epithelial cells, and immune cells (Taschuk and Griebel, 2012). Intestinal epithelial cells possess the ability to respond to stimuli and partially adjust barrier function (Peterson and Artis, 2014) through altered absorption and secretion of antimicrobial peptides, hormones, and mucus (Steele

et al., 2016). Small intestine and hindgut epithelial cells are coated by two distinct mucus layers which act as the first line of defence (Steele et al., 2016; Camilleri et al., 2019) by preventing pathogen interaction with epithelial cells (Johansson et al., 2008), exerting robust antimicrobial (Ouellette, 2010), and immunoglobulin A activity (Pabst, 2012). Immunoglobulin A has high affinity for neutralizing toxins and pathogenic microbes, but low affinity for commensal microbes within the GIT lumen (MacPherson et al., 2008; Taschuk and Griebel, 2012).

In addition to the host-defence mechanisms, symbiotic microbes throughout the GIT lumen also contribute to maintaining barrier function, with the small intestine and hindgut containing greater microbial diversity than the rumen (Lavker and Motoltsy, 1970). The microbial community promotes gut health by modulating immune cell abundance (Rothkotter and Pabst, 1989) and proliferation (Yasuda et al., 2004; Chung et al., 2012), and maintaining a commensal population that allows symbiotic microbes to exist (MacPherson et al., 2012), while acting as a barrier to pathogens (Petersson et al., 2011), and supplying nutritional and physiological benefits (Walter and Ley, 2011; Taschuk and Griebel, 2012). Flora are not only beneficial, but also essential in mucosal inflammatory protection, which is demonstrated by a study that reported mice with no microflora were unable to survive intestinal injury (Rakoff-Nahoum et al., 2004).

Overall, the bovine GIT contains a number of compartments, each with a unique structure, function, and microbial population, each of which contribute to digesting and absorbing nutrients, while maintaining gut barrier function.

1.2 Calving Transition Period

Throughout the calving transition period, cows face a great number of metabolic, physiologic, and anatomical changes as they prepare and adjust to the high energy demands and

metabolic disorders with the onset of lactation, with approximately 50% of cow morbidity occurring during this period (Bradford et al., 2015; Trevisi and Minuti, 2018; Horst et al., 2021). Additionally, DMI is often reduced during the transition period due to changes in physiology, hormones, anatomy and behavior (Hayirli and Grummer, 2004; Mikula et al., 2021). As cows begin to lactate, the rapid increase in energy demands induces a state of negative energy balance, which is exacerbated by decreased DMI during this period (Coppock, 1985; Hayirli and Grummer, 2004). The large energy demands, reduced DMI, and rapid metabolic and physiologic changes each contribute to the high incidence of metabolic disorders and culling during this period (Roberts et al., 2012; Sundrum, 2015; Probo et al., 2018), thus management of cows during the transition period is critical.

1.2.1 Physiology

During the prepartum portion of the transition period, a series of coordinated hormonal changes prepare the cow to calve and begin her lactating period (Ehrhardt et al., 2016). In the prepartum period, insulin resistance occurs which allows glucose to be allocated towards the developing fetus and mammary, and reduces glucose utilization in muscle and adipose tissue (Bell and Bauman, 1997; De Koster and Opsomer, 2013; Pascottini et al., 2020). Glucose, synthesized in the liver from propionate through gluconeogenesis (Lager and Jordan, 1997), maintains a consistent blood concentration until calving, during which concentrations are rapidly increased (Kunz et al., 1985; Vazquez-Anon et al., 1994), perhaps due to increased glucagon and glucocorticoid concentrations during calving that induce glycogenolysis of glycogen stores to increase glucose availability to the cow (Vazquez-Anon et al., 1994). Following calving, blood glucose concentrations are greatly decreased (Kunz et al., 1985; Vazquez-Anon et al., 1994) mainly due to energy demands of lactation (Aschenbach et al., 2010), and concentrations remain

low until about 14 days postpartum when glycogen stores begin to replete (Vazquez-Anon et al., 1994). Additionally, glucose production may be further inhibited by increased concentrations of blood β-hydroxybutyrate (BHB) at calving due to the inhibitory effect on BHB on gluconeogenesis, as BHB partially replaces glucose as an energy source, reducing the demand for glucose (Zarrin et al., 2014).

Shortly after calving the somatotropic axis uncouples in response to negative energy balance which results in reduced insulin-like growth factor 1 and increased growth hormone concentration (Knop and Cernescu, 2009; Lucy et al., 2009). Growth hormone increases hepatic gluconeogenesis (Pocius and Herbein, 1986), and insulin resistance which prevents glucose uptake by muscle or adipose tissue to increase glucose availability for milk production, and fatty acid (FA) mobilization to support fat synthesis in the mammary (Etherton and Bauman, 1998; Pires et al., 2007; De Koster and Opsomer, 2013), and energy demands in the postpartum period (Drackley, 1999)

Over the transition period, blood concentrations of FA and BHB increase, and remain high until approximately two and one week postpartum, respectively, with peak concentrations often reaching 0.8 mEq/L around calving (Grum et al., 1996; LeBlanc et al., 2005; Mikula et al., 2021). Increased concentrations of FA around calving are likely due to a combination of stress, hormonal controls, and rapid increases in energy requirements postpartum (NASEM, 2021). Increased growth hormone, decreased insulin, and high glucagon around calving increase blood FA concentration during negative energy balance (Holtenius and Holtenius, 1996) which is often increased further by decreased DMI around calving (Bobe et al., 2004; NASEM, 2021). To meet high energy needs, FA are mobilized from adipose tissues, and transported through the bloodstream to the liver (Lomax and Baird, 1983).

Fatty acids reaching the liver undergo one of four fates: 1) Complete oxidation, 2) incomplete oxidation, 3) exported from the liver, or 4) stored as fat in the liver (Grummer, 1993). In order for FA arriving at the liver to undergo complete oxidation, they must first be converted to acetyl CoA followed by entry to the TCA cycle which is facilitated by oxaloacetate (White, 2015). The availability of oxaloacetate to facilitate FA entry into the TCA cycle is dependent the oxalacetate precursor, propionate (Aschenbach et al., 2010). Thus, if sufficient propionate supply is not available, namely, in times of negative energy balance, oxaloacetate cannot be synthesized, and acetyl CoA cannot enter the TCA cycle (Baird et al., 1972). In such cases, acetyl CoA accumulates, and is instead converted to ketones, such as BHB (Grummer, 1993, Drackley et al., 2001; van Knegsel et al., 2005). Alternatively, some FA arriving at the liver may be exported from the liver through re-esterification to triglycerides (TG) and association with very low-density lipoproteins which can then enter blood circulation (Hoyumpa et al., 1975). However, the ability of the ruminant liver to export FA from the liver is limited, thus, little FA undergo this fate (Kleppe et al., 1998; Pullen et al., 1990; Puppel and Kucynska, 2016). Therefore, FA that are not completely oxidized, exported from the liver, or converted to ketones, are re-esterified to TG, and stored in the liver (Grummer, 1993, Drackley et al., 2001; van Knegsel et al., 2005). Transport of FA to the liver is proportional to the level of FA in the blood (Emery et al., 1992), thus some extent of ketone production (White, 2015) and TG deposition in the liver is expected in early postpartum cows due to the high blood FA concentrations during this period (Jorritsma et al., 2001; Bobe et al., 2004).

Alterations in immune status also occur over the transition period, which may result in immunosuppression (Goff and Horst, 1997, Hansen, 2013). Increases in blood concentration of cortisol, estradiol, prostaglandin and prolactin, and decreased progesterone in the week before

calving are thought to contribute to immunosuppression (Stevenson, 2007). During the calving transition period there is decreased function of lymphocytes (Burton et al., 1995; Dosogne et al., 1998;1999; Rinaldi et al., 2008), decreased concentrations of immunoglobulins (Herr et al., 2011), and altered types and numbers of immune cells (Hansen et al., 2013). Reduced DMI over the transition period may also contribute to reduced immune function (NASEM, 2021), perhaps as the immune system is energy-demanding, and energy supply is limited over this period (Bradford et al., 2015).

Changes in GIT anatomy, function, and absorptive capacity, primarily within the rumen, have been largely explored in dairy cattle over the calving transition period. The mass of rumen tissue increase 5% from 7 days prepartum, to 10 days postpartum (Reynolds et al., 2004), with continued growth until 120 days postpartum (Baldwin et al., 2004; Reynolds et al., 2004). Similarly, the small intestine mass increases from the pre- to postpartum periods, reaching peak mass around 90 days postpartum (Baldwin et al., 2004; Reynolds et al., 2004). The increase in mass of the rumen is mainly attributed to enhanced ruminal wall growth, particularly rumen papillae (Reynolds et al., 2004; Steele et al., 2015), as the volume of the rumen does not differ from pre- to postpartum (Reynolds et al., 2004). The increased mass of the GIT during the calving transition period likely reflects its increased absorptive capacity over this period (NASEM, 2021), perhaps to accommodate for greater nutrient demands in the postpartum period.

We do not know to what extent GIT changes over the transition period are influenced by changes in dietary intake during this period, compared to preprogrammed GIT changes associated with genetics and naturally occurring hormonal changes. The authors of NASEM (2021) note that significant dietary changes occur over the calving period, thus diet and

physiological factors are confounded, but these changes are most likely due to a combination of each. The dietary factors affecting changes in GIT physiology will be discussed further in section 1.2.2, 1.3.4, and 1.4.1.

1.2.2 Nutritional Management

To address the rapid increase in energy required by dairy cows in the postpartum period, dietary energy density is increased, primarily through increased dietary starch (Rabelo et al., 2003; McCarthy et al., 2015a; Albornoz et al., 2019). There is no clear consensus on optimal transition cow nutritional management; some producers choose to provide increased dietary starch during the closeup period, whereas other producers choose to wait until the fresh period to increase dietary starch. Regardless of when producers choose to increase dietary starch, a major consideration in increasing dietary starch is balancing VFA production with VFA absorption in the GIT to avoid GIT acidification and associated metabolic disorders (Aschenbach et al., 2011; Zebeli et al., 2015).

1.2.2.1 Prepartum

The previous NRC (2001) recommended feeding closeup cows diets with increased energy density, compared to far off cows, commonly referred to as a "step-up ration". These rations consist of an increased proportion of grain which is intended to increase prepartum energy intake to support postpartum energy demands, and promote ruminal adaptation to a high-grain postpartum diet through increased ruminal fermentation (NRC, 2001; NASEM 2021). A cow's rumen environment requires time to adapt to increased grain consumption; the ruminal microbial community must adjust to new feedstuffs, which results in an altered microbial profile and the associated fermentation products (NRC, 2001; Brown et al., 2006; NASEM 2021). As well, rumen papillae may require time after exposure to increased dietary starch to increase

surface area (Dirksen et al., 1985; Bannink et al., 2012; Dieho et al., 2016a) which may increase absorption of VFA (Melo et al., 2013), and rumen pH (Aschenbach et al., 2011). Despite the prior NRC recommendations, data does not consistently support these beliefs. Studies providing increased starch in the prepartum period have often reported no increases in ruminal papillae growth (Anderson et al., 1999; Reynolds et al., 2004). Additionally, VFA absorption has been reported to only have a weak relationship with papillae surface area during the transition period (Dieho et al., 2016b). Instead, offering a prepartum diet greater starch than required by prepartum cows may increase acidosis in prepartum cows, and increase acidosis susceptibility in fresh cows (Penner et al., 2007) as cows are more vulnerable to ruminal acidosis after experiencing it once (Dohme et al., 2008). In addition to increased ruminal acidosis risk, increased inflammatory markers have been reported in cows fed step up rations in the prepartum period, perhaps due to epithelial damage induced by ruminal acidosis (Shi et al., 2020). An additional concern of increasing dietary energy in the prepartum period is cows are at risk of over conditioning, insulin resistance (Holtenius et al., 2003; Dann et al., 2006) and related metabolic disorders (Mann et al., 2015). Relative to the postpartum cow, the prepartum cow has low energy requirements, thus, increasing dietary energy beyond their requirements could result in fat deposition and related metabolic disorders (Janovick et al., 2011; Mann et al., 2015).

Alternative to providing a step-up ration, many producers choose to provide “energy controlled” prepartum diets with energy levels close to the actual requirements of the prepartum cow. Previous research has reported that limiting energy intake in the dry period may result in reduced fat mobilization (Janovick et al., 2011; Mann et al., 2015, Zhang et al., 2015), reduced concentration of blood ketones (Vickers et al., 2013; Mann et al., 2015), and reduced incidence of ketosis (Doepel et al., 2004; Vickers et al., 2013) around calving, each of which may

contribute to increased postpartum DMI (Douglas et al., 2006) and energy balance (Dann et al., 2006, Hayirli et al., 2011). Postpartum DMI may also increase when energy controlled prepartum diets are fed as less prepartum energy reduces the extent of insulin resistance (Drackley et al., 2001; Douglas et al., 2006). In addition, cows fed rations with lower starch content have a lower risk of acidosis in the prepartum period (Penner et al., 2007).

Due to the conflicting results in literature, NASEM (2021) has stated that, within the scientific community, there is not strong evidence to support a step-up ration in the closeup period.

1.2.2.2 Postpartum

A major challenge of nutritional management for fresh cows is providing adequate starch in diets to meet high energy demands while avoiding GIT acidosis due to excessive starch fermentation. Dairy cows often experience GIT acidosis in early lactation due to a rapid increase in fermentable carbohydrate intake (Penner et al., 2007) which is associated with decreased DMI (Allen et al., 2009) and compromised health (Stone et al., 2004; Krause and Oetzel et al., 2006). However, fermentable carbohydrates must be included in the diet to provide adequate energy supply to cows with rapidly increasing energy requirements post-calving (Krause and Oetzel, 2006). Like prepartum nutritional management, optimal postpartum nutritional management is not established, and the extent to which dietary starch should be increased in the fresh diet compared to the closeup diet is also unknown (NASEM, 2021). Some studies report gradual increases in dietary starch from the prepartum to postpartum period result in greater milk production (Dieho et al., 2016a), whereas others reported higher milk yield from cows fed higher starch (Rabelo et al., 2003; McCarthy et al., 2015a; Piantoni et al., 2015).

Much of the research assessing the dietary starch in the fresh period has considered the closeup diet and its effect on cow response to fresh period starch. Shi et al. (2019) found when a low starch prepartum diet was fed, cows fed low starch postpartum diet produced more milk than cows fed a high starch postpartum diet. Conversely, studies that fed higher starch prepartum diets found cows fed higher starch postpartum diets had less BCS loss, higher DMI, and higher milk production compared to cows fed low starch postpartum diets (McCarthy et al., 2015a; Piantoni et al., 2015). It has been suggested that when cows are fed low starch diets in the prepartum period, the abrupt increase in dietary starch in the postpartum period induces GIT acidosis, compromising milk production (Krause and Oetzel, 2006; Penner et al., 2007). However, Haisan et al. (2021) reported cows fed low starch in the prepartum period had higher DMI and milk yield when fed high starch, compared to low starch, in the postpartum period, and Rabelo et al. (2003; 2005) reported prepartum dietary starch content did not affect postpartum metabolic status or milk production, and postpartum DMI and milk yield were higher for cows fed a high starch postpartum diet, compared to a low starch postpartum diet, regardless of prepartum dietary starch. Therefore, the interaction between prepartum and postpartum starch and the effect on cow health and performance is unclear.

Regardless of whether dietary starch is increased in the prepartum period or the postpartum period, cows fed the same diet have been reported to have great variation in diet digestibility (Tharangani et al., 2020), rumen fermentation (Cabezas-Garcia et al., 2017), feeding behavior (Rumpohorst et al., 2022), DMI, milk yield, and feed efficiency (Guinguina et al., 2020a,b). Similarly, amongst cows fed the same ration, GIT acidosis severity varies substantially (Gao and Oba, 2014), perhaps due to intrinsic cow factors such as differing rumen wall characteristics (Bannink et al., 2012), GIT metabolic efficiency and VFA absorption, thus influencing ruminal

acidosis susceptibility (Penner et al., 2009a). Thus, there is likely no single diet formulation in either the prepartum or postpartum period that optimally balances animal health and performance for all cows. Further research must investigate how best to manage nutrition of cows with varying requirements, and if it is possible to simultaneously meet the variable needs of many individual cows.

1.2.2.3 Offering Free Choice Hay

Perhaps between-cow variation in nutritional requirements can be managed through providing free choice hay in addition to a high starch TMR; Haisan et al. (2021) found fresh cows produced more milk when fed a high starch diet (32.8% starch), in addition to free choice hay, whereas Shi et al. (2019) found fresh cows produced more milk when fed a low starch diet (22.1 % starch), when free choice hay was not available. It is possible this discrepancy in cow performance may be due to free choice hay offered alongside TMR for the first 3 days after calving in the study by Haisan et al. (2021), but not in the study of Shi et al. (2019). Offering free choice hay in the study by Haisan et al. (2021) may have allowed cows to increase fiber consumption voluntarily, and decrease negative effects associated with rapid starch fermentation.

Forage consumption stimulates rumination in cows which leads to greater salivary bicarbonate secretion (Erdman, 1988) which buffers drops in rumen pH (Counotte et al., 1979), perhaps ameliorating consequences of high starch diets, such as inflammation. Forage consumption also contributes to reducing the dietary proportion of rapidly degraded starch and to developing the rumen mat (Zebeli et al., 2012). Previous research has shown that acidotic cows will sort their rations to consume forage that induce rumination and salivary buffer production (Keunen et al., 2002; DeVries et al., 2008). Physiological feedback in cows may affect feed choice based on rumen environment changes, such as declining pH following grain consumption

(Provenza, 1995; Keunen et al., 2002). Therefore, cows may be motivated to consume more forage in the fresh period when they experience sudden increases in rumen fermentation, as hay consumption may prevent inflammation associated with acidosis. However, increasing dietary fiber content often decreases DMI, especially in fresh cows (Weiss, 2015), which must be avoided due to the energy deficit already experienced by transition cows.

In the study by Haisan et al. (2021), TMR forage content was not increased, but rather, free choice hay was offered alongside TMR. This may have allowed cows to voluntarily consume hay on an as-needed basis, and increase dietary fiber intake, however, this study did not measure free choice hay intake. As cows appear to have highly variable responses when fed the same diet (Penner et al., 2009a,b; Gao and Oba, 2014; Guinguina et al., 2020a,b), free choice hay provision may allow a high starch ration to be available to more acidosis-tolerant cows to meet high energy requirements, while allowing less acidosis-tolerant cows to increase forage consumption, potentially reducing the incidence of acidosis and related conditions. Others have found offering free choice hay in addition to TMR may prevent DMI reductions during acidosis (Kmicikewycz and Heinrichs, 2014) and increase milk yield (Cavallini et al., 2018). However, both studies were conducted in early to mid-lactation cows, and not in transition cows. It appears there is no previous research investigating the effects of free choice hay for fresh cows and further research is warranted.

In summary, the numerous changes experienced by dairy cows during the calving transition period present considerable challenges in managing dairy cow health and productivity. We have much to learn regarding physiology and nutrition, and the complex interactions between them during the calving transition.

1.3 Metabolic Dysfunction, Disorders, and Infectious Diseases

1.3.1 Ketosis and Fatty Liver

Ketosis is defined as the abundance of ketones in blood circulation, with blood BHB concentration >1.4 mmol/L in dairy cows (Oetzel, 2004). Although ketones aside from BHB are produced following beta oxidation in the liver, BHB is the primary circulating ketone body (Kauppinen, 1983) and the ketone most frequently used by standard diagnostic tests, thus ketosis is defined according to BHB concentration (Duffield et al., 2009; Tatone et al., 2016; NASEM 2021). Ketosis may be clinical, with symptoms including reduced appetite and milk production, increased forage intake, acetone odor in the mouth and urine, (Guliński, 2021), increased laying time, and blood ketone concentrations often exceeding 2.9 mmol/L (Itle et al., 2015) or it may be subclinical, where cows do not display clinical signs, but blood concentration of ketones exceeds the threshold of 1.4 mmol/L (Oetzel et al., 2004). Subclinical ketosis is reported to affect approximately 54% of cows, with some herds reporting up to 80% of cows affected (Duffield, 2000). Ketosis is negatively associated with cow performance as cows with elevated blood BHB are 20% less likely to become pregnant after first insemination (Walsh et al., 2007), are more likely to have metritis (Ospina et al., 2010), have reduced immune function (Sato et al., 1995), and have an increased susceptibility to inflammation (Hoeben et al., 1997).

Ketosis is commonly categorized as type 1 or type 2, with type 1 resulting from a shortage of oxaloacetate in the TCA cycle, and type 2 occurring when there is excessive acetyl CoA (Oetzel, 2007). As previously described, FA are mobilized to a great extent following calving and the associated increased energy demands. As FA are mobilized and transported to the liver, they are converted to acetyl CoA and can undergo complete oxidation to energy when oxaloacetate is present (White, 2015). However, in times of low DMI and negative energy

balance, such as after calving, there is a shortage gluconeogenic precursors (Aschenbach et al., 2010; NASEM, 2021). Thus, existing oxaloacetate is converted to glucose, and additional oxaloacetate cannot be synthesized at the rate of FA arriving at the liver. This results in acetyl CoA conversion to ketones, and excessive ketone concentration in blood which is defined as type 1 ketosis. Type 1 ketosis may be at least partially alleviated by providing propylene glycol, a gluconeogenetic substrate and precursor of oxaloacetate (Nielsen and Ingvartsen, 2004). Alternatively, energy dense postpartum diets that reduce blood BHB and increase glucose may reduce type 1 ketosis incidence (Rabelo et al., 2005; McCarthy et al., 2015a; Zhang et al., 2015) as these diets provide additional oxaloacetate substrate which can allow acetyl CoA's entry to the TCA cycle. However, it should be noted that not all dietary energy will alleviate ketosis; rather, the energy supplied must be from glucose or propionate-producing energy sources, as these are oxaloacetate precursors, whereas energy sources such as fat, will not result in oxaloacetate production (Bach, 1978).

Type 2 ketosis occurs when FA mobilization remains high for an extended period of time, such as days or weeks after calving. During type 2 ketosis, excessive amounts of acetyl CoA are synthesized from the high volumes of FA arriving at the liver (Holtenius and Holtenius, 1996). As acetyl CoA is produced in such high quantities, not all can enter the TCA cycle, and instead, excessive ketones are synthesized from acetyl CoA (Holtenius and Holtenius, 1996). During type 2 ketosis, dietary supplementation cannot mitigate ketosis as excessive hepatic acetyl CoA synthesis will not be prevented following high degrees of fat mobilization. Thus, instead, prepartum BCS should be managed; high BCS (≥ 4.0) can result in excessive FA mobilization post-calving, thus by ensuring cows are not over conditioned, excessive FA

mobilization will be reduced (Guliński, 2021), reducing FA arrival at the liver and subsequent hepatic acetyl CoA synthesis and ketone production.

Similar to ketosis, fatty liver typically occurs following great fat mobilization in response to negative energy balance. High levels of FA in blood travel to the liver, where the liver has a limited ability to fully oxidize or export FA, thus if FA are not converted to ketones, they are esterified and accumulate as TG in the liver (Bobe et al., 2004; Grummer, 2008; Mulligan, and Dohetry, 2008). Consequences of fatty liver include inflammation, and reduced hepatic gluconeogenesis, each of which can further contribute to negative energy balance (Bobe et al., 2004; Bradford et al., 2015). It is estimated that when the ratio of stored FA: glycogen exceeds 2:1, or when liver weight of TG exceeds 5 to 10%, additional metabolic disorders and dysfunction may develop (Drackley et al., 2001), including displaced abomasum, immune dysfunction, and ketosis (Bobe et al., 2004). Each of these conditions may also predispose the cow to fatty liver as they can reduce DMI, increasing FA mobilization and transport to the liver (NASEM, 2021), thus fatty liver may cause further metabolic disorders, or the reciprocal may also occur. Similar to type 2 ketosis, managing BCS in the prepartum period is essential to preventing fatty liver as BCS $\geq$ 4.0 is a major predisposing characteristic in cows with fatty liver (Bobe et al., 2004; Roche et al., 2013). Further, over conditioned cows have greater reductions in DMI which increases the extent of fat mobilization (Stockdale, 2001), thus increasing fatty liver risk.

Reproductive dysfunction and reduced reproductive efficiency are also associated with elevated blood FA and BHB concentrations, which have been associated with inflammation, uterine disorders, and impaired immune cell function (Hammon et al., 2006). Cows with subclinical endometritis or puerperal metritis have been reported to have higher FA and BHB

blood concentrations (Hammon et al., 2006). It does not appear clear however, if this is a cause-and-effect relationship or if the general state of negative energy balance is affecting both blood metabolites and reproductive performance concurrently.

Increased blood concentration of FA following lipomobilization is also associated with inflammation (Sordillo et al., 1995; Contreras et al., 2010; Haisan et al., 2021) and immune system dysfunction (Bradford et al., 2015; Contreras et al., 2018) in dairy cows. Inflammation occurring during high FA blood concentration may be associated with fatty liver (Yoshino et al., 1992; Katoh and Nakagawa, 1999), or low feed intake (Kvidera et al., 2017a,b). Based on the current literature available, it is difficult to differentiate if low feed intake increases fat mobilization, and subsequent inflammation, or if inflammation reduces feed intake which increases fat mobilization (Haisan et al., 2021). Inflammation is an energy demanding state (Kvidera et al., 2017c) which may increase mobilization of fat to be used as an energy source, thus we do not currently know if the onset of various inflammatory states are responsible for reduced intake and increased blood FA concentration, or if the inverse is true (Haisan et al., 2021).

1.3.2 Mastitis

Mastitis, or inflammation of the mammary, occurs primarily due to microbial infection, but may also occur following physical injury to the mammary (Cheng and Han, 2020). Infection of the mammary occurs almost exclusively through bacterial entry to the mammary through the teat canal, typically when cows have milk in their udder as the pressure from the milk can cause leakage in the gland, opening the teat end and allowing entry of pathogens (Ballou, 2012). Mastitis is a frequently occurring disease affecting dairy cattle and is associated with substantial economic, cow welfare, and human health concerns (Martin et al., 2018).

Following entry of the pathogen to the mammary, an array of local and recruited immune cells initiate a coordinated inflammatory response, with the goal of eliminating the pathogen (Thompson-Crispi et al., 2014). Neutrophils are the most abundant and active cells during mastitis, and they are largely responsible for abnormal appearing milk as well as increased somatic cell count (SCC) in milk (Khan and Khan, 2006). During mastitis, neutrophils migrate to infected areas via vasodilation of blood vessels (Dhondt et al., 1977) and increased blood flow, which largely contributes to swelling and heat of the udder during mastitis (Ballou, 2012). Through detection of pathogen-associated molecular patterns and a chemokine concentration gradients produced by resident macrophage and epithelial cells, neutrophils are able to locate the pathogens in the mammary, phagocytose the pathogen, and initiate enzymatic or oxidative death of the pathogen (Alnakip et al., 2014). Additionally, pro-inflammatory cytokines produced by macrophages recruit acute phase proteins (APP), such as haptoglobin (Hp), to the mammary to further assist in pathogen death (Quesnell et al., 2012).

Mastitis may be clinical, with signs of redness and swelling in the udder as well as abnormal milk secretions (Kelton et al., 1998), or it may be subclinical where clinical signs are not present but milk production is decreased, as is the case with clinical mastitis. Clinical mastitis in dairy cows in Canada is estimated to affect 23% of cows (Olde Riekerink et al., 2008), with *Staphylococcus aureus* being the most commonly isolated pathogen, followed by *Escherichia coli* and *Enterococcus* species (Reyher et al., 2011). Both clinical and subclinical mastitis increase SCC in milk and are of significant concern to producers due to negative health high SCC milk that cannot be sold for human consumption (Lescourret and Coulon, 1994; Hogeveen et al., 2011).

1.3.3 Metritis

Metritis is another common inflammatory condition in cattle that encompasses any inflammation of the uterus, including puerperal metritis, toxic puerperal metritis, pyometra, and endometritis (Smith and Risco, 2002; Sheldon et al., 2006). Each type of metritis is most prevalent during the post-calving period due to the dilated state of the cervix which allows the entry of pathogens and subsequent infection, especially in cases of dystocia, retained fetal membranes, and milk fever (Rosales and Ametaj, 2021). In addition to activating the cow's immune system, increasing energy requirements and compromising cow welfare (Sheldon et al., 2019), metritis negatively impacts herd profitability due to decreased milk production, reproductive efficiency, and increased risk of early culling (Pérez-Báez et al., 2021).

Cows that experience calving difficulties are subject to metritis as they often incur more vaginal lacerations and tearing in the process, which, in and of itself causes inflammation, in addition to the increasing access of pathogens to the cow's internal environment (Vieira-Neto et al., 2016). Most cows will experience a degree of microbial uterine contamination (Sheldon et al., 2002), which is eventually resolved in healthy cows (Cheong et al., 2017). However, cows that have calving difficulties or milk fever may have retained fetal membranes, which greatly increase the risk of bacterial contamination of the uterus (Laven and Peters, 1996). Cows with retained fetal membranes have a much greater chance of developing metritis (Han and Ki, 2005), due to the "bridge" for pathogens from the outside environment to the cow's uterus (Smith and Risco, 2002). Fetal membranes are typically expelled within 8 hours of delivery, and failure to do so within 12 hours of delivery is considered as retained fetal membranes (Hillman and Gilbert, 2008).

During infection of the uterus, inflammation is initiated by a neutrophil response, similar to that during mastitis, where a combination of local resident immune cells and recruited immune cells work together to kill and neutralize pathogens (Machado and Silva, 2020). Polymorphonuclear leukocytes (PMN) are among the first immune cells to response to uterine inflammation (Gilbert and Santos, 2016), and the proportion of PMN in uterine samples may be evaluated through cytology to assess the degree of inflammation (Kasimanickam et al., 2004; Gilbert et al., 2005). While metritis typically occurs in the first three weeks postpartum, clinical endometritis is defined as purulent uterine discharge beyond three weeks postpartum (Sheldon and Owens, 2017). Reduced DMI and increased blood FA concentration are associated with reduced immune function and increased metritis in the postpartum period, which may predispose cows to further health disorders (Hammon et al., 2006).

1.3.4 Gastrointestinal Acidosis

Gastrointestinal acidosis occurs following excessive fermentation in the GIT, primarily in the rumen and hindgut, where VFA accumulate, reducing the pH of the GIT, which alters the local microbial population, and may cause damage to the GIT epithelium (Gressley et al., 2011). Typically, fermentation products in the GIT are absorbed by the GIT epithelium, however, following sudden or large intakes of rapidly fermentable carbohydrates, grain in particular, the VFA production within the GIT exceeds the absorptive capacity of the epithelium, resulting in accumulation of VFA (Beauchemin and Penner, 2009). As the proportion of grain intake increases, the proportion of forage intake is typically decreased, which further reduces rumen pH due to less rumination and less salivary buffer production (Nordlund et al., 1995; Mertens, 1997). Sudden increases in fermentable carbohydrate intake is common in transition cows as these cows are fed increased allocations of grain, starting either in the closeup period or in the fresh period,

to meet the large energy demands of milk production post-calving (Useni et al., 2018). As dry cows are fed low grain, high forage diets, dry cows experience less GIT fermentation, and the transition to high grain intake requires time to facilitate adequate GIT growth that can absorb large quantities of fermentation products (Dieho et al., 2016a; Bannink et al., 2012). In instances where GIT fermentation exceeds the ability of the GIT to absorb or neutralize fermentation products, a plethora of negative consequences may occur (Aschenbach et al., 2011), including GIT and systemic inflammation (Khafipour et al., 2009a).

The precise threshold for acute ruminal acidosis varies, ranging from pH < 4.8 (Beauchemin and Penner, 2009) to pH < 5.2 (Owens et al., 1998), and includes clinical signs of reduced or no feed intake, increased heartbeat and respiration rate, diarrhea, lethargy, and even death (Krause and Oetzel, 2006). Acute ruminal acidosis is of great concern as it has the potential to progress to metabolic acidosis (Brown et al., 2000), with blood pH < 7.4 (van Gastelen et al., 2021a), following increased acid in blood and depletion of blood bicarbonate (< 25.0 mmol/L; Aschenbach et al., 2009; van Gastelen et al., 2021a). Subacute ruminal acidosis (SARA) is categorized by repeated bouts of decreased rumen pH, lasting several minutes to several hours, with each bout returning to baseline pH, rather than remaining low like acute acidosis (Beauchemin and Penner, 2009). Although SARA is not as severe as acute ruminal acidosis, SARA still negatively affects cows as SARA reduces fiber digestion (Russel and Wilson, 1996) and absorptive capacity of the GIT (Harmon et al., 1985), and may damage the GIT epithelium (Beauchemin and Penner, 2009).

Historically, ruminal acidosis has received the vast majority of attention, compared to hindgut acidosis. In the last fifteen years, research surrounding hindgut acidosis has increased, improving our understanding of the etiology and impact of hindgut acidosis, although much is

still unknown. Hindgut-derived VFA from microbial fermentation of carbohydrates are generally absorbed through the hindgut epithelium (Argenzio et al., 1975; Gressley et al., 2011), but like ruminal acidosis, following excessive fermentation, VFA production may exceed absorption capacity leading to VFA accumulation (Gressley et al., 2011) and reduction of the pH in the hindgut lumen, around 6.0 (van Gastele et al., 2021a,b). Hindgut acidosis typically occurs following grain overload in the rumen, poor rumen mat function, increased feed intake and thus reduced rumen retention time, or a combination of these, which increase the arrival of feedstuff and subsequent fermentation in the hindgut (Gressley et al., 2011; Plaizer et al., 2018). Under typical conditions, epithelial cells can withstand regular fermentation in the hindgut which is limited by a steady flow of fermentable substrates (Bayley, 1978). However, during hindgut acidosis, a combination of decreased pH and osmotic pressure from excessive fermentation may damage the GIT epithelium and induce local inflammation (Owens et al., 1998; Plaizier et al., 2008; Gressley et al., 2011). As a consequence of the damaged GIT epithelium, barrier function may be reduced in the GIT, especially in the hindgut due to the monolayer of epithelial cells, rather than multiple layers in the rumen, which can allow translocation of microbes and toxins from the GIT lumen to the bloodstream, potentially inducing systemic inflammation (Khafipour et al., 2009a; Gressley et al., 2011).

Overall, dairy cows commonly experience an array of disorders and metabolic dysfunction during their lactation, often during the transition period. These disorders and metabolic dysfunction are commonly associated with additional energy expenditure towards to immune system, inflammation, compromised health status, and reduced performance. As such, preventing and management of disorders and metabolic dysfunction is crucial to dairy cow health and farm productivity.

1.4 Inflammation

Inflammation is the coordinated response of the body's immune system following stimuli such as tissue injury, infection, physiological events, or certain environmental conditions (Medzhitov, 2008; Bertoni et al., 2015). Inflammation usually occurs localized to one tissue or region initially, but unresolved inflammation or pathogenic entry to the bloodstream following inflammation may result in systemic inflammation (Zotova et al., 2016). Local inflammation begins with the activation of resident immune cells and receptors, such as macrophages and TLR, which recognize various toxins in the gut lumen, activating an inflammatory cascade, including activation of inflammatory transcription factors and cytokine production (Chen et al., 2018). If the injury or infection cannot be resolved by local immune cells alone, additional immune cells are recruited to the affected site, executing an integrated immune response in tandem with local immune cells to promote healing in the area, and in the case of infection-induced inflammation, kill or neutralize pathogens present (Medzhitov 2008; Bertoni et al., 2015). Common types of local inflammation in dairy cattle include mastitis, metritis, and gastrointestinal inflammation, each of which have the potential to progress to systemic inflammation (Bradford et al., 2015) and is commonly assessed through cytokine and immune cell production and activity, gene expression, and APP, hormone, and metabolite concentrations (Mezzetti et al., 2020).

1.4.1 Gastrointestinal Inflammation

Research assessing GIT inflammation in ruminants has historically focused on ruminal acidosis-induced GIT inflammation, although research assessing inflammation following hindgut acidosis is increasing. It should also be acknowledged that there are other sources of GIT inflammation, aside from acidosis, such as GIT damage associated with reduced feed intake

(Bertoni et al., 2015), infection (Holschbach et al., 2018; Mallikarjunappa et al., 2021), or stress (Plaizier et al., 2018).

1.4.1.1 Rumen vs. Hindgut

The rumen and hindgut may both undergo local inflammation following epithelial cell damage during acidosis (Nocek, 1997; Kleen et al., 2003; Steele et al., 2009), reduced intake (Bertoni et al., 2018), infection, or stress (Plaizer et al., 2018). Epithelial cell damage in the GIT during acidosis is at least partially attributed to reduced pH, osmotic pressure, hypoxia, parakeratosis, and the increased presence of toxins such as lipopolysaccharide (LPS) and histamine (Aschenbach et al., 2019), although the extent of each factor's contribution remains to be determined, and may differ in the hindgut compared to the rumen. Moreover, during infectious disease or metabolic disorders (Khalil et al., 2022), including GIT acidosis, microbial population composition may be altered (Plaizer et al., 2017), which can lead to microbial dysbiosis (Neubauer et al., 2020) and inflammation in the GIT (Krogstad and Bradford et al., 2023).

During GIT inflammation, the rumen epithelium appears to be more resilient to damage due to its structure and physiological conditions, compared to the monolayered hindgut epithelium (Gressley et al., 2011; Sanz-Fernandez et al., 2020). Rumen inflammation, also known as rumenitis, can be induced following ruminal acidosis, although the precise pathogenesis is not well described in current literature (Zhao et al., 2018). As described in section 1.1.4, the stratified squamous epithelium of the rumen, particularly the keratinized outermost cell layer, provides protection of the epithelium against microbial and fermentation-related damage (Graham and Simmons, 2005; Steele et al., 2011a; Steele et al., 2016). During excessive fermentation or increased pathogen concentration in the rumen, however, the outer

keratinized cell layer may be damaged including alterations to cell morphology, cell sloughing, and weakened cells (Steele et al., 2009), leading to localized inflammation of the rumen epithelium (Owens et al., 1998; Plaizier et al., 2008). Similar to the rumen, the hindgut epithelial may slough or weaken following excessive fermentation and increased pathogen presence (Gressley et al., 2011). However, as the hindgut only contains a single-layered epithelium and does not have multiple layers of protection, damage to the hindgut can be more severe, resulting in blood and mucin casts observed in feces (Bissell and Hall, 2010).

Both the rumen and hindgut contain a diverse array of microbes within its mucosa that contribute to barrier function (Mao et al., 2015), and can adapt to some extent of increased flow of fermentable substrates by increasing microbial numbers, altering VFA proportions, and decreasing pH as VFAs are produced (Ørskov et al., 1970; Mann and Ørskov, 1973). However, microbiota may be altered in composition and function during prolonged periods of reduced pH, which is of particular concern in the hindgut due to the monolayer of cells (Gressley et al., 2011). Additionally, the hindgut does not have protozoa or salivary bicarbonate present to modulate digesta pH (Immig, 1996; Gressley et al., 2011), thus making the hindgut more vulnerable to inflammation and barrier damage compared to the rumen (Tao et al., 2014a; Steele et al., 2016).

1.4.1.2 Leaky Gut

Leaky gut, also known as reduced GIT barrier function or GIT hyperpermeability, may occur following environmental stressors, infection, reduced feed intake, or physical trauma to the GIT (Stewart et al., 2017). In healthy cows, the GIT epithelial maintains a barrier between gut lumen contents and systemic blood circulation through microbial, immunological, and mechanical means (Steele et al., 2016). However, during any of the aforementioned stressors, leaky gut involves disruption of the GIT epithelial barrier through morphological changes or

destruction of epithelial cells (Pearce et al., 2013; Kvidera et al., 2017a), resulting in translocation of microbes and toxins into blood circulation (Twardowska et al., 2022).

Heat stress, an environmental stressor, is one of the most widely studied causes for leaky gut in dairy cattle. During heat stress, blood flow to gastrointestinal epithelial cells is reduced as blood is redirected to the periphery of the body to dissipate heat, resulting in intestinal hypoxia and nutrient restriction (Hall et al., 2001; Rollwagen et al., 2006), altering the morphology and function of epithelial cells, leading to leaky gut (Lambert et al., 2002; Pearce et al., 2013). Leaky gut may also occur following reductions in feed intake, which may occur during heat stress, weaning, transportation, overcrowding, restraint, social isolation or social mixing (Chen et al., 2015). Stress itself may induce leaky gut due to increased hormone secretion, related to stress, that appears to compromise gut barrier integrity (Wallon et al., 2008; Vanuytsel et al., 2014; Rodiño-Janeiro et al., 2015) and due to decreased feed intake that reduces cell proliferation, increasing GIT permeability (Kvidera et al., 2017b).

Physical damage to the GIT, such as during GIT acidosis or microbial infection and dysbiosis, also can induce leaky gut (Stewart et al., 2017). Leaky gut is not induced solely by low pH (Meissner et al., 2017) or hyperosmolarity during acidosis, but rather, inflammation, damage, and sloughing of epithelial cells and associated tight junctions precedes leaky gut (Steele et al., 2009; Penner et al., 2010). Thus, due to a combination of inflamed and damaged epithelial cells, and reduced tight junction activity between cells, gut barrier function is compromised during acidosis, which may allow translocation of microbes and toxins, such as LPS, from the gut lumen to blood circulation (Stewart et al., 2017). Lipopolysaccharide has received immense attention in leaky gut and systemic inflammation research, perhaps because

LPS is the most abundant and prolific in the GIT and because of its well established role in immune system activation (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997).

1.4.2 Systemic Inflammation

Systemic inflammation differs from local inflammation, in which local inflammatory events and subsequent immune responses are isolated to a single tissue, whereas systemic inflammation is characterized by increased presence of pro-inflammatory proteins and cytokines in blood, indicating inflammation is "systemic" (Brøchner and Toft, 2009). Systemic inflammation may occur when microbes or toxins from local inflammatory events are introduced into blood circulation, or it may occur following dysregulation of the immune system (Hakansson and Molin, 2011).

Microbial uterine infections in the postpartum period are often associated with local uterine inflammation, however, in some cows the local inflammation can advance to systemic inflammation (Huzzey et al., 2009). Systemic inflammation has been reported to following metritis and mastitis as toxins, LPS in particular, from the uterus (Peter et al., 1990; Cheong et al., 2017), or mammary glands (Ohtsuka et al., 2001) translocate into surrounding blood vessels, and enter systemic blood circulation (Mani et al., 2012). Gastrointestinal tract-derived LPS may also translocate from the gut lumen to the bloodstream during leaky gut, and travel to the mammary tissue, inducing mastitis (Zhang et al., 2016; Hu et al., 2022), or to the lamellae of the hooves, inducing laminitis (Boosman et al., 1991), thus demonstrating the potential of local inflammation to progress to systemic inflammation, whereby multiple tissues or systems are affected.

Rumen or hindgut acidosis are also commonly studied causal factors for systemic inflammation. The onset and extent of inflammation following GIT acidosis and leaky gut is inconsistent in research; however, production losses following GIT acidosis are proposed to be at least partially due to inflammation following epithelial damage and LPS transport into the bloodstream (Gozho, et al., 2005; Khafipour, et al., 2009a). Leaky gut associated with GIT acidosis is described as a major entry point for microbes or toxins, LPS, in particular, into the bloodstream (Plaizier et al., 2018). During GIT acidosis, pH is reduced causing lysis of Gram-negative bacteria and LPS release (Hurley, 1995; Wells and Russell, 1996), which when coupled with leaky gut, may result in LPS translocation from the rumen or hindgut (Aschenbach et al., 2019). When LPS travels through the gut epithelial barrier and enters blood circulation, immune activation occurs where immune cells within the bloodstream bind and detoxify LPS (Ceciliani et al., 2012). Additionally, LPS within the bloodstream can be detoxified by the liver and Kupffer cells, however, if the concentration of LPS surpasses the detoxification capacity of the various immune cells, systemic inflammation will follow (Plaizer et al., 2018).

There is some debate regarding the origin of circulating LPS following GIT acidosis; some have proposed the rumen as a point of entry for LPS (Minuti et al., 2014; Emmanuel et al., 2007; Guo et al., 2017), whereas others have suggested the hindgut is the primary site of LPS translocation due to the hindgut's apparent greater susceptibility to inflammation and cell damage (Khafipour et al., 2009a,b; Gressley et al., 2011; Li et al., 2012a). As well, it appears GIT acidosis does not consistently cause LPS detection in the bloodstream (Plaizer et al. 2018) or systemic inflammation, despite confirming acidosis in the rumen (Khafipour et al., 2009b; Li et al., 2012a) or hindgut (Pianotoni et al., 2018; van Gastele et al., 2021a,b). The relationship between GIT acidosis, leaky gut, and systemic inflammation requires further exploration, and

factors explaining why systemic inflammation occurs in some instances of GIT acidosis, but not in others, remains to be elucidated.

1.4.3 Consequences of Inflammation

Inflammation is an energy-demanding process (Mireles et al., 2005; DiAngelo et al., 2009; Tang et al., 2010), thus when cows are experiencing inflammation, energy is allocated towards the immune system, rather than towards milk production or reproduction (Bradford et al., 2015). It is suggested that the magnitude and persistency of inflammation affects future cow performance, especially during the transition period when inflammatory conditions are most prevalent (Bertoni et al., 2015; Bradford et al., 2015; Trevisi and Minuti, 2018). Even in the absence of clinical inflammatory signs, energy efficiency was reduced by 15% in cows with subclinical inflammation (Trevisi et al. 2010). To support large energy demands during inflammation, tissue metabolism is also altered to spare energy for the immune system (Wolowczuk et al., 2008; Bradford et al., 2015), compromising the function of various tissues and organs. Glucose is a primary energy source of various immune cells, thus its availability for normal physiological functions as well as milk production is reduced during inflammation (Bradford et al., 2015). This was confirmed by Bertoni et al. (2008) who found transition cows experiencing inflammation not only had reduced milk production, but also experience more severe negative energy balance, had greater reductions in BCS, and greater ketone production. Similarly, reproduction is negatively affected due to less energy availability and altered hormone concentrations, which prolongs follicle formation and ovulation (Beam and Butler, 1997; Butler et al., 2006; Cheong et al., 2015), and increases the number of inseminations per pregnancy and open days (Bertoni et al., 2008).

In addition to altered glucose metabolism, insulin-resistance often accompanies inflammation to further increase energy supply during inflammation (Odegaard and Chawla, 2012). Insulin resistance may increase FA mobilization to serve as an additional energy supply, however, high blood FA can result in metabolic disorders, as previously described (Ohtsuka et al., 2001; Zachut et al., 2013). Reduced intake is often reported alongside inflammation in cows (Kushibiki et al., 2003; Yuan et al., 2013), which can further increase FA mobilization and inflammation (Sordillo et al., 2016). In general, cows with higher inflammation are suggested to have compromised welfare (Calamari et al., 2008), further emphasizing the impact of inflammation in cattle.

1.4.4 Monitoring and Management of Inflammation

1.4.4.1 Markers of Inflammation

Acute phase proteins are the most extensively used markers of inflammation in dairy cattle research due to their significant changes in serum concentration during inflammation (Schmitt et al., 2021). During inflammation, the liver adjusts synthesis and release of APP, which aid in blood coagulation, infection defense, circulation of metabolites, nutrients, and hormones, and maintaining homeostasis during infection (Gabay and Kushner, 1999; Devaraj et al., 2009). Acute phase proteins are classified as negative or positive; negative APP include albumin, transferrin, retinol-binding protein, and antithrombin, each of which decrease in concentration during inflammation (Fleck, 1989; Gruys et al., 2005). Conversely, positive APP include Hp and serum amyloid A (SAA; Powanda, 1980), each of which increases in concentration more than 25% during inflammation (Eckersall and Bell, 2010), infection, and trauma (Murata et al., 2004; Petersen et al., 2004; Ceron et al., 2005). In dairy cattle research, Hp and SAA are the most frequently used APP for identifying inflammation such as mastitis (Eckersall et al., 2001;

Grönlund et al., 2005; Nazifi et al., 2010), metritis, (Huzzey et al., 2009; Burke et al., 2010a; Kaya et al., 2016) laminitis (Ilievska et al., 2019), and gastrointestinal inflammation (Gozho et al., 2007; Khafipour et al., 2009a). When released, Hp acts as an antioxidant, antimicrobial, and anti-inflammatory agent (El Ghmati et al., 1996), whereas SAA recruits immune cells, modulates inflammatory response, and binds LPS (Schroedl et al., 2001; Gulhar et al., 2022).

While APP are used as inflammatory markers for an array of conditions, due to their increase during numerous events, their specificity is limited and it may be difficult to identify specific sources of inflammation (Humblet et al., 2008). This is particularly true when identifying post-calving inflammation, as calving appear to increase the concentration of APP, even in healthy cows (Tóthová et al., 2008; Qu et al., 2014), perhaps due to stress (Giannetto et al., 2011; Schmitt et al., 2021) and decreased feed intake (Cappellozza et al., 2011; Marques et al., 2012), which are also reported to increase APP. During clinical inflammatory conditions, such as clinical mastitis, clinical signs of inflammation such as heat, swelling, or redness (Morin et al., 1998), may be easily identified, thus when increased APP are accompanied by clinical signs, it may be more accurate to attribute increased APP to a specific source of inflammation. However, in the absence of clinical symptoms, during subclinical inflammation, it can be difficult to decipher the specific inflammatory event that is causing increased APP concentrations. In addition, during GIT inflammation, clinical signs exhibited, such as reduced DMI or milk production, are not exclusive to GIT inflammation and occur during numerous conditions, as previously described, thus precisely identifying the occurrence of GIT inflammation is difficult, if not impossible.

Dairy cattle research investigating gastrointestinal inflammation has traditionally used APP concentration or GIT biopsies to assess the extent of inflammation occurring in the GIT. As

highlighted, increases in APP may occur during an array of inflammatory conditions or other stressors, and do not occur solely during GIT inflammation, thus the accuracy of interpreting GIT inflammation based on APP concentration alone is limited. Collecting biopsies from the GIT tract and analyzing for histology or inflammatory genes or proteins present in tissue is more accurate and representative of the extent of GIT inflammation (Washabau et al., 2010; Geboes et al., 2013; van Niekerk et al., 2018). However, collecting biopsies is a more invasive procedure that is not practical on-farm, and the collection and analysis of samples requires substantial time, effort, and money. Therefore, identifying a marker specific to GIT inflammation in dairy cattle is imperative to better understanding of GIT inflammation. Without an accurate measure of GIT inflammation, we are limited in our ability to manage it as we cannot accurately identify when it is occurring, and we cannot evaluate the efficacy in treatments for GIT inflammation.

As there is a strong need for a marker specific to GIT inflammation in cows, fecal calprotectin has been identified as a potential marker for GIT inflammation in cows; calprotectin is a protein released by neutrophils along the GIT during inflammation and binds calcium and zinc to starve pathogens in the GIT (Bjarnason, 2017). Fecal calprotectin is widely used in human medicine to quantify intestinal inflammation (Alibrahim et al., 2015; D'Angelo et al., 2017) as the amount of calprotectin present reflects active neutrophils in the GIT during inflammation (Bjarnason, 2017). The accuracy of calprotectin in identifying GIT inflammation is further confirmed by its correlation with endoscopic and histological assessments of GIT inflammation in human medicine (Konikoff and Denson, 2006). In humans, calprotectin is made up of two protein subunits, A8 and A9 (accession # P05109.1 and #P06702.1, respectively), which are also encoded in the bovine genome (accession #P28782.2 and P28783.3, respectively), indicating calprotectin is also present in bovines (NCBI, 2021). Therefore, fecal calprotectin may

exert a similar role in GIT inflammation in cows and may be a suitable marker for gastrointestinal inflammation.

1.4.4.2 Butyrate

Butyrate, a VFA produced following microbial fermentation in the GIT (Bergman, 1990), has received much attention in the past fifteen years as a feed additive for gastrointestinal development, barrier function, and inflammation modulation (Górka et al., 2018). Butyrate can be used as an energy source for epithelial cells along the GIT, with 90% of butyrate being metabolized by colonocytes (Hamer et al., 2008). The effects of supplemental butyrate have been assessed in an array of species for its effects in the GIT in regards to antimicrobial activity (Kabara et al., 1972, Thormar et al., 2006; Guilloteau et al., 2009), gene expression regulation (Canani et al., 2011; Guilloteau et al., 2009), increased cell growth (Sakata and Tamate, 1978; Guilloteau et al., 2009; Górka et al., 2011 a,b), increased absorption of nutrients (Ruppin et al., 1980), and reduction in inflammation (Vinolo et al., 2011).

In cows, butyrate is proposed to be essential in restoring GIT function following damage (Dionissopoulos et al., 2013), which may support the supplementation of dietary butyrate during the calving transition period when GIT inflammation commonly occurs, as described in sections 1.2.2, 1.3.4, and 1.4.1.

Local GIT inflammation may also be reduced with butyrate supplementation as butyrate may reduce the activity of enzymes responsible for inflammation induction (Sauer et al., 2007). Butyrate supplementation may also improve barrier function through increased epithelial cell proliferation (Blottiere et al., 2003), growth (Guilloteau et al., 2010; Górka et al., 2011a,b; Kowalski et al., 2015), and tight junction formation (Peng et al., 2009; Baldwin et al., 2012).

Increased barrier function of the GIT may decrease systemic inflammation due to the prevention of absorption of toxins from the gut lumen to blood circulation (Lewis et al., 2010), which has been reported following butyrate supplementation in goats (Chang et al., 2018). In cows, butyrate improves barrier function and reduces inflammation by strengthening the stratum corneum of the rumen epithelium, reducing immune cell recruitment, preventing LPS-induced inflammation, and decreasing inflammatory gene expression (Dai et al., 2017). An additional way butyrate may reduce inflammation in dairy cows is through the increased production of glucagon-like peptide 2 (Taylor-Edwards et al., 2011; Fukumori et al., 2020), which is a hormone produced in the intestine that increases growth, digestion, absorption, barrier function, and blood flow, and may reduce inflammation (Brubaker, 2018), both locally and systemically.

Butyrate may also play a role in modulating FA metabolism as dairy cows supplemented with butyrate had reduced blood FA concentration (Herrick et al., 2017; Halfen et al., 2021), perhaps due to butyrate's role in reducing lipolysis of adipocytes as reported in humans (Yan and Ajuwon, 2015). Similar findings in humans reveal BHB supplementation, a metabolite of butyrate, also reduced blood FA concentration (Metz and Bergh, 1972; Mielenz, 2017). As elevated blood concentrations of FA are associated with increased inflammation in dairy cows, perhaps butyrate partially reduces inflammation through its reduction in circulating FA. As FA concentrations are increasing during the calving transition period, it is possible butyrate supplementation may reduce blood FA concentration and associated inflammation.

1.4.4.3 NSAID

The administration of non-steroidal anti-inflammatory drugs (NSAID) has been explored as a method of reducing inflammation in dairy cattle (Trimboli et al., 2020). Administration of NSAID reduced inflammation through inhibition of the cyclooxygenase (COX) enzyme activity

which subsequently prevents the conversion of arachidonic acid to prostaglandins (Vane et al., 1996). Within the NSAID category, there are multiple types of drugs, each with differing mechanisms, half-lives, and dosages, potentially differing in their efficacy in affecting animal physiology and inflammation, but the exact differences have not been extensively explored in dairy cattle. Moreover, how NSAID administration affects transition cows, compared to cows at later stages of lactation, is not well understood.

Treatment with NSAID has shown reductions in inflammation and improvements in performance for cows with metritis (Amiridis et al., 2002), mastitis (Königsson et al., 2002; Vangroenweghe et al., 2005), and general postpartum inflammation (Yuan et al., 2001). Reduced inflammation following NSAID treatment in dairy cows may increase milk yield (Bertoni et al., 2006; Trevisi and Bertoni, 2008), and milk fat and protein (Farney et al., 2013a), as well as reducing the number of cows exiting the herd (McDougall et al., 2009). However, conflicting results exist with some studies finding NSAID treatment did not improve reproductive function (Drillich et al., 2007), or milk production (Schwartz et al., 2009). In fact, administration of NSAID has also been reported to cause negative side effects in cows treated such as decreased intake (Carpenter et al., 2018), fever, stillbirth, decreased productivity, retained placenta and metritis (Schwartz et al., 2009; Newby et al., 2017).

As most NSAID reduce or prevent inflammation via prostaglandin inhibition, placental expulsion may be impaired; prostaglandins play an essential role in placental tissue expulsion (Laven and Peters, 1996), thus administering NSAID soon after parturition, retention of fetal membranes may occur, which may lead to metritis further inflammation (Bradford et al., 2015). Duffield et al. (2009) administered NSAID two hours postpartum and found an increased incidence in retained fetal membrane, and Waelchli et al. (1999) administered flunixin

meglumine to cows immediately following a caesarean section and found cows given the NSAID were more likely to retain fetal membranes. These results may be due to impaired prostaglandin production due to NSAID administration thus impairing uterine contractions and fetal membrane expulsion (Horta, 1984).

Additionally, NSAID administration may block the onset of inflammation in the short term, but may actually increase overall inflammation and reduce cow productivity in the long term (Schwartz et al., 2009; Newby et al., 2017). Inflammation following infection is necessary to some extent to clear pathogens from the body and promote healing (Bradford et al., 2015), especially following parturition when inflammation in clinically healthy cattle is postulated to contribute to homeorhetic adaptions to lactation (Bionaz et al., 2007; Graugnard et al., 2012; Mullins et al., 2012). When NSAID are used to block initial inflammation, "rebound inflammation" may occur when NSAID effects subside, and inflammation may proceed and interfere with other physiological processes (Bradford et al., 2015). Therefore, the timing of NSAID administration (Meier et al., 2014a; Trimboli et al., 2020), and potential negative side effects of NSAID must be further evaluated in transition cows.

In summary, inflammation, regardless of the source, involves a coordinated immune response which reduces energy available for other processes such as milk production and reproduction. Gastrointestinal inflammation is of particular concern due to its potential relationship with leaky gut and systemic inflammation, although the precise relationship is not well understood. Extensive research has assessed how to prevent and treat inflammation in dairy cows, however, much is still unknown about how to best manage inflammation.

1.5 Knowledge Gap

A knowledge gap remains regarding the optimal management of inflammation in dairy cows, specifically during the calving transition period. To improve understanding and management of inflammation, further research is required in transition cows investigating nutritional management strategies, NSAID administration, factors related to gut permeability, factors associated with variation in voluntary hay intake, and identification of a biomarker specific to GIT inflammation.

Butyrate supplementation may be a nutritional management strategy to reduce inflammation in fresh cows through its anti-inflammatory properties and improvement of the gut barrier function, but additional research in fresh cows is required to determine butyrate's efficacy. Inflammation in postpartum cows may also be reduced through administration of NSAID, however, the timing NSAID is given to fresh cows must be assessed. Research has never evaluated the concurrent use of dietary butyrate supplementation and NSAID administration to reduce inflammation and improve performance in dairy cows, thus this remains a knowledge gap.

An additional area requiring research attention is the optimal dietary starch and NDF content, and the effects of increasing dietary starch content on cow performance and health. Cow response to increasing dietary starch appears inconsistent throughout literature, but the precise rationale behind this unknown. Similarly, the relationship between dietary starch content, gut permeability, and inflammation is not well understood, and requires further investigation. As well, substantial inter-cow variation, even when cows are fed the same diet and are of similar DIM, presents an additional challenge and knowledge gap in dairy cow management; when dietary starch is increased during the transition period, some cows respond positively with

increased milk yield, whereas others may decrease milk yield and experience metabolic disorders, thus further research is required to determine how to best accommodate inter-cow variation. Offering free choice hay to fresh cows alongside TMR may allow provision of a high starch diet to meet energy needs, while allowing cows experiencing metabolic dysfunction to reduce starch intake from TMR and increase fiber intake. However, the effects of offering free choice hay to fresh cows have never been evaluated.

A further opportunity for future research is the identification of markers unique to specific inflammatory events, which is especially important in GIT inflammation; GIT inflammation has been frequently described to compromise health and performance of dairy cows, but there is no marker that can precisely detect when it is occurring, thus limiting our ability to diagnose GIT inflammation, or to evaluate the efficacy of anti-inflammatory treatments in the GIT. Therefore, there is a strong need for validation of a GIT-specific inflammatory marker, such as calprotectin. Calprotectin has been extensively researched and proven as an accurate marker of GIT inflammation in human medicine, but no research has been conducted in ruminants.

Therefore, the objectives of this research were to 1) evaluate the effects of dietary butyrate supplementation and oral NSAID drug administration on inflammation and performance in transition dairy cow, 2) assess the effects of offering free choice hay to cows for the first five days postpartum on inflammation, gut permeability, and performance, and 3) evaluate the efficacy of calprotectin as a marker of gastrointestinal inflammation.

Chapter 2. Effects of dietary butyrate supplementation and oral nonsteroidal anti-inflammatory drug administration on serum inflammatory markers and productivity of dairy cows during the calving transition

2.1 Introduction

Dairy cattle experience inflammation during the calving transition period, which decreases energy available for milk production (Bradford et al., 2015). Some inflammation occurs in the gastrointestinal tract (GIT) when fresh cows are fed high-grain diets to meet the high energy requirements associated with lactation. As grain rapidly ferments in the GIT, local inflammation of the GIT can occur, compromising gut barrier function and allowing absorption of toxins into circulation, which can induce systemic inflammation (Plaizier et al., 2012). Prolonged or slowly-resolved inflammation can increase the likelihood of cows developing metabolic disorders during the calving transition period (Sordillo and Raphael, 2013), increasing economic costs (Kerslake et al., 2018) and decreasing animal production (Bertoni et al., 2008) and welfare (Abeni and Bertoni, 2009). Therefore, it is imperative to prevent pathological inflammation when possible.

Butyrate is a VFA produced by microbial fermentation (Bergman, 1990) and one of the primary energy sources for the rumen and hindgut (Hamer et al., 2008). Butyrate is expected to reduce GIT and systemic inflammation as it stimulates proliferation of epithelial cells (Blottière et al., 2003), increases epithelial cell growth in GIT (Guilloteau et al., 2010; Górka et al., 2011a,b), and increases tight junction formation (Bordin et al., 2004; Peng et al., 2009), ultimately reducing pathogen translocation across colon epithelium (Lewis et al., 2010). Butyrate can also reduce inflammation by reducing cyclooxygenase (COX)-2 enzyme activity (Sauer et al., 2007), which mediates inflammation (Simon, 1999). Much previous research evaluated the

effects of butyrate in lactating cows (Herrick et al., 2017; Fukumori et al., 2020; Halfen et al., 2021). However, there is little research evaluating the effects of butyrate supplementation during the calving transition period on performance and inflammation. Although Kowalski et al. (2015) evaluated the effects of butyrate supplementation in cows during the calving transition, butyrate was only supplemented in the close-up period; thus, research assessing the effects of butyrate in the fresh period is lacking.

Administration of nonsteroidal anti-inflammatory drugs (NSAID) is another approach to reduce inflammation in the calving transition period. Meloxicam, an NSAID, inhibits the action of COX-2 (Lees et al., 2004; Beretta et al., 2005), and has been suggested to be an effective method of alleviating inflammation (Bogado Pascottini et al., 2020) and increasing milk production (Carpenter et al., 2016; Shock et al., 2018; Swartz et al., 2018). Both butyrate and NSAID are expected to reduce inflammation that occurs during the calving transition period through inhibition of COX-2; however, it is unknown if using butyrate and NSAID in conjunction will exert an additive effect as their efficacy has not been evaluated together and their interactions are unknown.

We hypothesized that dietary butyrate supplementation and oral NSAID administration would reduce inflammation during the calving transition period and increase milk yield. The objective of the present study was to evaluate the effects of dietary butyrate supplementation during the entire calving transition period and oral NSAID administration after calving on serum inflammatory markers, plasma metabolites, and production performance in cows during the calving transition period.

2.2 Materials and Methods

This study was conducted at the University of Alberta Dairy Research and Technology Centre, a 146-cow tiestall barn (Edmonton, Alberta, Canada), in 2019 to 2020. All procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP00003364).

2.2.1 Animals, Diets, and Experimental Design

Eighty-three (47 primiparous, 36 multiparous) Holstein cows were used to evaluate the effects of dietary butyrate supplementation and oral NSAID administration in a 2 × 2 factorial arrangement of treatment. Cows were blocked by parity and calving date and randomly assigned to treatment. Cows were assigned to 1 of 2 isoenergetic diets containing fatty acid (FA)- coated calcium butyrate (1.0% butyrate, 0.24% calcium, and 0.18% fatty acids; Probiotech International) or a control supplement [1.04% commercial fat supplement (Jefo Dairy Fat; 85% palmitic acid; Jefo Nutrition Inc.) and 0.38% calcium carbonate] in TMR. The butyrate supplementation rate was determined based on previous research to maximize its supply without causing feed intake reduction. As reductions in feed intake were reported when lactating cows were fed 2.0% butyrate in feed (Urrutia et al., 2019), but not when 1.1% butyrate was supplemented (Izumi et al., 2019), we fed butyrate at 1.0% (as calcium butyrate at 1.42%) of diet DM. Assuming 0.5 mol of basal butyrate production per kilogram of DMI (Sutton et al., 2003), our supplementation rate was expected to increase butyrate supply to the animal by more than 20%.

Cows received an oral NSAID (meloxicam 15 mg/mL oral suspension; Solvet) at 1 mL/15 kg of BW in carrier solution (equivalent to 1 mg of meloxicam/kg of BW; as recommended by the product label and determined by Shock et al., 2018, 2019) or a placebo (food dye at 1 mL/15 kg of BW in carrier solution) at 12 to 24 h after calving. Cows were

assigned to drug treatment randomly, but all study personnel were blinded to NSAID treatment until completion of data analysis. To administer the NSAID or placebo, cows were restrained in a chute, and an oral drench gun was used to deposit treatment into the back of the mouth while their heads tipped slightly upwards with a halter to prevent the spillage. Cows were kept immobilized until drug had been swallowed.

The experimental close-up TMR contained 13.3% starch and 42.4% NDF and were fed from 28 d before expected calving date to calving (Table 1). The experimental fresh cow TMR contained 22.1% starch and 34.1% NDF on a DM basis and was fed from calving until 24 DIM. Day 1 was defined as the first day cows received experimental fresh cow diets. Diets were formulated using the Nutritional Dynamic System (NDS; CNCPS version 6.55, RUM&N) to meet or exceed nutrient requirements for a 650-kg cow producing 31 kg/d of milk with 3.8% milk fat and 3.0% milk protein. Cows were fed individually in mangers once daily at 0800 h at 105 to 110% of actual intake of the previous day and had ad libitum water access. Cows were milked daily at 0500 and 1600 h.

2.2.2 Data and Sample Collection

Daily feed provision and orts were weighed to calculate daily intake. Samples of ration ingredients were collected once per week and dried in a forced-air oven at 55°C for 48 h to calculate feed DM, and the ration was adjusted if feed DM deviated more than 2%. Samples were then ground in a Wiley mill (Thomas Scientific) with a 1-mm screen and saved for later nutrient analysis.

Milk yield was recorded at each milking. Milk samples were collected once a week from consecutive morning and afternoon milkings for each cow at 7, 14, and 21 DIM ± 3 d. Body condition score was recorded at −28 d (± 3 d) relative to expected calving date, on the day of

calving, and at 24 DIM (± 3 d) by 2 trained individuals using the 5-point scale (Wildman et al., 1982), and the average BCS was recorded. Body weight was recorded at enrolment at −28 d (± 3 d) relative to expected calving date, on the day of calving and at 1 DIM (averaged for d-1 BW), and at 24 and 25 DIM (± 3 d; averaged for d-24 BW). Body weight was always measured after the morning milking but before feeding. Changes in BW and BCS from d 1 to d 24 were calculated.

Blood samples were collected before feeding, at approximately 0700 h on d −28 ± 3 from expected calving date before the experimental diets were fed, twice a week starting at d −10 relative to expected calving date until calving, and d 4, 7, and 21 ± 3 relative to calving. Additional blood samples were collected 12 to 24 h after calving, immediately before the administration of NSAID or placebo. The prepartum blood sample closest to d −4 relative to actual calving date was selected for each cow and analyzed. Blood was collected from the coccygeal blood vessels into 2 Vacutainers (Becton Dickinson and Co.), one containing sodium heparin for plasma, and the other without an anticoagulant for serum. The sodium heparin–containing tube was placed on ice immediately after collection until centrifuging. The serum tube was kept at room temperature for a minimum of 30 min or until the blood was clotted. Blood was centrifuged at 3,000 × g (20 min, 4°C) and stored at −20°C until analysis.

2.2.3 Sample Analyses

Feed samples were sent to Cumberland Valley Analytical Services (Hagerstown, MD), and analyzed for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), CP (AOAC International, 2000; method 990.03), ADF (AOAC International, 2000; method 973.18), NDF (Van Soest et al., 1991), starch (Hall, 2009), and ether extract (AOAC International, 2006; method 954.02).

Milk samples were analyzed at the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada) for fat, CP, lactose, MUN, BHB, and SCC by infrared spectroscopy (AOAC International, 2002; method 972.16; MilkoScan 605, Foss North America). As described by Tyrrell and Reid (1965), 3.5% FCM was calculated using the following formula: 0.432 × milk yield + 16.23 × fat yield.

A plate reader (SpectraMax 190; Molecular Devices Crop.) was used to determine concentrations of plasma glucose, BHB, and FA, and serum amyloid A (SAA) and haptoglobin (Hp). Commercial kits were used to determine plasma concentrations of glucose (Autokit glucose; Wako Chemicals USA Inc.), FA (NEFA HR2; Wako Chemicals USA Inc.), and BHB (No. H6501; Roche), and serum concentrations of Hp (Phase Haptoglobin Assay, Tridelta Development Ltd.) and SAA (Multispecies SAA ELISA Kit, Tridelta Development Ltd.).

2.2.4 Statistical Analysis

Statistical analysis was conducted using JMP Pro 14.3 (SAS Institute Inc.) using the Fit Model procedure. The following covariance structures were tested: unequal variance, autoregressive 1, compound symmetry, antedependent equal variance, and compound symmetry unequal variance. The covariance structure with the lowest Akaike information criterion value was used for each response variable with repeated measures. Prepartum data were analyzed for the fixed effect of supplement, parity, day, and full factorial interactions; block nested in parity; and cow nested within block as a random effect. Postpartum data were analyzed for the fixed effect of supplement, drug, parity, day, and full factorial interactions; block nested in parity; and cow nested within block as a random effect. Significance was declared at $P \leq 0.05$, and tendencies were declared when $0.10 \geq P > 0.05$.

No significant differences or tendencies existed for plasma metabolites before experimental diets were fed on d −28. However, at 12 to 24 h, before NSAID or placebo administration, differences between NSAID and placebo-assigned cows were present for glucose ($P = 0.10$), FA ($P = 0.10$), and SAA ($P = 0.07$); therefore, these values were included as a covariate for the respective analysis. In addition, as prepartum DMI happened to be lower for cows assigned to the NSAID treatment ($P = 0.02$), prepartum DMI was also used as a covariate for postpartum data analyses.

One cow in the butyrate group did not receive the NSAID due to postcalving disease; as such, her prepartum data were included, but postpartum data were removed from statistical analysis. Cow assignment to each treatment combination was as follows: butyrate + NSAID (n = 21), butyrate + placebo (n = 20), control + NSAID (n = 20), or control + placebo (n = 21). In the postpartum period, 20 cows were treated with antibiotics or NSAID for health disorders. Data after cows were treated were removed from statistical analysis as follows: 8 for butyrate + NSAID, 5 for butyrate + placebo, 3 for control + NSAID, and 4 for control + placebo.

2.3 Results

In the prepartum period, butyrate supplementation did not affect DMI, plasma glucose or BHB concentration, or SAA concentration (Table 2). Multiparous cows had higher prepartum DMI compared with primiparous cows (13.0 vs. 10.9 kg/d; $P < 0.0001$).

On d −4 relative to calving, butyrate-fed cows had greater plasma FA concentration (501 vs. 340 μEq/L; $P = 0.05$) and tended to have greater serum Hp concentration (0.23 vs. 0.10 mg/mL; $P = 0.10$). Multiparous cows had higher SAA concentration than primiparous cows on d −4 (135 vs. 75 μg/mL; $P = 0.02$). At 12 to 24 h, multiparous cows had a lower plasma

concentration of glucose (66.5 vs. 79.1 mg/dL; $P < 0.01$) and a higher plasma concentration of FA (659 vs. 429 μEq/L; $P < 0.01$) compared with primiparous cows.

In the postpartum period, butyrate supplementation and NSAID administration did not affect plasma FA or BHB, or serum inflammatory marker concentrations (Table 3). However, a supplement × drug × day interaction ($P = 0.02$) was observed for plasma glucose concentration; there was a supplement × drug effect on d 4 ($P = 0.02$), where butyrate supplementation decreased plasma glucose concentration only for cows administered placebo (62.8 vs. 70.1 mg/dL; $P < 0.05$) but not for those administered NSAID. Butyrate supplementation and NSAID administration did not affect plasma glucose concentration on d 7 or 21.

Neither butyrate supplementation nor NSAID administration affected DMI, BW or BCS change, milk (Table 4). However, milk CP yield tended to be lower in cows fed butyrate compared with control-fed cows (1.21 vs. 1.27 kg/d; $P = 0.06$).

Multiparous cows had greater postpartum DMI than primiparous cows (17.9 vs. 15.1 kg/d; $P < 0.0001$), but multiparous cows lost more BCS (−0.32 vs. −0.19; $P < 0.01$) and BW (−3.66 vs. −2.30 kg/d; $P = 0.02$) compared with primiparous cows. Compared with primiparous cows, multiparous cows also had greater milk yield (41.9 vs. 27.7 kg/d; $P < 0.05$) and plasma concentrations of FA (785 vs. 705 μEq/L; $P = 0.02$) and BHB (13.4 vs. 9.4 mg/dL; $P < 0.0001$), but a lower concentration of SAA (135 vs. 271 μg/mL; $P < 0.01$).

2.4 Discussion

2.4.1 Effect of Butyrate Supplementation

Butyrate is a primary energy source for GIT cells (Bergman, 1990) and promotes cell growth (Guilloteau et al., 2010; Górka et al., 2011a,b), improving gut barrier function (Peng et al., 2009) and limiting the absorption of toxins that may induce systemic inflammation (Dai et

al., 2017). Additionally, butyrate may downregulate genes involved in inflammation (Dionissopoulos et al., 2013); thus, we had anticipated dietary butyrate supplementation would reduce inflammation. In contrast, we detected higher serum Hp concentration at d −4 relative to calving in butyrate-fed cows, and no postpartum differences in concentrations of the serum inflammatory markers, SAA and Hp. Fukumori et al. (2020) also reported higher serum Hp concentration in cows fed butyrate and suggested that this may be due to gut-specific inflammation as butyrate increases epithelial expression of TLR4, activates the nuclear factor-κB signal pathway, and increases proinflammatory response (Andoh et al., 1999; Hýzd'alová et al., 2008; Xiao et al., 2018). Alternatively, the higher plasma FA concentration on d −4 in butyrate-fed cows may have contributed to increased serum Hp concentration in these cows; elevated serum Hp concentration has been reported alongside increased plasma FA concentrations (Stengärde et al., 2008; Kvidera et al., 2017b; Haisain et al., 2021), perhaps due to their associations with fatty liver (Katoh and Nakagawa, 1999).

There was also no difference in milk yield in the present study. We had anticipated that butyrate would reduce inflammation and associated energy expenditures, thus allowing more energy to be allocated toward milk production. However, there were no differences in milk production between treatments, and this may be attributed to the lack of differences in inflammation.

The lack of butyrate effect on concentrations of postpartum serum inflammatory markers is consistent with findings of Dionissopoulos et al. (2013), who also reported no difference in concentrations of serum inflammatory markers following butyrate supplementation in lactating cows. Conversely, Wu et al. (2021) found that butyrate supplementation reduced plasma concentration of acute phase proteins, Hp and C-reactive protein, in cows fed a high-concentrate

diet (concentrate: forage = 6:4) compared with those fed the same basal diet without butyrate. The discrepancy in the findings of the present study and that of Dionissopoulos et al. (2013) compared with the findings of Wu et al. (2021) may be attributed to differences in basal diet; in the present study, we fed 22.1% starch in the fresh period, which may not have been high enough to elicit positive effects of butyrate. It has been suggested that effects of butyrate supplementation on inflammation may be affected by dietary starch content (Fukumori et al., 2020). High-concentrate diets lead to a higher degree of GIT inflammation and subsequent systemic inflammation (Gozho et al., 2006, 2007; Plaizier et al., 2008), thus allowing anti-inflammatory action of butyrate to take effect, whereas when cows are fed a low concentrate diet, there may be little gut-derived inflammation to mitigate.

We cannot exclude the possibility that the concentration of inflammatory markers used in the present study could be inflated with other sources of inflammation; the concentration of Hp and SAA may increase during many types of inflammatory events such as metritis (Chan et al., 2010), mastitis (Grönlund et al., 2003), or calving-related injuries (Tóthová et al., 2014; Pohl et al., 2015; Schmitt et al., 2021). Thus, the elevated serum concentrations of Hp and SAA may not be associated with the extent of GIT inflammation or systemic inflammation induced following a breach in gut barrier function. Dionissopoulos et al. (2013) and Dai et al. (2017) reported that butyrate downregulated gene expression of proinflammatory markers in the rumen epithelium, suggesting that butyrate exerts local anti-inflammatory effects, but these effects may not be detected by systemic serum inflammatory markers as there was no change in SAA concentration in the study by Dionissopoulos et al. (2013), despite local inflammation being reduced.

Butyrate supplementation did not affect plasma BHB concentration in the present study, which contrasts studies where butyrate increased plasma BHB (Izumi et al., 2019; Urrutia et al.,

2019; Halfen et al., 2021). This may be explained by the difference in physiological state; from a few days before calving to the fresh period, plasma BHB increases from incomplete oxidation of FA in the liver (Grummer, 2008), reducing the relative contribution of supplemental butyrate to overall plasma BHB. In the studies reporting that butyrate supplementation increased plasma BHB, cows were approximately 60 (Izumi et al., 2019), 150 (Halfen et al., 2021) or 180 DIM (Urrutia et al., 2019), when excessive FA mobilization likely did not occur (Grum et al., 1996; Nogalski et al., 2012; García-Roche et al., 2021) and BHB production in the liver was reduced, which increased the relative contribution of supplemental butyrate to plasma BHB.

On d −4 relative to calving, cows fed butyrate had greater concentration of plasma FA. This is in contrast to others that have found butyrate decreases lipolysis (Ohira et al., 2013; Yan and Ajuwon, 2015; Halfen et al., 2021) or reduces plasma FA by 18% (Urrutia et al., 2019). Urrutia et al. (2019) suggested this was due to inhibition of lipolysis induced by BHB (Metz and van den Bergh, 1972; Mielenz, 2017). However, Herrick et al. (2018) reported sodium butyrate infusion increased plasma BHB without affecting plasma FA concentration. Similarly, Urrutia and Harvatine (2017) reported that sodium acetate infusion increased plasma BHB, but it did not reduce plasma FA concentration. Taken together, effects of butyrate or BHB on lipolysis are not consistent, and further research is needed.

On d 4 after calving, butyrate supplementation decreased plasma glucose concentration in cows that received the placebo treatment. Decreased plasma glucose concentrations from increased butyrate supply has also been reported in other studies (Krehbiel et al., 1992; Huhtanen et al., 1993; Herrick et al., 2018). Butyrate increased glucose uptake into adipocytes in vitro (Yan and Ajuwon, 2015), and reduced propionate absorption and conversion to glucose in vitro (Aiello et al., 1989) and in vivo (Krehbiel et al., 1992; Huhtanen et al., 1993), explaining the

decreased concentration in circulating glucose. However, the decrease in plasma glucose on d 4 in butyrate-fed cows was not observed in NSAID-treated cows. It is possible that NSAID administration mitigated the decrease in plasma glucose in butyrate-fed cows as NSAID treatment has been previously reported to increase blood glucose concentrations (Carpenter et al., 2016; Bogado Pascottini et al., 2020); however, the mechanisms explaining this have not been elucidated. Although NSAID administration in butyrate-fed cows appeared to increase plasma glucose on d 4, this effect was not observed in control-fed cows, possibly because there was no decrease in plasma glucose concentration for the NSAID to ameliorate.

Butyrate-fed cows tended to decrease milk protein yield in the current study. Urrutia et al. (2019) also reported butyrate supplementation decreased milk protein yield. This might be due to altered rumen microbial composition (Li et al., 2012b), nitrogen use, and decreased ammonia in the rumen (Herrick et al., 2017), reducing the nitrogen available for microbial protein production, and thus available for milk CP. Agarwal et al. (2015) also suggested that butyrate may increase epithelial tissue protein synthesis, limiting protein available for milk, which may explain the decreased protein output in milk we observed.

2.4.2 Effect of NSAID Administration

We found minimal effects of oral NSAID administration on cow performance or concentration of serum inflammation markers. Conflicting results about the effects of NSAID on milk production have been reported with some studies observing an increase (Bertoni et al., 2004; Carpenter et al., 2016; Swartz et al., 2018), some observing no change (Duffield et al., 2009; Shwartz et al., 2009; Newby et al., 2013), and one study observing a decrease (Newby et al., 2017).

In the present study, we administered the NSAID 12 to 24 h postpartum, and no changes in milk production or inflammatory markers were found. Mainau et al. (2014) also administered NSAID shortly after calving and found little effect of NSAID on cow performance or inflammation. The timing of NSAID administration may have affected cow response; Swartz et al. (2018) found that cows administered meloxicam boluses prepartum (6–48 h before calving) produced more milk than cows administered meloxicam postpartum (within 12 h of calving). Additionally, Newby et al. (2013) speculated that their lack of milk production response may be due to waiting 24 h postpartum to treat cows, and suggested that earlier administration may have different results. Perhaps the delay of NSAID administration postpartum, even if the NSAID is administered shortly after calving (within 6 h of calving), reduces NSAID efficacy because inflammatory mechanisms are initiated during calving and NSAID administration may be too late or insufficient in reducing inflammation once the inflammatory cascade has begun (Swartz et al., 2018). The NSAID administration inhibits the COX-2 enzyme, preventing prostaglandin synthesis (Zarghi and Arfaei, 2011), and thus any prostaglandin synthesis and subsequent inflammation that occurs prior to NSAID administration is unlikely to be subdued by NSAID administration. It is possible that by 12 to 24 h postcalving, when the NSAID was administered in the present study, sufficient prostaglandin synthesis may have already occurred to induce inflammation in the cow; therefore, NSAID administration was ineffective. This may suggest that NSAID administration prepartum, as in Swartz et al. (2018), is advantageous as it suppresses inflammation before the onset of calving-related inflammation. If NSAID administration occurs postpartum, multiple doses may be necessary to effectively reduce active inflammatory pathways inflammation. This is supported by Bertoni et al. (2004), who found lysine acetylsalicylate treatment consecutively for the first 5 DIM increased milk production, and Farney et al. (2013),

who found sodium salicylate treatment consecutively for the first 7 DIM reduced the expression of genes involved in the inflammatory response.

However, multiple administrations of NSAID do not explain the increase in whole-lactation milk production reported by Carpenter et al. (2016), where they administered only a single dose of meloxicam 12 to 36 h after calving. Studies that observed an increase in milk yield (Carpenter et al., 2016; Swartz et al., 2018) measured milk production for a longer duration (52 and 15 wk, respectively) and suggested that a key factor in identifying an NSAID response may be duration of measurement. This is because NSAID responses may not be present until later in lactation; namely, at peak lactation (Carpenter et al., 2016; Swartz et al., 2018). Therefore, the present study and others (Duffield et al., 2009; Shwartz et al., 2009; Newby et al., 2013) may not have monitored milk yield for a long enough duration to detect differences in milk yield, if any, between NSAID treated and untreated cows. However, the underlying mechanisms explaining increased milk production over a longer duration compared with a shorter duration have not been sufficiently described; thus, further research is needed to confirm this speculation. We cannot exclude the possibility that the type of NSAID administered may have influenced animal response and subsequent results, as there are various drugs classified as NSAID, each with differing modes of action (Meier et al., 2014b).

Further research on the ideal timing of NSAID administration during the calving transition period is warranted. Administering NSAID too early may have harmful effects on animal physiology (Meier et al., 2014b) because inflammation may assist in homeorhetic adaptations to support increased milk yield (Farney et al., 2013b) and pathogen clearance (LeBlanc, 2012; Bradford et al., 2015). However, dysregulated inflammation beyond what is necessary for homeorhetic adaptations to lactation can contribute to metabolic disorders and have

long lasting effects on cow performance during lactation (Trevisi et al., 2012; Sordillo and Raphael, 2013). It is difficult to determine at what point inflammation goes from adaptive to maladaptive, and if limiting inflammation at certain points in the calving transition period is helpful or harmful. Farney et al. (2013b) hypothesized that NSAID administration would limit metabolic disease prevalence in fresh cows, but surprisingly they found that inflammation appears to be necessary in homeorhetic adaptations to lactation. Therefore, limiting inflammation is not necessarily advantageous. The same group suggested "rebound inflammation" may follow after blocking inflammatory pathways (Farney et al., 2013b). This may explain the lack of difference in inflammatory markers in the present study. It is possible inflammatory markers were reduced initially when the NSAID was administered 12 to 24 h postpartum, but rebound inflammation occurred and no difference in inflammatory markers was found when acute phase proteins were assessed on d 4 and 7 postpartum.

2.5 Conclusions

Dietary butyrate supplementation during the calving transition period increased plasma FA concentration and tended to increase serum Hp concentration on d −4 relative to calving, and tended to increase milk CP yield in the postpartum period. There was a supplement × drug interaction on d 4, where butyrate supplementation decreased plasma glucose concentration in cows that did not receive the NSAID. Contrary to our hypothesis, dietary butyrate supplementation and oral NSAID administration did not reduce concentrations of postpartum serum concentrations of inflammatory markers and did not increase milk yield.

2.6 Tables

Table 2.6.1 Ingredient and chemical composition (% DM unless otherwise noted) of experimental diets[1]

Item	Prepartum			Postpartum		
	Butyrate	Control	SD	Butyrate	Control	SD
Ingredient, % DM						
Barley silage	60.3	60.3		44.2	44.2	
Alfalfa hay	-	-		7.2	7.2	
Corn grain, ground	5.8	5.8		12.2	12.2	
Barley grain, ground	3.8	3.8		13.3	13.3	
Beet pulp	5.7	5.7		1.3	1.3	
Canola meal	6.3	6.3		8.8	8.8	
Corn gluten meal	0.9	0.9		1.5	1.5	
Bypass soy[2]	5.1	5.1		6.9	6.9	
Soybean hulls	3.6	3.6		0.5	0.5	
Corn distillers	2.1	2.1		-	-	
Minerals and vitamins supplement[3]	4.9	4.9		2.5	2.5	
Calcium butyrate[4]	1.4	-		1.4	-	
Commercial fat supplement[5]	-	1.0		-	1.0	
Calcium carbonate	-	0.4		-	0.4	
Diet Nutrient Content						
% DM	40.2	40.2	4.2	41.1	41.1	2.1
CP, % DM	15.9	15.9	0.6	18.1	18.1	0.6
ADF, % DM	27.5	27.5	0.7	22.3	22.3	0.6
NDF, %DM	42.4	42.4	1.5	34.1	34.1	1.0
Forage NDF, %DM	31.0	31.0	1.6	25.8	25.8	1.4
Starch, %DM	13.3	13.3	2.0	22.1	22.1	1.0
Ether extract, %DM	3.4	4.3	0.6	3.2	4.1	0.4
NFC, %DM	29.0	29.0	1.8	37.4	37.4	1.2
NE_L Mcal/kg of DM	1.47	1.47		1.70	1.70	
ME allowable milk[6], kg/d	-	-		31.5	31.5	
MP allowable milk[6], kg/d	-	-		33.2	33.2	

[1] The prepartum diet was fed from 28 ± 3 days before expected calving date to calving, and the postpartum diet was fed from calving until 24 ± 3 days after calving

[2] SoyPlus® (Landus), manufactured with a mechanical expeller process

[3] Prepartum supplement contained 25.2% Ca, 5.5% Mg, 2.7% P, 1.7% S, 0.1% K, 0.1% Na, 2,500 mg/kg Zn, 2,148 mg/kg Mn, 1,423 mg/kg Fe mg/kg, 368 mg/kg Cu, 42 mg/kg Co, 35 mg/kg I, 33mg/kg Se,338 KIU vitamin A, 69 KIU vitamin D, 5,725 KIU vitamin E. Postpartum supplement contained 19.0% Ca, 3.0% Mg, 1.8% P, 1.0% S, 0.3% K, 9.3% Na, 2,114 mg/kg Zn, 1,824 mg/kg Mn, 976 mg/kg Fe, 336 mg/kg Cu, 36 mg/kg Co, 29 mg/kg I, 9 mg/kg Se, 208 KIU vitamin A, 49 kIU/kg of vitamin D, and 1,481 kIU/kg of vitamin E

[4] Calcium butyrate contained 70.4% butyrate, 16.9% calcium, and 12.7% fatty acids, and contributed 1.0% butyrate, 0.24% calcium, and 0.18% fatty acids to the diet; Probiotech International.

[5] Commercial fat supplement contained a minimum of 85% palmitic acid; Jefo Nutrition Inc.

[6] Estimated using the Nutritional Dynamic System (NDS; CNCPS version 6.55, RUM&N).

Table 2.6.2 Effect of dietary butyrate supplementation[1] on prepartum DMI, and plasma metabolite and serum acute phase protein concentration on d -4 and h 12 to 24 after calving.

Variable	Butyrate	Control	SEM	*P*-value
Prepartum DMI, kg/d	11.9	12.0	0.24	0.77
Glucose mg/dL				
d -4	72.1	77.9	2.60	0.14
h 12-24	75.0	71.1	2.4	0.25
Fatty acids, μEq/L				
d -4	501	340	52.4	0.05
h 12-24	503	535	30.5	0.58
BHB, mg/dL				
d -4	7.99	7.00	0.44	0.14
h 12-24	10.9	10.3	0.57	0.51
Haptoglobin, mg/mL				
d -4	0.23	0.10	0.05	0.10
h 12-24	0.39	0.39	0.07	0.97
Serum amyloid A, μg/mL				
d -4	81.8	89.4	1.56	0.81
h 12-24	259	233	33.6	0.60

[1] Cows received a dietary fatty acid-coated calcium butyrate supplement (1.0% butyrate, 0.24% calcium, and 0.18% fatty acids; Probiotech International) or a control supplement (1.04% commercial fat supplement [Jefo Dairy Fat; 85% palmitic acid; Jefo Nutrition Inc.] and 0.38% calcium carbonate) at 1.42% of diet DM) from -28 d (± 3) before expected calving date, to 24 d (± 3) after calving.

Table 2.6.3 The effect of dietary butyrate supplementation[1] and oral non-steroidal anti-inflammatory drug administration (NSAID)[2] on postpartum plasma metabolite and serum acute phase protein concentration

	Butyrate		Control			*P*-value					
	NSAID	Placebo	NSAID	Placebo	SEM	Supplement	Drug	Supplement × Drug	Supplement × Day	Drug × Day	Supplement × Drug × Day
Glucose, mg/dL									0.08	0.79	0.02
d 4	70.9[a]	62.8[b]	69.5[ab]	70.1[a]	2.0	0.14	0.08	0.03			
d 7	68.8	63.5	65.8	64.3	2.6	0.67	0.25	0.49			
d 21	70.6	66.2	68.9	66.2	2.75	0.75	0.25	0.78			
Fatty acids, µEq/L	768	785	814	884	52.0	0.15	0.44	0.60	0.11	0.85	0.99
BHB, mg/dL	10.7	11.0	12.5	11.4	0.68	0.32	0.13	0.21	0.77	0.95	0.57
Haptoglobin, mg/mL	0.63	0.58	0.61	0.67	0.17	0.83	0.97	0.76	0.14	0.95	0.20
Serum amyloid A, µg/mL	199	219	171	207	47	0.63	0.55	0.84	0.55	0.90	0.27

[ab] Least square means in the raw differ ($P < 0.05$) if superscripts differ.

[1] Cows received a dietary fatty acid-coated calcium butyrate supplement (1.0% butyrate, 0.24% calcium, and 0.18% fatty acids; Probiotech International) or a control supplement (1.04% commercial fat supplement [Jefo Dairy Fat; 85% palmitic acid; Jefo Nutrition Inc.] and 0.38% calcium carbonate) at 1.42% of diet DM) from -28 d (± 3) before expected calving date, to 24 d (± 3) after calving.

[2] Cows received an oral NSAID (meloxicam [15 mg/mL] oral suspension, USP; Solvet, Calgary, AB, Canada) administered at 1 mL/15 kg BW in carrier solution (as recommended by the product label and determined by Shock et al., 2018; 2019; equivalent to 1 mg meloxicam/kg BW) or a placebo (food dye at 1 mL/15 kg BW in carrier solution) at 12 to 24 h after calving.

Table 2.6.4 The effect of dietary butyrate supplementation and oral non-steroidal anti-inflammatory drug administration (NSAID) on postpartum DMI, changes in BW and BCS, and yield and composition of milk during the fresh period (d 1 to 24 ± 3 relative to calving)

	Butyrate		Control			*P*-value					
	NSAID	Placebo	NSAID	Placebo	SEM	Supplement	Drug	Supplement × Drug	Supplement × Day	Drug × Day	Supplement × Drug × Day
DMI, kg/d	16.6	16.2	16.6	16.9	0.37	0.36	0.95	0.30	0.41	0.59	0.99
BW change, kg/d	-2.07	-2.8	-2.8	-2.8	0.27	0.56	0.55	0.22			
BCS change, /21d	-0.33	-0.23	-0.21	-0.21	0.04	0.45	0.94	0.92			
Yield, kg/d											
Milk	33.9	35.2	33.9	35.8	0.61	0.95	0.90	0.99	0.61	0.92	0.07
Fat	1.54	1.59	1.56	1.61	0.03	0.72	0.42	0.96	0.24	0.32	0.91
CP	1.20	1.21	1.26	1.27	0.02	0.06	0.71	0.99	0.23	0.18	0.85
Lactose	1.64	1.72	1.68	1.73	0.03	0.35	0.35	0.89	0.18	0.25	0.76
Total solids	4.75	4.83	4.86	4.98	0.07	0.26	0.34	0.82	0.12	0.40	0.99
3.5% FCM	40.6	41.9	41.4	42.6	0.71	0.85	0.83	0.97	0.17	0.34	0.97
Composition											
Fat, %	4.30	4.32	4.17	4.36	0.06	0.90	0.63	0.56	0.52	0.19	0.75
CP, %	3.35	3.32	3.41	3.43	0.03	0.33	0.41	0.68	0.72	0.62	0.05
Lactose, %	4.51	4.50	4.48	4.60	0.0	0.28	0.20	0.07	0.99	0.87	0.74
MUN, mg/dL	14.4	15.6	14.1	14.8	0.73	0.50	0.45	0.69	0.11	0.46	0.22
BHB, mM	0.135	0.115	0.130	0.131	0.006	0.46	0.73	0.29	0.14	0.60	0.12
SCC, 10^3 cells/mL	126	121	387	113	21.0	0.31	0.16	0.25	0.25	0.59	0.39

[1] Cows received a dietary fatty acid-coated calcium butyrate supplement (1.0% butyrate, 0.24% calcium, and 0.18% fatty acids; Probiotech International) or a control supplement (1.04% commercial fat supplement [Jefo Dairy Fat; 85% palmitic acid; Jefo

Nutrition Inc.] and 0.38% calcium carbonate) at 1.42% of diet DM) from -28 d (± 3) before expected calving date, to 24 d (± 3) after calving.

[2] Cows received an oral NSAID (meloxicam [15 mg/mL] oral suspension, USP; Solvet, Calgary, AB, Canada) administered at 1 mL/15 kg BW in carrier solution (as recommended by the product label and determined by Shock et al., 2018; 2019; equivalent to 1 mg meloxicam/kg BW) or a placebo (food dye at 1 mL/15 kg BW in carrier solution) at 12 to 24 h after calving.

Chapter 3. Effects of dietary butyrate supplementation and oral nonsteroidal antiinflammatory drug administration on uterine inflammation and interval to first ovulation in postpartum dairy cows

3.1 Introduction

Postpartum dairy cattle regularly experience microbial invasion of the uterus, characterized by increased PMN in the uterine lumen (Kluciński et al., 1990). Although some uterine inflammation is necessary for homeorhetic adaption to lactation and return to estrus, dysregulated inflammation can result in endometritis (>18% PMN; Kasimanickam et al., 2004). Reproductive performance has been reported to decrease in cows with clinical (Pleticha et al., 2009) and subclinical endometritis (Kaufmann et al., 2009). Uterine inflammation has been suggested to delay return to ovarian cyclicity due to inhibited growth and function of the dominant ovarian follicle (Sheldon et al., 2002) and reduced reproductive hormones (Williams et al., 2007).

Short-chain fatty acids, such as butyrate, activate and regulate the immune system (Cox et al., 2009), and cows with lower concentrations of butyrate in utero-placental tissues are more likely to retain fetal membranes (Boro et al., 2014), which can predispose cows to endometritis (Potter et al., 2010). Therefore, adjusting dietary levels of short-chain fatty acids during the transition period may improve uterine health (Boro et al., 2014).

Parenteral administration of nonsteroidal antiinflammatory drugs (NSAID), such as acetylsalicylate, carprofen, and meloxicam, has been explored to manage endometritis, and NSAID have been reported to reduce inflammation (Pascottini et al., 2020) and increase pregnancy rate in dairy cattle (Priest et al., 2013). More recently, oral administration of meloxicam has been proposed to mitigate inflammation and pain in cattle (Shock et al., 2019)

due to its relatively long half-life compared with injectable Metacam (Coetzee et al., 2009), and reportedly improved milk production (Carpenter et al., 2016). However, oral meloxicam has not been evaluated on uterine inflammation or ovarian function. Therefore, our objectives were to evaluate the effects of dietary butyrate supplementation and oral NSAID administration on uterine inflammation and the interval from calving to first ovulation in dairy cows (ICFO; in days).

3.2 Materials and Methods

3.2.1 Animals, Diets, and Experimental Design

This study was conducted at the University of Alberta Dairy Research and Technology Centre, a 146-cow tiestall barn (Edmonton, Alberta, Canada) in 2019–2020. All procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP00003364). This experiment was conducted in tandem with Engelking et al. (2022).

Sixty-five (40 primiparous, 25 multiparous) Holstein cows were blocked by parity and expected calving date and randomly assigned to 1 of 2 iso-energetic diets containing fatty acid-coated calcium butyrate (1.0% butyrate, 0.24% calcium, and 0.18% fatty acids; Probiotech International) or a control supplement [1.04% commercial fat (Jefo Dairy Fat; 85% palmitic acid; Jefo Nutrition Inc.) and 0.38% calcium carbonate] at 1.42% of diet DM. Prepartum and postpartum diets, and details describing diet formulation and dietary butyrate concentration determination, are included in Engelking et al. (2022). Experimental diets were fed ad libitum through individual mangers at 0800 h, from 28 ± 3 d before expected calving date to 24 ± 3 d after calving. Day 1 was defined as the first day cows received experimental fresh cow diets.

Cows received an oral NSAID [meloxicam (15 mg/mL) suspension, USP; Solvet] administered at 1 mL/15 kg of BW in carrier solution, equivalent to 1 mg of meloxicam/kg of BW) or a placebo (food dye at 1 mL/15 kg of BW in carrier solution) at 12 to 24 h after calving using an oral drench gun. Study personnel were blinded to NSAID treatment until completion of data analysis. Treatment groups were (1) butyrate + NSAID (n = 15), (2) butyrate + placebo (n = 18), (3) control + NSAID (n = 14), and (4) control + placebo (n = 18).

3.2.2 Data Collection and Analysis

Ovarian structures were evaluated once weekly by transrectal ultrasonography from 14 ± 3 d postpartum (DPP) until the first ovulation was confirmed, or until a maximum of 56 ± 3 d. Locations of major ovarian structures (follicles >5 mm diameter and luteal tissue) were recorded, and ovulation was confirmed by the appearance of a corpus luteum in the ovary. Vaginal mucus samples were collected using a Metricheck device (Simcro Tech Ltd.) at 14 and 28 d, and its appearance and odor were scored as per Williams et al. (2005) with the addition of an odor score category. Mucus appearance scores: clear or translucent = 0; white or off-white flecks of pus = 1; ≤50% white or off-white mucopurulent material = 2; and >50% purulent material, usually white or sanguineous = 3. Mucus odor scores: no odor = 0, faint nonfetid odor = 1, strong fetid odor = 2.

Samples for endometrial cytology were obtained at 28 d using a cytobrush (Medscand Medical) modified for use in large animals as described by Kasimanickam et al. (2004). In 3 of 65 cows (1 control + NSAID, 2 control + placebo), cytology samples could not be collected because the cervix was not passable. Cytological samples were smeared on microscope slides and fixed with cytofixative (Cytoprep, Fisher Scientific). Slides were then stained for a minimum of 8 min (Wright-Giemsa Stain; Fisher Scientific), washed with distilled water, dried, and

examined under 400× magnification. Total cell and PMN counts were assessed to calculate % PMN based on at least 200 cells/slide. High and low PMN were defined as >18% and ≤18% PMN, respectively (Kasimanickam et al., 2004).

3.2.3 Statistical Analysis

Statistical analyses were conducted using SAS (Statistical Analysis System, version 9.4 for Windows; SAS Institute Inc.). Normality of data was determined using the UNIVARIATE procedure. Binomial and continuous dependent variables were modeled against the fixed effects of independent variables (i.e., parity, dietary treatment, drug treatment, and their interactions) and analyzed using GLIMMIX procedure of SAS. For binomial dependent outcomes, the model was specified as binomial (dist = binary link logit), and the ilink option with Tukey's adjustment used to obtain corresponding least squares means by parity, dietary, and drug treatment groups. As none of the interactions was significant, the fixed effects of interactions were removed from the final model. The differences in the proportions of cows by vaginal mucus characteristics at 14 and 28 d were also analyzed by GLIMMIX procedure with Tukey's adjustment to obtain LSM by parity, dietary, and drug treatment groups. In addition to determining the proportion of cows that ovulated at 14, 21, 28, 35, 42, 49, and 56 DPP using the aforesaid GLIMMIX procedure, the probability of ovulation up to 56 d DPP was evaluated by the Kaplan-Meier survival analysis (LIFETEST procedure) and tested by a Cox proportional-hazards model (PHREG procedure). During data collection, 7 cows (butyrate + NSAID, n = 2; butyrate + placebo, n = 1; control + NSAID, n = 2; and control + placebo, n = 2) were treated with antibiotics or NSAID (other than the experimental treatment) for health disorders. Data for these cows were included up to when the above interventions occurred but removed from any

statistical analysis after treatment. Significance was declared at $P \leq 0.05$ and tendencies were declared when $P > 0.05$ but ≤ 0.10.

3.3 Results and Discussion

Contrary to our hypothesis, the proportions of cows with high (>18%) endometrial PMN did not differ by dietary (Figure 1; butyrate vs. control; 33 ± 9 vs. 35 ± 9%; $P = 0.90$) or drug treatment (NSAID vs. placebo; 43 ± 9 vs. 26 ± 9%; $P = 0.17$). The proportions of cows distributed by vaginal mucus characteristics on d 14 and 28 also did not differ (Table 1), but a smaller proportion of NSAID (3 vs. 17%) tended to have a vaginal mucus appearance score of 2 (≤50% mucopurulent material) at 28 DPP, and a larger proportion of NSAID had no vaginal mucus odor on d 28, compared with placebo (97 vs. 82%). While vaginal discharge can be associated with the growth of certain bacteria in the uterus (Williams et al., 2005), meloxicam reportedly does not affect uterine bacterial composition in early postpartum cows (Pascottini et al., 2021).

The cumulative proportions of cows that ovulated at each weekly interval (Figure 2) and the mean ICFO did not differ between butyrate and control (27.5 ± 2.1 vs. 25.3 ± 2.0 d; $P = 0.44$) or between NSAID and placebo (28.5 ± 2.0 vs. 24.3 ± 2.1 d; $P = 0.14$). The ovulation rate up to 56 DPP by survival analysis did not differ between butyrate and control (hazard ratio of 0.76; 95% CI 0.45 to 1.28; $P = 0.30$). However, the ovulation rate up to 56 DPP tended to be lower in NSAID than in placebo (hazard ratio of 0.61; 95% CI 0.35 to 1.04; $P = 0.07$). There were no differences in the interval from calving to the first detection of ovarian follicles of 10-mm diameter in butyrate compared with control (14.9 ± 0.7 vs. 15.1 ± 0.6 d; $P = 0.76$) or in NSAID compared with placebo (15.2 ± 0.6 vs. 14.8 ± 0.6 d; $P = 0.63$). Similarly, the interval from calving to the first detection of 16-mm diameter follicles did not differ in butyrate

compared with control (19.4 ± 2.3 vs. 20.6 ± 2.2 d; $P = 0.71$) or in NSAID compared with placebo (20.8 ± 1.9 vs. 19.2 ± 2.6 d; $P = 0.63$). The absence of treatment differences in the intervals from calving to detection of 10- and 16-mm follicles indicates that the tendency for a lower ovulation rate up to 56 DIM in NSAID was not because of impeded recruitment of preovulatory size follicles.

Butyrate has been proposed to improve reproduction in cattle as it is an energy source (Ulfina et al., 2015); however, because rations were iso-energetic in the present study, additional energy from butyrate supplementation was unlikely. Overall, we did not observe any beneficial effects of supplemental butyrate.

Limited data are available on the effects of NSAID on uterine inflammation in dairy cattle; however, those who have assessed it found no changes (Priest et al., 2013; Meier et al., 2014b; Pascottini et al., 2020), similar to our findings. Perhaps administration of NSAID 12 to 24 h postpartum was too early for the treatment to reduce inflammation at 28 DPP. Alternatively, it is possible NSAID administration, regardless of timing, does not affect endometrial PMN proportion. Additionally, Priest et al. (2013) found NSAID treatment for cows with subclinical endometritis improved pregnancy rate; thus, NSAID may be efficacious in cows with high PMN in the uterus (that is, those experiencing endometritis), but not necessarily in cows with low uterine PMN.

In the present study, NSAID was administered orally, whereas in much of the existing research NSAID was given through s.c. or i.m. routes. Although oral meloxicam has a similar therapeutic onset to the injectable form, the oral formulation has a longer half-life (27.5 h in calves) and a significantly longer lasting action (Coetzee et al., 2009). Injectable meloxicam may

be cleared faster than oral meloxicam (14.33 vs. 3.20 h; Karademir et al., 2016); thus, the prolonged action of oral meloxicam may have interfered with natural postcalving inflammation, contributing to the tendency for delayed ovulation in NSAID. Some degree of uterine inflammation is required for uterine remodeling; thus, perhaps the initial treatment with NSAID delayed inflammation, resulting in postponed "rebound inflammation" (Farney et al., 2013b), extending the duration of uterine recovery. This in turn may have reduced the ovulation rate in NSAID. This speculation is consistent with numerically greater proportion of high PMN cows in the NSAID group, but it must be noted that uterine inflammation was only assessed on d 28, and it was not significantly different between NSAID and placebo, so we cannot definitively say if this was a contributing factor.

We acknowledge that the differences in the modes of action of different NSAID formulations may have also contributed to the differences among studies. The present study used meloxicam, whereas other studies have evaluated carprofen (Priest et al., 2013; Meier et al., 2014b) and sodium salicylate (Farney et al., 2013b). Although it is possible that the lack of treatment effect on inflammation is due to timing, route of administration, or the type of NSAID, it is also possible that NSAID is not effective at mitigating endometrial inflammation in postpartum dairy cows.

Cows given the NSAID tended to have a reduced ovulation rate up to 56 DIM compared with cows given placebo in the present study. Administration of NSAID has been reported to delay ovulation in rodents (Gaytán et al., 2006) and humans (Sirois et al., 2004) primarily due to NSAID inhibition of prostaglandin. Increased endometrial PMN reportedly increases ICFO in cattle (Burke et al., 2010b; Dourey et al., 2011; Green et al., 2011). This evidence is consistent with our findings that NSAID had numerically greater proportion (43 vs. 26%) of high PMN and

slower rate of first ovulation up to 56 DIM. Cows with endometritis have impaired reproductive hormone production and slower ovarian follicle growth (Sheldon et al., 2010). Although reproductive hormones were not measured, ovarian follicular growth up to 10- and 16-mm diameter sizes was not affected in the present study, indicating that adequate gonadotropin support was available to sustain follicle growth and dominance.

It has been suggested that NSAID are likely more effective for cows experiencing calving difficulties (Laven et al., 2012) or inflammation, but when given to healthy cows, it may suppress inflammatory signaling in the immune system and lead to infections (Trimboli et al., 2020). Overall, it appears that there are risks associated with administering NSAID as a blanket treatment to all transition cows as inflammation is necessary to adapt to lactation; thus, it may be advisable to only provide NSAID to cows experiencing excessive inflammation following a difficult calving or other inflammatory conditions. As previously described, data used for analysis were obtained from cows without clinical diseases. The inclusion of only "healthy" cows may have reduced the efficacy of NSAID and could be a limitation of the present study. Another limitation is the lack of adequate statistical power in our study. Though originally planned with 120 cows, due to the limited availability of cows and for other reasons beyond our control, this was not possible.

3.4 Conclusion

In conclusion, neither dietary butyrate supplementation nor oral NSAID administration reduced endometrial inflammation or reduced the mean ICFO. However, NSAID-treated cows tended to have a lower ovulation rate up to 56 DPP than cows given placebo. Considering the lack of power in the present study, further research with a larger sample size is warranted to understand the effects of NSAID on uterine inflammation and ovarian function.

3.5 Tables and Figures

Table 3.5.1 Proportions (LSM %) of cows distributed by scores for vaginal mucus appearance (0, 1, 2, 3) and vaginal mucus odor (0, 1, 2) and by dietary[1] (butyrate vs. control) or non-steroidal anti-inflammatory drug[2] (NSAID vs. placebo) treatment at 14 and 28 d postpartum.

	Vaginal mucus appearance score[3]				Vaginal mucus odor score[4]		
Item	0	1	2	3	0	1	2
Day 14 postpartum							
Butyrate, % (n)	10 (3)	14 (4)	31 (9)	44 (13)	72 (20)	21 (7)	6 (2)
Control, % (n)	14 (5)	24 (8)	23 (8)	39 (15)	84 (29)	8 (4)	7 (3)
NSAID, % (n)	9 (3)	18 (6)	28 (9)	44 (15)	72 (23)	14 (6)	13 (4)
Placebo, % (n)	15 (5)	19 (6)	26 (8)	39 (13)	83 (26)	12 (5)	4 (1)
Day 28 postpartum	0	1	2	3	0	1	2
Butyrate, % (n)	31 (9)	59 (17)	5 (2)	3 (1)	95 (27)	3 (1)	1 (1)
Control, % (n)	29 (10)	44 (16)	10 (5)	11 (5)	89 (31)	9 (4)	1 (1)
NSAID, % (n)	31 (10)	58 (19)	3[a] (1)	6 (3)	97[c] (32)	2 (1)	0 (0)
Placebo, % (n)	29 (9)	44 (14)	17[b] (6)	6 (3)	82[d] (26)	10 (4)	7 (2)

[1] Cows received butyrate (fatty acid coated calcium butyrate supplement) or a control (commercial fat and calcium carbonate supplement mixture) at 1.42% of diet DM

[2] Cows received oral NSAID (1 mg meloxicam/kg BW) or placebo (food dye)

[3] Mucus appearance scoring: 0 = clear or translucent, 1 = off-white or white flecks, 2 ≤ 50% white or off-white mucopurulent material, 3 ≥ 50% purulent material, usually white or sanguineous (Williams et al., 2005)

[4] Mucus odor scoring: 0 = no odor, 1= faint odor, 2 = strong fetid odor (Modified from Williams et al., 2005)

[a,b]NSAID vs. Placebo, $P = 0.07$

[c,d]NSAID vs. Placebo, $P = 0.08$

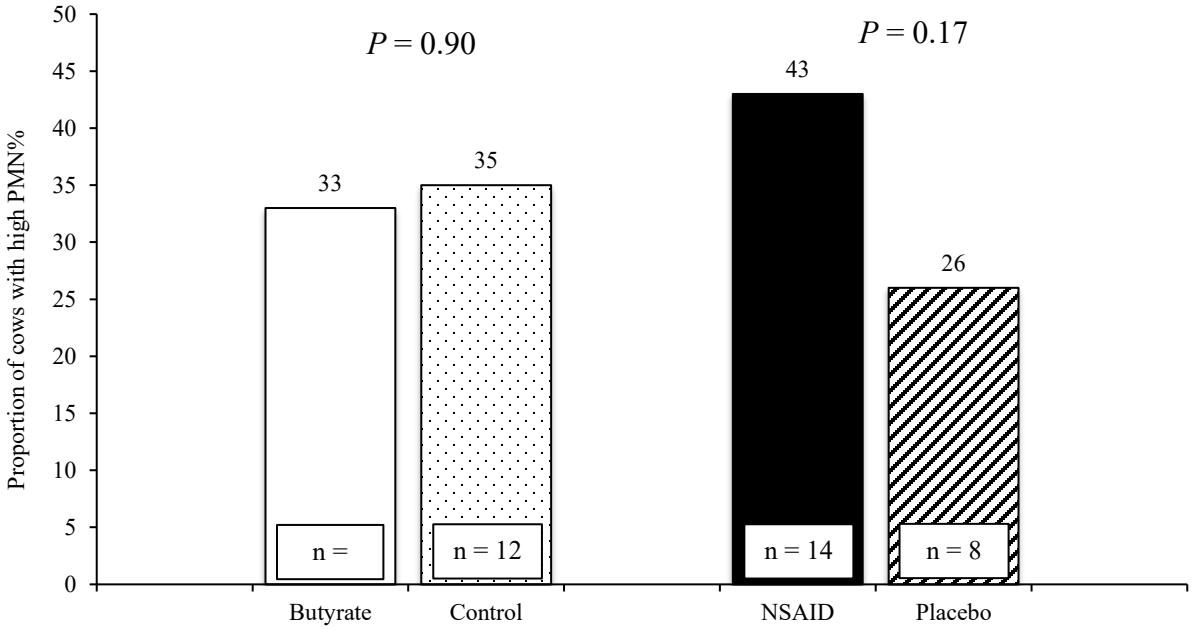

Figure 3.5.1 Proportions of cows with high uterine polymorphonuclear (PMN) leukocytes (PMN > 18%; n = 22) by dietary butyrate (fatty acid coated calcium butyrate supplement vs. control [commercial dairy fat and calcium carbonate supplement mixture] at 1.42% diet DM), and non-steroidal anti-inflammatory drug (NSAID; 1 mg meloxicam/kg BW vs. placebo [food dye]) treatment. High PMN was defined as >18% PMN based on endometrial cytology performed at 28 ± 3 DIM.

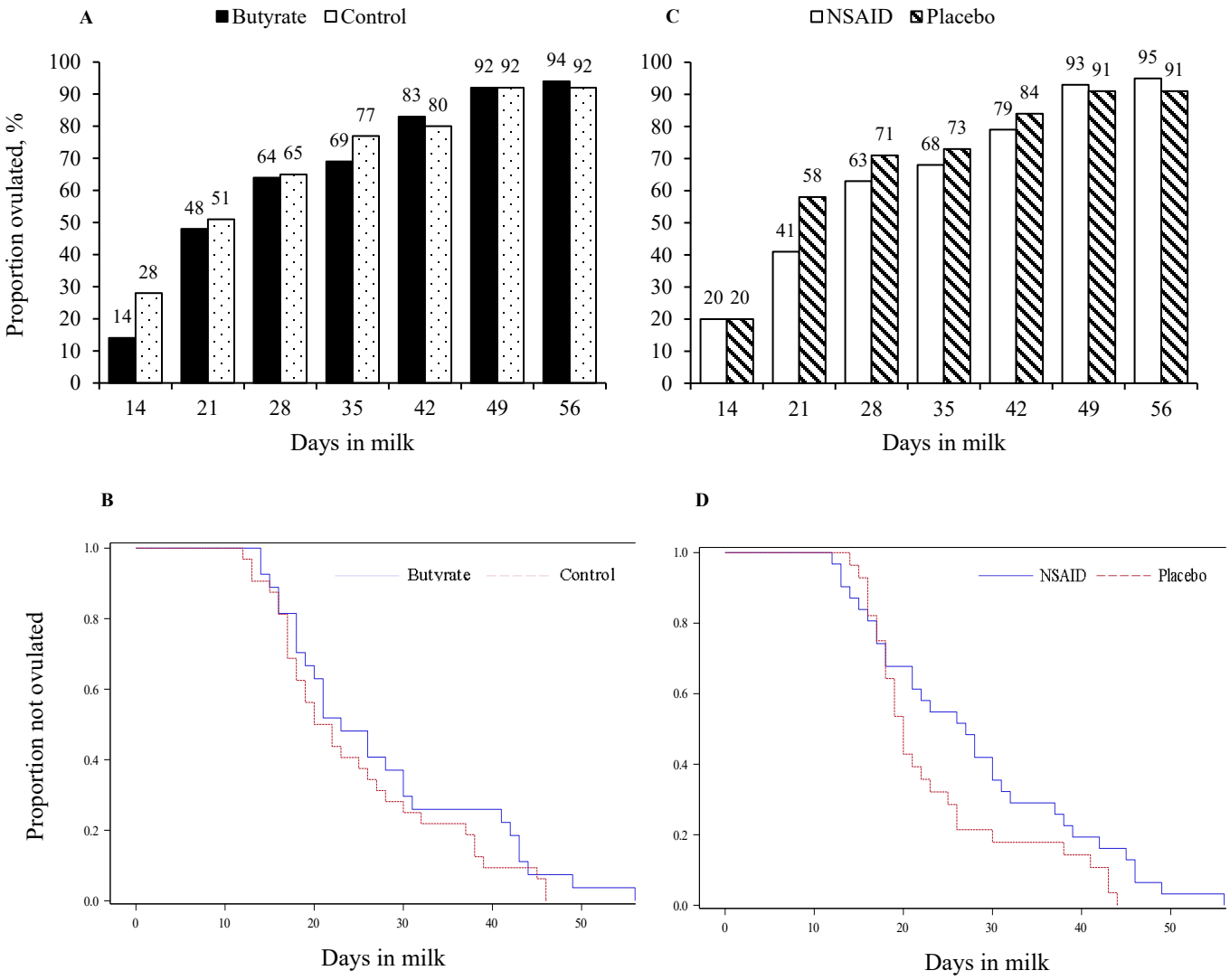

Figure 3.5.2 Proportions of cows ovulated by DIM given butyrate (fatty acid coated calcium butyrate supplement; A, B) vs. control (commercial fat and calcium carbonate supplement mixture) at 1.42% of diet DM, or when administered a non-steroidal anti-inflammatory drug (NSAID; 1 mg meloxicam/kg BW; C, D) vs. placebo (food dye). Ovulation was detected by weekly transrectal ultrasonography and results were analyzed either by GLIMMIX procedure and plotted as bar charts (A, C), or by Kaplan-Meier survival analysis approach and plotted as survival function graphs (B, D). The proportions of cows that ovulated at each DIM did not differ between diet and drug treatments. The rate of ovulation up to 56 DIM tended to differ, with NSAID-treated cows having a lower hazard for ovulation compared to control cows (hazard ratio: 0.61; 95% CI: 0.35 to 1.04; $P = 0.07$). The rate of ovulation up to 56 DIM did not differ between butyrate and control cows (hazard ratio: 0.75; 95% CI: 0.44 to 1.28; $P = 0.29$).

Chapter 4. Peripartum factors associated with variation in voluntary postpartum hay intake in dairy cows.

4.1 Introduction

It has been suggested that ruminants have "nutritional wisdom" (Provenza, 1995; Forbes and Provenza 2000; Miller-Cushon and DeVries, 2017) as they voluntarily adjust dietary intake of fermentable carbohydrates (Villalba and Provenza, 1999) and physically effective fiber (Yang and Beauchemin, 2006), perhaps in a manner to support their nutritional and metabolic needs (Giane et al., 2015). It has been postulated that animals will adjust feeding behavior to maximize energy intake (Breed and Moore, 2016), which is supported by reports of dairy cattle voluntarily increasing consumption of energy-dense grain compared to forage (Leonardi and Armentano, 2003; DeVries et al., 2008; Greter et al., 2008;), with cows experiencing greater negative energy balance sorting to a greater extent for energy-dense particles (Moore and DeVries, 2020). However, in some instances when dairy cows are given the choice between forage and energy-dense feeds, cows will voluntarily increase forage consumption (Yang and Beauchemin, 2006; Kmicikewycz and Heinrichs 2014), despite the lower energy content in forage compared to grain, and it has been proposed that factors beyond nutritional requirements may influence forage intake in ruminants (Forbes and Kyriazakis, 1995; Miller-Cushon and DeVries, 2017).

Forage intake appears to be very variable in cows, even when cows are fed the same diet and are at similar DIM (Leonardi and Armentano, 2003). The large variation in forage intake of cows with relatively similar nutrient requirements may be due to variable physiology and metabolism of animals (Launchbaugh and Howery, 2005; Manteca et al., 2008); cows may individually adjust the feed type consumed according to individual differences in physiology and metabolism (Cooper et al., 1995; Kyriazakis et al., 1999). Previous studies have evaluated factors

that may influence voluntary forage selection in beef calves (Atwood et al., 2001), beef cattle (DeVries et al., 2014a, b) dairy calves (Miller-Cushon et al., 2013a ,b; Costa et al., 2016), and acidosis-challenged mid-lactation cows (Keunen et al., 2002, DeVries et al., 2008; Kmicikewycz and Heinrichs et al., 2014). Havekes et al. (2020) reported that cows sorted for long forage particles in the first week after calving, but research with dairy cows immediately after calving is limited. Understanding factors that influence feed selection and intake is important as it would affect metabolism and health, and selective feed consumption may allow us to better manage individual cow health (Launchbaugh and Howery, 2005; Miller-Cushon and DeVries, 2017), particularly during the transition period when the majority of metabolic disorders occur (Drackley, 1999; Van Saun, 2016). As such, the objective of this research was to assess the extent of variation in hay intake amongst fresh cows offered free choice hay alongside TMR, and identify factors related to the hay intake.

4.2 Materials and Methods

4.2.1 Animals, Diets, and Experimental Design

This study was conducted at the University of Alberta Dairy Research and Technology Centre, a tie stall barn (Edmonton, Alberta, Canada), from January through August of 2021. All procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP00003716). Twenty multiparous Holstein cows were enrolled in the present study. All cows were fed a closeup TMR (21.5% starch, 16.3% CP, 39.1% NDF, 32.1% forage NDF on a DM basis; NE_L = 1.47 Mcal/kg DM) in individual mangers from 21 ± 3 d before expected calving date until calving. For 5 d after calving, cows were offered free choice timothy hay (1.0% starch, 9.6% CP, 61.6% NDF) in addition to a fresh cow TMR (26.8% starch, 18.1% CP, 33.0% NDF, 23.4% forage NDF on a DM basis; NE_L = 1.63 Mcal/kg DM; barley silage was

the sole forage source). Free choice timothy hay was 2.54-cm theoretical length of cut with a particle size distribution of 16.8, 11.8, 33.0, and 38.4% for the first (>19.0 mm), second (8.0–19.0 mm), third (1.18–8.0 mm) and fourth (<1.18 mm) sieves of the Penn State Particle Separator (Kononoff et al., 2003), respectively. Fresh cow TMR had a particle size distribution of 9.6, 31.0, 40.3, and 19.1%, on an as fed basis, for the first (>19.0 mm), second (8.0–19.0 mm), third (1.18–8.0 mm) and fourth (<1.18 mm) sieves of the Penn State Particle Separator (Kononoff et al., 2003), respectively.

Cows were fed individually with separate mangers for TMR and hay (approximately 99 cm and 61 cm wide, respectively), each offered ad libitum in addition to ad libitum water access. Total mixed ration was fed once daily at 0800 h at 105 to 110% of actual intake of the previous day. Hay was fed daily at 0800 h and was replenished throughout the day as needed. The fresh cow TMR was formulated using the Nutritional Dynamic System (NDS; CNCPS version 6.55, RUM&N) to meet or exceed nutrient requirements for a 650-kg cow producing 31 kg/d of milk with 3.8% milk fat and 3.0% milk protein. Cows were milked twice daily at 0500 and 1600 h.

4.2.2 Feed Intake and Analysis

Hay and TMR offered and refused were weighed daily to calculate daily intake. Samples of free choice hay and closeup and fresh cow TMR ingredients were collected weekly to determine their nutrient content and particle size distribution. Wet feed samples were dried at 55°C for 48 h in a forced-air oven. Wet and dried sample weights were used to calculate DM of feeds, and rations were adjusted if feed DM changed more than 2% units. Dried feed samples were ground with a Wiley mill (Thomas Scientific), using a 1-mm screen, and sent to Cumberland Valley Analytical Services (Hagerstown, MD), and analyzed for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), CP

(AOAC International, 2000; method 990.03), NDF (Van Soest et al., 1991), and starch (Hall, 2009). Wet closeup and fresh cow TMR ingredients and timothy hay samples were assessed weekly for particle size distribution using the Penn State Particle Separator according to Kononoff et al. (2003).

4.2.3 Blood Collection and Analysis

Baseline blood samples were collected at 0700 h, before the first feeding of fresh cow TMR and hay at 0800 h; for cows calved after daily feed delivery at 0800 h, the baseline blood samples were collected at 0700 h on the following day. Blood was collected from the coccygeal blood vessel into two evacuated tubes (Vacutainer, Becton Dickinson and Co.), one containing sodium heparin to harvest plasma, and the other with no additives to harvest serum. The tube with sodium heparin was placed immediately on ice after collection until centrifuging. The serum tube was kept at room temperature for a minimum of 30 min or until the blood was clotted, whichever was later. Blood was centrifuged at 3,000 × *g* (20 min, 4°C) and plasma and serum were stored at -20°C until analysis. Commercial kits were used to determine plasma concentrations of glucose (Autokit glucose; Wako Chemicals USA Inc.), fatty acids (FA; NEFA HR2; Wako Chemicals USA Inc.), and BHB (No. H6501; Roche), and serum concentrations of haptoglobin (Hp; Phase Haptoglobin Assay, Tridelta Development Ltd.) and serum amyloid A (Multispecies SAA ELISA Kit, Tridelta Development Ltd.) using a plate reader (SpectraMax 190; Molecular Devices, LLC).

4.2.4 Statistical Analysis

Data reported in the present study is from 2 d before actual calving date until D 5; D 1 was defined as the first day cows were provided fresh cow TMR and free choice hay. Daily hay intake was assessed as kg/d and as % of total DMI, and a Tukey-Kramer adjustment test was

used to compare daily hay intake. Hay intake (% of total DMI; 5-d mean values) for each cow was used to evaluate its relationship with DMI at 2 d before calving, and concentrations of plasma metabolites and serum inflammation markers right after calving, but before the first feeding. Statistical analysis was conducted using bivariate regression analysis in JMP 16.1.0 (SAS Institute, Inc), and Pearson Correlation coefficients were determined using the MULTIVARIATE procedure. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$. A post-hoc power analysis for Pearson Correlation was performed using the POWER procedure of SAS 9.4 (SAS Institute, Inc.); the sample size of 20 allowed for detection of significant relationships between variables with a correlation coefficient of 0.58 ($\alpha = 0.05$; 80% power).

4.3 Results and Discussion

In the present study, cows had large variation in daily free choice hay intake, ranging from 0 to 4.7 kg/d. Mean (range) hay intake was 1.17 kg (0.15 - 2.8 kg), 1.37 kg (0 - 4.7 kg), 0.62 kg (0 - 2.5 kg), 0.50 kg (0 - 2.4 kg) and 0.58 (0 - 2.1 kg) on d 1, 2, 3, 4, and 5 postpartum, respectively (Figure 1). Hay intake on d 2 was higher than d 3, 4, and 5 ($P < 0.0001$). Mean (range) hay intake (% of total DMI) was 8.8% (1.0 - 21.1%), 8.0% (0 - 30.6%), 5.9% (0 - 47.0%), 6.1% (0 - 55.2%), and 3.7 (0 - 16.7%) on d 1, 2, 3, 4, and 5 postpartum, respectively. Intake of fresh cow TMR was 14.1 kg (6.4 - 22.1 kg), 13.9 kg (4.9 - 24.5 kg), 15.4 kg (2.8 - 24.6 kg), 14.7 kg (0.7 - 23.6 kg), and 16.2 kg (6.2 - 26.0 kg) d 1, 2, 3, 4, and 5 postpartum, respectively. Due to variable hay intake, starch content of consumed diets (TMR + hay) varied among cows; mean (range) dietary starch content, on a DM basis, was 24.6 (21.4 - 26.6), 24.9 (18.9 - 26.8), 25.3 (14.7 - 26.8), 25.3 (12.6 - 26.8), and 25.9 (22.5 - 26.8) on d 1, 2, 3, 4, and 5 postpartum, respectively. Nonetheless, these data should be interpreted with caution as orts were

not analyzed for nutrient composition in the present study, and the starch content of consumed diets was calculated assuming no sorting. Additionally, we acknowledge that particle size of the fresh cow TMR was smaller than intended; thus, less inclusion of physically effective fiber in the fresh cow TMR may have affected voluntary free choice hay intake in the fresh period.

Free choice hay intake was not associated with baseline concentration of plasma glucose ($P = 0.64$, r = -0.12) or serum amyloid A ($P = 0.35$, r = 0.26). Similarly, postpartum TMR intake was not associated with baseline concentration of plasma glucose ($P = 0.61$, r = 0.12), serum amyloid A ($P = 0.43$, r = -0.19) or haptoglobin ($P = 0.17$, r = -0.32). However, cows with higher baseline concentration of serum inflammatory marker, Hp, (after calving, before fresh cow TMR and hay was fed) consumed more hay (% of total DMI) from d 1 to 5 ($P = 0.01$; r = 0.60; Figure 2), suggesting inflammatory markers at calving are positively associated with voluntary hay consumption in the fresh period. Little research has assessed factors associated with voluntary forage intake in dairy cows, aside from acidosis research; when cows are fed high starch diets and experience acidosis, they are reported to increase consumption of long forage particles (DeVries et al., 2014a), with greater amounts of forage being consumed in cases of more severe acidosis (DeVries et al., 2014b). While ruminal acidosis is often associated with increased inflammation (Plaizier et al., 2012; Zebeli et al., 2015), in the present study, serum Hp concentration was measured before fresh cow TMR was offered, thus cows had not yet consumed high starch diets, thus inflammation may not be related to acidosis and another mechanism may exist. The present study did not measure rumen pH, however, therefore we cannot definitively say if it influenced hay intake.

In monogastrics, it has been suggested that forage intake is motivated by gastrointestinal discomfort (Sueda et al., 2008; Shurkin, 2014), as it may heal gastrointestinal damage and have

an anti-inflammatory effect (Fruth et al., 2014). As monogastric animals increase forage intake during gastrointestinal disturbances, it is possible that factors associated with gastrointestinal discomfort, beyond rumen pH, influence forage intake. A similar phenomenon has been proposed in cattle termed “nutritional wisdom”, in the context of cows altering the type of feed consumed based on their internal metabolic state (Provenza 1995; Forbes and Provenza 2000; Miller-Cushon and DeVries, 2017), which includes sensorial, metabolic, and physiological signalling (Ginane et al., 2015). Alterations in feed preferences in cows following internal feedback have been described as adaptive because it may allow cows to meet metabolic needs, maintain homeostasis (Ginane et al., 2015), and relieve metabolic discomfort (Gregorini et al., 2015). Feed preference also varies greatly among cows, perhaps due to differences in individual internal metabolic state (Ginane et al., 2015; Gregorini et al., 2015), which may explain the great variation in free choice hay intake in the present study. Providing free choice hay may have allowed cows to adjust their hay intake individually to respond to internal signalling related to inflammation, metabolism, and energy balance, however, additional research is necessary to assess this speculation.

Cows with lower DMI 2 d before calving consumed less TMR (kg/d; $P < 0.01$; r = 0.71) and consumed more hay (% of total DMI; $P < 0.01$, r = 0.63) from d 1 to 5 after calving. In addition, cows with higher baseline plasma BHB concentration ($P < 0.01$; r = 0.68) consumed less TMR (kg/d; $P = 0.02$, r = -0.51), and more hay (% of total DMI) from d 1-5. Cows with higher baseline plasma FA concentration tended to consume more hay (% of total DMI; $P = 0.06$; r = 0.41) over the 5-d period, however, baseline plasma FA concentration was not associated with 5-d postpartum TMR intake (kg/d; $P = 0.18$, r = -0.31). Plasma FA and BHB are indicators of energy balance, and often increase following reduced intake leading to fat

mobilization and subsequent ketone production (LeBlanc, 2006; Djoković et al., 2017). In the present study, higher BHB and FA was associated with lower pre-calving DMI; cows with lower DMI 2 d before calving had higher plasma FA ($P < 0.0001$; r = -0.67) and BHB concentration ($P < 0.001$; r = -0.58). In addition, cows with lower DMI 2 d before calving had greater baseline serum Hp concentration ($P = 0.03$; r = -0.64), indicating reduced pre-calving DMI is associated with increased concentration of inflammatory markers at calving, as described previously by Kuhla (2020). Taken together, greater reductions in pre-calving DMI may be positively associated with markers of fat mobilization, ketone production, and inflammation, each of which were associated with greater voluntary hay intake in fresh cows in the present study.

It is possible that postpartum hay intake is directly related to pre-calving intake or post-calving metabolism and inflammation. However, postpartum hay intake might have been influenced by another factor associated with lower pre-calving DMI and greater post-calving concentration of plasma BHB and FA, and serum Hp. Metabolic disorders are often associated with reduced intake (Sundrum et al., 2015), increased inflammatory markers (Esposito et al., 2014; Pohl et al., 2015), and increased blood FA and BHB concentration (Ospina et al., 2010). Therefore, reduced pre-calving intake and increased post-calving inflammation and circulating FA and BHB may occur concurrently with, or as consequence of metabolic disorders, which may increase free choice hay consumption in fresh cows. By providing free choice hay in addition to TMR rather than including hay in TMR, fresh cows experiencing metabolic disorders can alter what they consume based on their needs while other cows consume only TMR to meet high energy demands associated with milk production.

4.4 Conclusion

Overall, when provided free choice hay for 5 days after calving, there was substantial variation in voluntary hay intake amongst cows. Our findings suggest that reduced pre-calving intake and greater post-calving ketone production and inflammation are associated with greater voluntary hay intake in fresh cows.

4.5 Figures

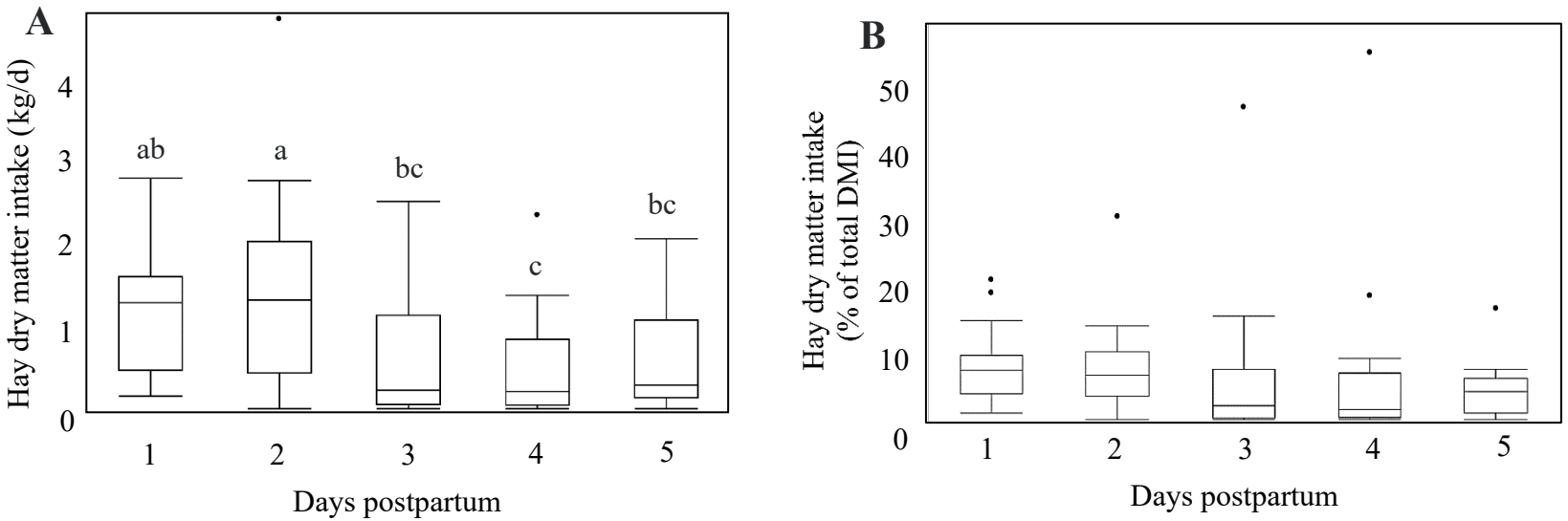

Figure 4.5.1 (A) Absolute hay intake (kg/d) and (B) hay intake (% total DMI) for cows offered ad libitum free choice hay for the first 5-d postpartum. Day 1 was defined as the first day cows were provided fresh cow TMR and free choice hay. The horizontal black line within each box denotes median value; the boxes extend from the 1st to 3rd quartile, representing the interquartile range; the whisker extending from the bottom of box represents 1.5 times below the interquartile range; the whisker extending from the top of box represents 1.5 times above the interquartile range; dots beyond the upper whisker denote outliers.

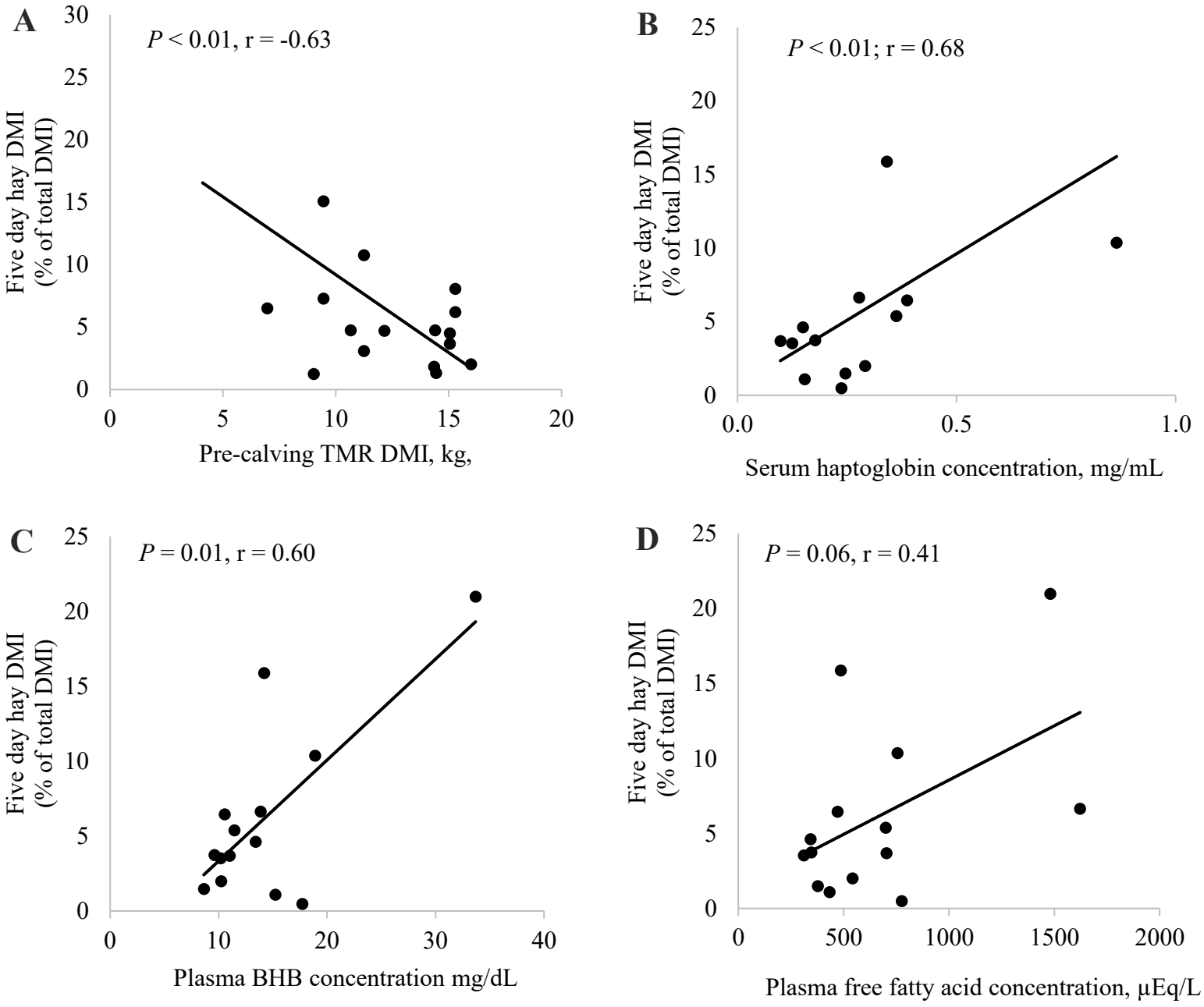

Figure 4.5.2 The correlation between 5-d hay dry matter intake (DMI; % of total DMI) and pre-calving DMI (2 d before calving; A), the baseline concentration of serum haptoglobin (B), plasma β-hydroxybutyrate (BHB; C), and plasma fatty acids (C) for cows offered ad libitum free choice hay for the first five days postpartum. Baseline plasma and serum samples were collected after calving, but before fresh cow feed and hay were offered to cows.

Chapter 5. The effects of offering free choice hay for the first five days postpartum on productivity, serum inflammatory markers, gut permeability, and colon gene expression in fresh dairy cows.

5.1 Introduction

The fresh period, defined as 1 to 21 d postpartum, is a challenging time for dairy cattle as their energy demands exceed their energy intake, thus putting them in a state of negative energy balance. Producers often provide high starch diets to fresh cows to help meet energy demands. However, high starch diets may increase metabolic disorder risk (Allen and Piantoni, 2013), compromise gastrointestinal tract (**GIT**) barrier function (Steele et al., 2009; Tao et al., 2014a Bach et al., 2018), and increase proinflammatory gene expression in the rumen (Liu et al., 2013) and hindgut (Tao et al., 2014b), and positive acute phase protein concentration in blood (Gozho et al., 2007; Emmanuel et al., 2008), each of which can subsequently reduce milk production (Zebeli and Metzler-Zebeli, 2012). There have been conflicting results within literature regarding the response of fresh cows to high starch diets; some have reported high starch diets increased milk production (Rabelo et al., 2003; McCarthy et al., 2015a; Haisan et al., 2021) whereas others have observed a decrease in milk production (Dieho et al., 2016; Shi et al., 2019). Similarly, the effect of high starch diets on inflammatory markers in fresh cows appears inconsistent, with some studies reporting increased serum haptoglobin (**Hp**) concentration with increased dietary starch content (McCarthy et al., 2015b; Albornoz et al., 2020) and others reporting decreased serum Hp concentration (Haisan et al., 2021).

The discrepancy of results reported in Haisan et al. (2021) may be due to offering up to 3 kg/d of free choice hay, separately from TMR, for the first 3 d postpartum; Haisan et al. (2021) found cows fed a high starch postpartum diet had reduced serum Hp concentration and increased milk production, compared to cows fed a lower starch postpartum diet. Increasing dietary forage

NDF can reduce consequences associated with high starch diets (NASEM, 2021); reductions in the proportion of rapidly fermentable carbohydrates in the GIT (Beauchemin and Penner, 2009) may improve gut barrier function (Tao et al., 2014a) and gut integrity, and reduce the expression of pro-inflammatory genes and proteins in the rumen (Liu et al., 2013) and hindgut (Tao et al., 2014b).

However, cows vary substantially in their nutrient requirements and in their response to dietary changes (Sundrum, 2015). Cows with high milk production, and thus high energy requirements, may be negatively affected by increased fiber in the TMR as fiber increases rumen fill and may decrease DMI, whereas lower producing cows can consume more fiber without a reduction in milk production (Allen, 2000; Voelker et al., 2002; Boerman et al., 2015). Conversely, increases in dietary starch content may increase milk production in high producing cows, but not in low producing cows (Boerman et al., 2015). Variation in cow response to dietary starch and forage NDF creates a dilemma for producers who feed a single TMR to fresh cows; a high starch diet may increase milk yield in some cows, while inducing metabolic disorders in others, whereas a low starch diet may lower metabolic disorder risk for some cows, but reduce milk yield in high producing cows. Many dairy farms formulate a single ration for fresh cows, despite large variation in animal requirements and response, which consequently affects individual response and production (Provenza et al., 2003; Gregorini et al., 2015).

Providing free choice hay outside of the TMR, may allow some cows to increase forage NDF intake on an individual basis, while other cows still have access to a high starch TMR to support high milk production. To our knowledge there has been no research evaluating the effects of offering free choice hay to fresh cows. As such, the objective of the present study was to evaluate the effects of offering free choice hay to cows from d 1 to 5 postpartum, on feed

intake, milk yield, plasma metabolites, serum inflammatory markers, rumination, gut permeability, and colon gene expression in the fresh period. It was hypothesized that cows offered free choice hay would have lower gut permeability, lower inflammation, and higher milk production, compared to cows not offered hay.

5.2 Materials and Methods

This study was conducted at the University of Alberta Dairy Research and Technology Centre, a 146-cow tiestall barn (Edmonton, Alberta, Canada), in 2021. A portion of data from this study, evaluating peripartum factors associated with variation in voluntary postpartum hay intake, is presented in Engelking and Oba (unpublished data). All procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP00003716).

5.2.1 Animals, Diets, and Experimental Design

Thirty-two multiparous Holstein cows were blocked by expected calving date, BCS, and parity, and assigned randomly to 1 of 2 treatments: offering free choice hay for the first 5 d postpartum (**HAY**; n = 20) or no free choice hay (**NH**; n = 12). A greater number of cows were assigned to the HAY treatment to account for anticipated greater variation associated with hay intake and maintain statistical power. Using the POWER procedure of SAS 9.4 (SAS Institute, Inc), a post-hoc analysis was conducted using TMR intake data; using sample size of 20 with 15% variation in TMR intake for HAY, and a sample size of 12 with 10% variation in TMR intake for NH, at least a 10% difference in TMR intake could be detected between treatments ($\alpha = 0.05$, power = 80%).

Cows in the HAY treatment were provided ad libitum free choice timothy hay (61.6% NDF, 9.6% CP; Table 1) for the first 5 d postpartum. Hay was offered outside of the TMR, in a

manger (approximately 61 cm wide) separate from their TMR manger (approximately 99 cm wide; Figure 1). Timothy hay was chopped at 2.54 cm theoretical length of cut and had a measured particle size distribution of 16.8, 11.8, 33.0, and 38.4% for the first sieve (>19.0 mm), second sieve (8.0–19.0 mm), third sieve (1.18–8.0 mm), and pan (<1.18 mm) of the Penn State Particle Separator (Kononoff et al., 2003), respectively. Hay was replaced each day at 0800 h and replenished throughout the day as needed.

All cows were fed a common closeup TMR containing 21.5% starch and 32.1% forage NDF on a DM basis from 21 ± 3 d before expected calving date to calving (Table 2). All cows were fed a common fresh cow TMR containing 26.8% starch and 23.4% forage NDF on a DM basis after calving. Day 1 was defined as the first day cows received fresh cow diets, and the fresh period was defined as d 1 to 21 postpartum. Particle size distribution of the postpartum TMR was 9.6, 31.0, 40.3, and 19.1%, o for the first sieve (>19.0 mm), second sieve (8.0–19.0 mm), third sieve (1.18–8.0 mm), and pan (<1.18 mm) of Penn State Particle Separator, respectively. The postpartum TMR was formulated using the Nutritional Dynamic System (NDS; CNCPS version 6.55, RUM&N) to meet or exceed nutrient requirements for a 650-kg cow producing 31 kg/d of milk with 3.8% milk fat and 3.0% milk protein. Cows were fed TMR individually in mangers once daily at 0800 h at 105 to 110% of actual intake of the previous day and had ad libitum water access.

5.2.2 Feed Intake and Analysis

Hay and TMR offered and refused were weighed daily to calculate daily TMR and total DMI (TMR + hay). Samples of free choice hay and ingredients of closeup and fresh cow TMR were collected weekly, and dried at 55 °C for 48 h in a forced-air oven. Weekly wet and dry sample weights were used to calculate DM of feeds, and rations were adjusted if feed DM

changed more than 2% units. Dried feed samples were ground with a Wiley mill (Thomas Scientific), using a 1-mm screen, and sent to Cumberland Valley Analytical Services (Hagerstown, MD), and analyzed for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), CP (AOAC International, 2000; method 990.03), NDF (Van Soest et al., 1991), undigested NDF (Goering and Van Soest, 1970), and starch (Hall, 2009). Particle size of the closeup TMR, fresh cow TMR, and timothy hay was assessed weekly using the Penn State Particle Separator according to Kononoff et al. (2003). Particles were separated by size into 4 fractions; long (>19 mm), medium (<19 to >8 mm), short (<8 to >1.18 mm), and fine (<1.18 mm) particles.

5.2.3 Milk Yield, Body Condition Score, Body Weight, and Rumination Data

Cows were milked twice daily at 0500 and 1600 h. Milk yield was recorded at morning and afternoon milkings, and the total daily milk yield was calculated. Daily feed efficiency was calculated by dividing daily milk yield by total daily DMI (TMR + hay DMI). Body condition score was recorded at 1 and 21 DIM (± 1 d) by two trained individuals using the 5-point scale (Wildman et al., 1982), and the average BCS was recorded. Body weight was recorded at 1 and 2 DIM (averaged for d 1 BW) and at 21 and 22 DIM (±1; averaged for d 21 BW). Body weight was measured after the morning milking, but before feeding. Changes in BW and BCS from d 1 to d 21 were calculated.

Rumination data were collected using CowManager SensOor (Agis Automatisering) tags clipped to radio frequency identification ear tags already present on cows. CowManager tags were attached 7 to 10 d before expected calving date to allow tags to configure to cow movements. Data from d 1 to 21 were recorded for analysis. As described by Bikker at al. (2014), movements of the cow's ear were registered by a 3-dimensional accelerometer in the

SensOor, and data were sent wirelessly to a router, which then transmitted data to a coordinator connected to a central computer. Data recorded by the SensOor system provided rumination minutes per hour, which were then used to calculate rumination minutes per day.

5.2.4 Blood Collection and Analysis

Blood samples were collected after the morning milking, but before feeding, at approximately 0700 h at d 1 and 3. Samples collected at d 1 were considered baseline samples as they were taken after calving, but before free choice hay or TMR was offered. D 1 data were used to assess change in blood metabolites from d 1 to 3, and as a covariate for d 3 data, when necessary. Day 3, rather than d 5, was selected for the second blood collection to avoid confounding effects of urinary catheterization procedure, including non-steroidal anti-inflammatory drug (**NSAID**) administration, on d 3, after blood collection. Blood was collected from the coccygeal blood vessel into two evacuated tubes (Vacutainer, Becton Dickinson and Co.), one containing sodium heparin to harvest plasma, and the other with no additives to harvest serum. The plasma collection tube containing sodium heparin was placed immediately on ice after collection until centrifuging. The serum tube was kept at room temperature for a minimum of 30 min or until the blood was clotted, whichever was later. Blood was centrifuged at 3,000 × *g* (20 min, 4°C) and plasma and serum were stored at -20°C until analysis. Commercial kits were used to determine plasma concentrations of glucose (Autokit glucose; Wako Chemicals USA Inc.), fatty acids (**FA**; NEFA HR2; Wako Chemicals USA Inc.), and BHB (No. H6501; Roche), and serum concentrations of haptoglobin (Phase Haptoglobin Assay, Tridelta Development Ltd.) and serum amyloid A (**SAA**; Multispecies SAA ELISA Kit, Tridelta Development Ltd.) using a plate reader (SpectraMax 190; Molecular Devices, LLC).

5.2.5 Gut Permeability Measurement

5.2.5.1 Lithium chromium EDTA preparation

Lithium chromium EDTA (Li·Cr-EDTA) was used as a marker to assess gut permeability. Chromium EDTA is a large (340 kDa), non-nutritive molecule (Wood et al., 2015), that can only be absorbed from the digesta via paracellular transport, thus its recovery in urine represents permeability of the gut (Hollander, 1992; Schweigel et al., 2005), and it was validated as a suitable marker in cattle (Zhang et al., 2013).

Cows were dosed with 1.8 L of 0.18 M chromium EDTA. Following the protocol outlined by Hall and Van Soest (2019), crystalized Li·Cr-EDTA was prepared using CrAcOH (MilliporeSigma), EDTA (MilliporeSigma), and lithium hydroxide monohydrate (MilliporeSigma) in equimolar amounts of Cr, Li, and EDTA. On the day of Li·Cr-EDTA dosing, homogenized Li·Cr-EDTA crystals were reconstituted with deionized water, pH was adjusted to approximately 6.0 using sodium hydroxide, and the solution was brought to an exact volume of 1.8 L/cow.

5.2.5.2 Urinary Catheterization

At 3 DIM, after morning milking, blood sample collection, and feeding, cows were restrained in a headgate for urinary catheter insertion for total urine collection. Before catheter insertion, cows were treated with the NSAID, Ketoprofen (10% Injectable Cattle Anafen, Merial Boehringer Ingelheim Animal Health), to reduce pain and discomfort associated with catheterization, at a dose of 3 mg/kg body weight.

Cows were thoroughly scrubbed from the anus past the vulva with an antiseptic iodine scrub solution (PREPODYNE HS, West Penetone) three times, followed by a surgical iodine

solution rinse (PREPODYNE GEN, West Penetone). Following aseptic surgical techniques for large animals (Ray Rajotte Surgical, Medical Research Institute, University of Alberta), the bladder was accessed through a sterile urethral catheter (Foley Catheters, LUBRICATH, 2-Way, Specialty, Medium Round Tip, Two Staggered Drainage Eyes, Bard). Once the catheter was placed in the bladder, the catheter balloon was filled with sterile saline to secure its position in the bladder. Through the catheter, a 2% povidone-iodine antiseptic solution (BETADINE) was injected into the bladder. The catheter was clamped with surgical forceps to ensure the solution remained in the bladder, and after 10 minutes, the clamp was removed to allow the animal to urinate.

5.2.5.3 Lithium Chromium EDTA Administration

Lithium chromium EDTA was administered to each cow following urinary catheterization. Cows remained restrained in the headgate and were also haltered to immobilize the head during administration. After the cow was secured, a Frick speculum (Drench-Mate) was inserted through the mouth, past the esophagus, and into the rumen. Once in the rumen, Nalgene tubing was inserting through the speculum into the rumen and the other end of the tube was fastened to a drench pump (Bovivet Rumen Drench Pump), and the Li·Cr-EDTA solution was pumped into the rumen.

5.2.5.4 Urine Collection

After dosing with Li·Cr-EDTA, the urinary catheter was connected to a collection jug using Nalgene tubing. The cow was then returned to her tie-stall along with the collection jug which was placed out of the cow's reach to prevent urine spillage. Urine was collected from the jug every 6 h, for a total of 48 h. Total urine volume was recorded and a representative sample was collected at each time point. After 48 h, the urine jug and Nalgene tubing were disconnected

from the urinary catheter and the cow's bladder was flushed with 2% povidone-iodine antiseptic solution for 10 min, as previously described, before removal of the catheter.

5.2.5.5 Urine Digestion and Chromium Analysis

A single representative urine sample was created for each cow by compositing a portion of each urine sample from each time point, according to urine collected at each time point. From each composited cow sample, 10 mL of urine was dried at 60°C for 3 d. After drying, urine samples were digested and analyzed for chromium content as described by Williams et al. (1962); urine was digested using phosphoric acid-manganese sulfate and potassium bromate on a hot plate at 300°C. Digested urine was allowed to cool prior to combining it with calcium chloride (4000 ppm calcium) and brought to a volume of 100 ml with deionized water. Chromium concentration of the samples was determined in triplicate using atomic absorption spectrophotometry at 541 nm (AA240 FS, Varian). Total chromium excretion in urine was calculated using chromium concentration of digested samples and total urine output of each cow. All samples were analyzed for chromium concentration in one run, and the intra-assay CV was 1.33%.

5.2.6 Colon Gene Expression

5.2.6.1 Colon Biopsy Collection

At 5 DIM, after the completion of the urine collection, when the urinary catheter was removed, cows were restrained in a headgate for collection of colon biopsies following protocols from Bach et al. (2018) and van Niekerk et al. (2018). Feces were manually removed from the rectum, and a lubricated endoscope (100 cm length, 9.8 mm diameter; GIF-Q140, Olympus) was inserted through the rectum to the colon, approximately 100 cm from the rectum. To visualize the biopsy site, the endoscope and a light source and processor (CLV-U40 and CV-140,

Olympus), were used to locate the distal colon, approximately 100 cm from the anus. Once the distal colon mucosa was successfully identified, Captura hot biopsy forceps (2.4 mm diameter; HDBF-2.4-230-S, Cook Medical) were threaded through the endoscope to the colon epithelium, and the biopsy forceps were used to obtain tissue samples. Ten tissue samples (approximately 12.6 mg/sample; van Niekerk et al., 2018) were collected per cow. Immediately after collection, tissue samples were rinsed with PBS and stored in RNAlater (Invitrogen) at 4°C for approximately 24 h, followed by storage at -80°C until further processing. The endoscope instrument was thoroughly cleaned between cows using specialized endoscope detergent to reduce risk of sample cross contamination (INTERCEPT Detergent, Medivators).

5.2.6.2 Tissue Homogenization, RNA Extraction, and Reverse Transcription

Biopsy tissues were combined with 1 mL of TRIzol (TRIzol Reagent, Invitrogen) and homogenized using a soft tissue homogenizing kit (Precellys CK14 Lysing Kit, Soft Tissue Homogenizing, Bertin) in a homogenizer (Precellys 24, Bertin Technologies) at 6300 rpm (2 × 30 sec with 10 seconds between). After tissue homogenization, RNA was extracted and precipitated with chloroform, a high salt solution, and isopropanol, followed by a wash step with ethanol according to the protocol described by Chomczynski and Sacchi (1987). Precipitated RNA was then dissolved in 50 µL of molecular grade water. Concentration and purity of RNA samples was determined using a spectrophotometer (NanoDrop 1000 Spectrophotometer; NanoDrop Technologies), and RNA integrity was assessed using the RNA integrity number (Agilent 4150 Tapestation, Agilent Technologies). Quality control for all samples was conducted by assessing the 260/280 (average = 2.0 ± 0.1) and 260/230 (average = 2.2 ± 0.1) absorbance ratios and RNA integrity number (average = 7.2 ± 1.4). Next, 1 µg of RNA from each sample underwent DNA digestion using a DNase treatment kit (DNase I, Amplification Grade;

Invitrogen) followed by reverse transcription using a cDNA synthesis kit (iScript Reverse Transcription Supermix for RT-qPCRl Bio-Rad).

5.2.6.3 Real-Time Quantitative PCR

On the day of real-time quantitative PCR (**qPCR**) cDNA underwent a 1:20 dilution. Each qPCR reaction well contained 2 µL of diluted cDNA, 5 µL of SYBR green (Fast SYBR Green Master Mix, Applied Biosystems), 1 µL of 20 µM forward primer, 1 µL of 20 µM reverse primer, and 1 µL of nuclease free water. Each sample was run in triplicate well reactions. Gene expression was quantified for claudin 1 (*CLDN1*), claudin 4 (*CLDN4*), occludin (*OCLN*), zonula occludens 1 (*ZO1*), toll-like receptor 4 (*TLR4*), and interleukin 10 (*IL10*). Forward and reverse primer sequences are listed in Table 3. All genes assessed with qPCR were ran according to the following program: initial denaturing for 20 seconds at 95°C, 40 cycles of 3 seconds at 95°C, and 30 seconds at 60°C. Melt curves were assessed for each primer, prior to qPCR analysis, and for each sample during qPCR analysis, to ensure amplification specificity. Relative expression of each gene in each sample was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), where β-actin (*ACTB*) was the housekeeping gene, and the mean NH gene expression for each gene was used as the calibrator. Intra-assay and inter-assay CV were 0.49 and 7.6%, respectively.

5.2.7 Statistical Analysis

Data with repeated measures including feed intake, rumination, milk yield and feed efficiency, were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute, Inc). Postpartum intake data were assessed in three periods: d 1 to 2 (when hay was offered to HAY, before NSAID administration and urinary catheterization), d 1 to 5 (when hay was offered to HAY), d 6 to 21 (when hay was no longer offered to HAY; carryover period). Multiple

covariance structures were tested for each parameter and the covariance structure with the lowest Akaike information criterion value was selected for each variable prior to running the model. The statistical model evaluated the fixed effects of treatment (HAY vs. NH), DIM, and the interaction of treatment × DIM. Days in milk was considered a repeated measure and the effect of cow nested within block was included as a random effect.

Plasma, serum, change in BW and BCS, chromium excretion, and gene expression data were analyzed using JMP 16.1.0 (SAS Institute, Inc). The relationship between variables was assessed using bivariate regression analysis and the MULTIVARIATE procedure; Pearson correlation coefficients were used for normally distributed data, and Spearman correlation coefficients were used when data were not normally distributed. Treatment differences (HAY vs. NH) were determined using the Fit Model procedure with treatment and block used as fixed effects. For all response variables, significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

5.3 Results

From d 1-5, there were 2 cases of pneumonia (Hay: n = 2), 1 case of mastitis (HAY: n = 1), 1 case of ketosis (NH: n = 1), 1 case of dystocia (NH: n = 1), and 1 case of retained placenta (NH: n = 1). From d 6-21, there were 4 cases of mastitis (HAY: n = 3; NH: n = 1), and 1 case of infection (NH: n = 1). However, all cows, regardless of health events, were included in statistical analysis.

On d 3 after calving, there were no differences between HAY and NH in concentration of plasma glucose (62.8 vs. 64.5 mg/dL; $P = 0.69$; Table 4), FA (632 vs. 632 µEq/L; $P = 0.99$), BHB (18.7 vs. 16.6 mg/dL; $P = 0.71$), or SAA (538 vs. 700 µg/mL; $P = 0.36$). However, serum Hp

tended to be lower in HAY compared to NH (0.95 vs. 1.52 mg/mL; $P = 0.08$). Within the HAY treatment, cows that consumed more hay on d 1 and 2 (kg/d) had a smaller increase in SAA from d 1 to 3 (d 3 concentration - d 1 concentration; $P = 0.05$, r = 0.37; Figure 2) and tended to have a smaller increase in serum Hp concentration from d 1 to 3 ($P = 0.08$, r = 0.36). Urinary chromium excretion from d 3 to 5, the indicator of gut permeability, did not differ between HAY and NH (633 vs. 487 mg; $P = 0.35$). On d 5, colon gene expression did not differ between treatments for any genes assessed (Table 5).

Dry matter intake in the prepartum period did not differ between HAY and NH (12.6 vs. 12.8 kg/d; $P = 0.57$). Postpartum intake data were assessed in three periods: d 1 to 2, d 1 to 5, d 6 to 21 (Table 6); d 1-2 data allowed evaluation of intake factors prior to d 3 blood collection and the d 3 to 5 permeability assay, d 1 to 5 data allowed evaluation of effects during hay availability, and d 6 to 21 allowed evaluation of carryover effects after hay was not offered. On d 1 to 2, HAY cows tended to have lower TMR DMI compared to NH (13.9 vs. 15.7 kg/d; $P = 0.06$), however, there were no differences in total DMI (TMR + hay DMI; 15.1 vs. 15.7 kg/d; $P = 0.64$). Forage NDF intake was similar between HAY and NH on d 1 to 2 (3.89 vs. 3.45 kg/d; $P = 0.33$), but proportional forage NDF intake (forage NDF intake as a % of total DMI) was greater for HAY, compared to NH (27.5 vs. 23.4%; $P < 0.01$). Conversely, HAY cows had lower starch intake (3.19 vs. 4.16 kg/d; $P < 0.01$), lower proportional starch intake (starch intake as a % of total DMI; 24.8 vs. 26.8%; $P < 0.0001$), and a lower starch:forage NDF ratio (starch intake (kg/d)/forage NDF (kg/d); 0.91 vs. 1.14; $P < 0.0001$), compared to NH.

From d 1 through 5, HAY cows had lower TMR DMI (15.0 vs. 17.1 kg/d; $P < 0.01$), total DMI (15.9 vs. 17.1 kg/d; $P = 0.05$), proportional starch intake, (25.2 vs. 26.8%; $P < 0.01$), and tended to have lower starch intake (3.63 vs. 4.48 kg/d; $P = 0.08$), compared to NH. From d 1

through 5, HAY cows had similar forage NDF intake (3.93 vs. 3.87 kg/d; $P = 0.87$), but had higher proportional forage NDF intake (26.2 vs. 23.4%; $P < 0.001$) and a lower starch:forage NDF ratio (0.98 vs. 1.14; $P < 0.001$) compared to NH. From day 6 through 21, when hay was no longer offered to HAY cows and all cows were fed the same TMR, HAY cows tended to have lower TMR DMI compared to NH (20.3 vs. 20.8 kg/d; $P = 0.06$).

There were no treatment effects from d 1 to 5 on milk yield (27.3 vs. 28.0 kg/d; $P = 0.38$; Table 7), feed efficiency (1.88 vs. 1.70 kg milk yield/kg DMI; $P = 0.18$), or rumination time (429 vs. 418 min/d; $P = 0.57$), for HAY vs. NH. From d 6 to 21, milk yield (44.5 vs. 44.8 kg/d; $P = 0.63$) and rumination time (579 vs. 589 min/d; $P = 0.19$) did not differ between HAY and NH, however, HAY cows tended to be more feed efficient compared to NH (2.23 vs. 2.17 kg milk yield/kg DMI; $P = 0.06$). There were no differences between HAY and NH for change in BCS (-0.20 vs. -0.30; $P = 0.34$) or BW (-3.49 vs. -4.0 kg/d; $P = 0.50$) over the first 21 DIM.

5.4 Discussion

A single ration is typically formulated for fresh cows, despite wide variation in intake (Forbes, 2003; Penner et al., 2009) and cow responses to the same diet (Weber et al., 2013; Gao and Oba, 2014; Guinguina et al., 2020). Due to this extensive variation, Gregorini et al. (2015) highlighted the need for dairy systems to allow more individualized feed selection, as feed preference is at least partially related to individual internal state and metabolism, and cows may select certain feedstuffs based on metabolic discomfort or metabolic needs (Ginane et al., 2015). In a separate component of this study, reported in Engelking and Oba (unpublished data), we found that cows with lower pre-calving DMI, and higher plasma BHB and serum Hp concentration at calving, consumed more free choice hay for the first 5 DIM.

In the present study, we intended to evaluate the effects of providing free choice hay, and thus allowing individual feed selection, on cow performance, physiology, and health, compared to cows not offered free choice hay. The fresh cow TMR in our study was formulated for a high dietary starch content (26.8%) in accordance with previous studies (Dann and Nelson, 2011; McCarthy et al., 2015a,b). During the transition to a highly fermentable diets, impairments in gut barrier function are reported due to low pH (Emmanuel et al., 2007) and reduced tight junction expression associated with rapid fermentation in the GIT, which may lead to leaky gut, damage to the gut epithelium, and an increase in pro-inflammatory gene and protein expression in the rumen and hindgut (Liu et al., 2013; Tao et al., 2014a,b). By providing free choice hay to cows and allowing them to choose between TMR and free choice hay, some cows may increase forage NDF intake on an as-needed basis, while other cows consume only a high starch TMR. We hypothesized that cows offered free choice hay would have lower gut permeability and inflammation, thus reducing related energy expenditure, ultimately increasing milk yield, compared to cows not offered hay.

We observed that HAY cows tended to have lower serum inflammatory marker, Hp, at d 3 after 2 days of free choice hay provision, compared to NH. Within the HAY treatment, cows that consumed more hay (d 1 and 2; kg/d) had a smaller increase in SAA, and tended to have a smaller increase in serum Hp concentration from d 1 to 3. These data suggest that free choice hay consumption may prevent increases in serum Hp concentration in postpartum period. We had hypothesized that free choice hay intake would reduce inflammation due to a reduced proportion of starch in the GIT, thus reducing gut permeability, as indicated by reduced urinary chromium excretion and increased gene expression of tight junctions. However, no treatment differences were detected for gut permeability from d 3 to 5, or colon gene expression at d 5, suggesting that

free choice hay does not affect gut permeability or colon gene expression. Therefore, alterations in gut permeability and colon gene expression do not explain reduced serum Hp concentration in HAY in the present study. We acknowledge that the present study administered NSAID to all cows immediately before urinary catheterization, which may have influenced permeability data; ketoprofen was selected for NSAID administration due to its very short half-life, relative to other NSAID, thus reducing side effects of ketoprofen (Newby et al., 2017; Trimboli et al., 2020) such as inhibition of cyclooxygenase enzymes associated with reduced gastrointestinal integrity (Cryer and Feldman, 1998). However, ketoprofen administration may still affect gut permeability (Legen and Kristl, 2002), and the data should be interpreted with caution.

The lower serum Hp concentration observed in HAY cows may be due to lower proportional starch intake (% of DMI), or a higher proportional forage NDF intake (% of DMI). Increased concentration of acute phase proteins in the blood may follow high starch intake due to reduced pH (Emmanuel et al., 2008), excessive fermentation, and subsequent damage in the rumen (Steele et al., 2011; Liu et al., 2013), and colon (Emmanuel et al., 2007), reducing barrier function and ultimately resulting in inflammation (Plaizier et al., 2008; Steele et al., 2016). However, in the present study, serum Hp concentration on d 3 was not correlated with starch (% of total DMI; $P = 0.28$, $r = 0.20$). While higher starch intake in NH may have reduced rumen and colon pH in the present study, reduced pH does not consistently result in inflammation (Khafipour et al., 2009b; Abeyta et al., 2023a). This was recently emphasized in a literature review that reported the inconsistences in the inflammatory response of dairy cows to high starch diets and low rumen or hindgut pH, especially when diets contain less than 30% starch (Krogstad and Bradford, 2023). Thus, while free choice hay intake resulted in a lower dietary starch content in HAY (25.2 vs. 26.8% of total DMI for HAY vs. NH, respectively), and perhaps higher rumen

or colon pH, lower starch intake does not appear to explain the lower serum Hp in HAY. Similarly, while HAY cows consumed greater proportional forage NDF (% of DMI), serum Hp concentration on d 3 was not correlated with proportional forage NDF (% of total DMI; $P = 0.93$, r = -0.02). We acknowledge, however, that we did not measure pH, thus we do not know how colon or rumen pH would influence these results.

The tendency for lower serum Hp concentration on d 3 in HAY cannot be explained by starch or forage NDF intake alone; however, it may be explained if both starch and forage NDF intake are considered together as a ratio of starch:forage NDF (starch intake (kg/d)/forage NDF (kg/d)). The HAY cows had a lower starch:forage NDF ratio, and starch:forage NDF tended to be positively correlated with the change in serum Hp concentration from d 1 to 3 ($P = 0.09$; r = 0.32), indicating that cows with a lower starch:forage NDF ratio had less of an increase in serum Hp concentration from d 1 to 3. Assessing the starch and forage NDF intake together as a ratio may be more important to consider, rather than either single factor. A cow may have high intake of starch, but if this is combined with high forage NDF intake, the proportion of rapidly fermentable carbohydrates in the GIT is "diluted" (Beauchemin and Penner, 2009), thus causing less inflammation, as indicated by acute phase protein concentration (Emmanuel et al., 2008), and health disorders associated with high starch diets (Lechartier and Peyraud, 2010). Conversely, even if a cow has lower starch intake, if her forage NDF intake is insufficient, she may experience health disorders (Yang and Beauchemin, 2009).

The present study did not observe any differences in milk yield, despite the tendency for lower Hp in HAY, contrary to what was hypothesized. Inflammation is an energy-demanding process, often associated with reduction in milk production (Bradford et al., 2015). Pascottini et al. (2020) reported when inflammation was reduced, as indicated by lower serum Hp

concentration, energy balance was improved. In addition, Huzzey et al. (2011) determined cows with serum Hp concentration > 1 mg/ml are more likely to experience a postpartum disorder, which are associated with greater energy costs (Sundrum, 2015). Thus, taken together, we anticipated lower serum Hp concentration in HAY would reduce energy expenditure towards inflammation, therefore sparing more energy for milk production and ultimately increasing milk yield (Bertoni et al., 2004; Trevisi and Bertoni, 2008; Horst et al., 2018). However, cows in the HAY treatment had lower TMR and total DMI from d 1 to 5, which may have negated any energy saved due to lower serum Hp concentration, thus resulting in no differences in milk yield or energy status, compared to NH.

The lack of difference detected between HAY and NH for plasma energy metabolites and BW and BCS change further supports the idea that energy status was similar between treatments; glucose, FA, and BHB concentration are frequently used as indicators of energy status, with higher glucose concentration typically representing higher energy balance, and higher FA and BHB concentration typically representing lower energy balance (Krnjaić et al., 2022). Additionally, changes in BCS and BW over the first 21 d of lactation are used to indicate the extent of fat and protein mobilization during negative energy balance in early lactation (Pires et al., 2013). As there were no differences between treatments in plasma energy metabolites at d 3, or change in BW or BCS over the 21 d fresh period, it appears fat mobilization and energy balance may not be affected by offering free choice hay, despite lower TMR and total DMI in HAY cows from d 1 to 5.

From d 1 to 5, total DMI may have been limited in HAY due to the higher proportional forage NDF intake by HAY. Although both treatments had similar forage NDF intake (kg/d), through voluntary hay intake, HAY cows increased their proportion of forage NDF intake (% of

total DMI), compared to NH. It has been well documented that fiber consumption increases rumen fill, leading to increased satiety via rumen distention and mechanoreceptors, ultimately decreasing intake (Allen, 2000). Both treatment groups may have stopped eating upon reaching their threshold of forage NDF, however, as HAY cows had a greater proportion of forage NDF intake through voluntary hay consumption, their TMR intake was reduced, resulting in less starch intake. Despite HAY cows consuming free choice hay, the additional forage consumption was not enough offset the reduction in TMR intake, resulting in lower total DMI, compared to NH. Cavallini et al. (2018) also found that when mid-lactation cows were offered free choice hay alongside TMR, a reduction of TMR intake was not compensated by free choice hay intake, and they speculated this was due to greater palatability of TMR vs. hay, which may also be a factor in the present study. It is worth noting that total DMI did not differ on d 1 and 2, while TMR intake tended to be lower for HAY, indicating that on d 1 and 2 cows were able to maintain similar total DMI through free choice hay consumption. This data suggests that offering free choice hay may not reduce total DMI for the first 2 d postpartum, but beyond 2 DIM, free choice hay may reduce total DMI.

Despite lower TMR and total DMI in HAY cows from d 1 to 5, there was no difference in rumination time between HAY and NH, similar to the findings of Cavallini et al. (2018) where mid-lactation cows offered free choice hay, in addition to ad libitum TMR, had no difference in rumination time, compared to cows not offered free choice hay. Rumination time is strongly related to forage NDF intake (Yang et al., 2001; Yang and Beauchemin, 2007, 2009), and rumination time (min/d) was positively correlated with forage NDF intake (kg/d; $P < 0.01$, r = 0.52) in the present study.

Although free choice hay was no longer offered after d 5, and both treatments were offered the same diet, HAY cows tended to have lower TMR DMI compared to NH from d 6 to 21. This was unexpected as both treatments received the same diet, and the exact cause is unknown. Proportional forage NDF intake, (% of DMI), from d 1 to 5, tended to be negatively associated with TMR DMI from d 6 to 21 ($P = 0.07$; r = -0.37), indicating that cows that consumed a greater proportion of forage NDF intake in the first five DIM tended to have lower TMR DMI from d 6 to 21. To our knowledge, no research has assessed carryover effects of offering free choice hay once the hay is removed. The reduced starch intake in HAY from d 1 to 5 may have delayed GIT adaption and reduced intake from d 6 to 21, compared to NH. An adaptation period to increased starch intake may be required for the GIT microbial population (Fernando et al., 2010; Grilli et al., 2016; Petri et al., 2018), and epithelium (Bannink et al., 2008; 2012) to facilitate VFA absorption (Dirksen et al., 1985; NRC, 2001; Melo et al., 2013), which subsequently may delay increases in DMI in the short term (Ricci et al., 2022). However, starch intake inconsistently affects GIT growth, morphology, and absorption capacity (Andersen et al., 1999; Reynolds et al., 2004), thus current data does not strongly support the role of starch in postpartum adaptation (NASEM, 2021), and another mechanism may be present.

Alternatively, the lower TMR DMI in HAY from d 6 to 21 may be of behavioral origin. Dairy cattle have been reported to preferentially select feed particles that they have previously been exposed to (Miller-Cushon and DeVries, 2011; Mitani et al., 2020), and animals may have "metabolic memory" whereby previous feeding experience influences feeding behavior (Ginane et al., 2015). While the TMR fed on d 6 to 21 was not novel as it was the same TMR fed on d 1 to 5, it is possible HAY cows were accustomed to consuming less TMR, relative to NH, and this lower selection of TMR remained in the carryover period. Despite the tendency for lower TMR

DMI in HAY, there were no differences in rumination or milk yield. As HAY had lower intake, but similar milk production compared to NH, HAY cows tended to be more feed efficient.

Although not the primary objective of the present research, we also evaluated the relationship of gut permeability with multiple response variables as existing research and understanding of gut permeability, particularly in fresh cows, is limited. We found gut permeability (from d 3 to 5) tended to be negatively correlated with expression of tight junction genes in the colon tissues, *CLDN4* ($P = 0.08$, r = -0.38) and *OCLN* ($P = 0.09$, r = -0.36), at d 5. This supports what is currently understood about the role of tight junctions in gut barrier function, with reduced tight junction gene expression typically resulting in increased leaky gut (Steele et al., 2016).

In addition, gut permeability tended to be negatively correlated with pre-calving DMI ($P = 0.07$, r = -0.36), and tended to be positively correlated with serum Hp concentration at 3 DIM ($P = 0.07$, r = 0.35), indicating gut permeability is related to lower pre-calving DMI and serum Hp concentration after calving. These findings are in alignment with others (Zhang et al., 2013; Kvidera et al., 2017a; Horst et al., 2020) that found when DMI was restricted in cattle, cattle had increased gut permeability. In the same study by Kvidera et al. (2017a), a second treatment group was administered gamma-secretase inhibitor to induce leaky gut, which resulted in lower DMI. These data indicate increased gut permeability and decreased feed intake are interrelated, both of which also increased concentration of serum Hp and SAA in Kvidera et al. (2017a). Reduced feed intake, and thus reduced nutrient supply to GIT epithelial cells, has been suggested to reduce gut barrier function due to alterations in epithelial cell morphology and reduced cell proliferation which can compromise gut barrier function (Kvidera et al., 2017b). Conversely, leaky gut appears to also reduce feed intake (Kvidera et al., 2017a), however, the mechanisms are less clear.

5.5. Conclusion

Cows offered free choice hay for the first 5 d postpartum tended to have lower serum Hp concentration, and cows that consumed more hay had less increase in serum inflammatory markers from d 1 to 3. However, cows offered free choice hay had lower TMR and total DMI from d 1 to 5. Despite lower intake in cows offered hay, there were no treatment differences detected for milk yield, rumination, plasma energy metabolites, or change in BCS or BW over the first 21 DIM. Similarly, there were no differences in gut permeability or colon gene expression. In conclusion, offering free choice hay to fresh cows may reduce serum inflammatory marker concentration, however, milk yield may not increase due to reduced intake in cows offered hay.

5.6 Tables and Figures

Table 5.6.1 Nutrient composition and particle size distribution of free choice timothy hay[1]

Item	Nutrient content	SD
% DM	95.7	0.4
CP, % DM	9.6	0.3
ADF, % DM	42.2	0.4
NDF, %DM	61.6	0.6
Starch, %DM	1.0	0.1
Ash, % DM	8.6	0.0
Particle size distribution[2] , %		
Long	16.8	5.7
Medium	11.8	4.1
Short	33.0	2.3
Fine	38.4	3.7

[1] Cows in the hay treatment received ad libitum free choice timothy hay for the first five days postpartum, alongside TMR.

[2] Particle size distribution determined by proportion of particles retained on each sieve of the Penn State Particle Separator (>19.0 mm, 8.0–19.0 mm, 1.18–8.0 mm, and <1.18 mm).

Table 5.6.2 Ingredient and chemical composition (% DM unless otherwise noted) of experimental closeup dry and fresh cow TMR[1]

Item	Closeup TMR	SD	Fresh cow TMR	SD
Ingredient, % DM				
Barley silage	60.3		48.0	
Barley straw	3.5		-	
Barley grain, ground, 3.2 mm	6.6		6.5	
Corn grain, ground, 3.2 mm	5.5		18.0	
Beet pulp	5.1		-	
Canola meal	5.1		12.5	
Bypass soy[2]	5.8		6.1	
Soybean hulls	-		0.9	
Corn distillers	2		3.3	
Anionic material[3]	2.9		-	
Rumen-protected choline[4]	0.4		-	
Canola oil	-		0.1	
Commercial fat supplement[5]	-		1.2	
Sugar fines	-		0.4	
Minerals and vitamins supplement[6]	2.8		3.0	
Diet nutrient content				
% DM	42.8		48.6	
CP, % DM	16.3	0.2	18.1	0.2
ADF, % DM	24.4	0.3	19.8	1.2
NDF, % DM	39.1	0.5	33.0	1.3
Forage NDF, % DM	32.1	0.4	23.4	0.4
Starch, % DM	21.5	0.4	26.8	1.8
Ash, % DM	10.1	0.3	8.5	0.3
Starch:forage NDF ratio	0.67		1.14	
NE_L[7], Mcal/kg of DM	1.47		1.63	
ME allowable milk[7], kg/d	-		31.0	
MP allowable milk[7], kg/d	-		30.0	
Particle size distribution[8], %				
Long	11.6	3.5	9.6	6.0
Medium	39.5	5.4	31.0	4.9
Short	38.6	4.3	40.3	3.0
Fine	10.3	2.2	19.1	4.1

[1] The closeup TMR was fed from 21 ± 3 d before expected calving date to calving, and the fresh cow TMR was fed from calving until 5 d after calving.

[2] SoyPlus manufactured with a mechanical expeller process (Landus).

[3] Animate (Phibro Animal Health Corporation).

[4] ReaShure Choline (Balchem Corporation).

[5] Commercial fat supplement contained a minimum of 85% palmitic acid (Jefo Nutrition Inc.).

[6] Closeup dry supplement contained 29.5% Ca, 4.7% Mg, 0.1% P, 1.0% S, 0.2% K, 0.1% Na, 2,129 mg/kg Zn, 1,817 mg/kg Mn, 540 mg/kg Fe mg/kg, 313 mg/kg Cu, 35 mg/kg Co, 29 mg/kg I, 16 mg/kg Se, 288 KIU vitamin A, 59 KIU vitamin D, 4,866 KIU vitamin E, and 1 mg/kg monensin sodium.

Fresh cow supplement contained 19.0% Ca, 3.3% Mg, 1.6% P, 0.2% S, 0.3% K, 10.1% Na, 2,006 mg/kg Zn, 1,731 mg/kg Mn, 943 mg/kg Fe, 319 mg/kg Cu, 34 mg/kg Co, 28 mg/kg I, 14 mg/kg Se, 197 KIU vitamin A, 46 vitamin D, 1,693 vitamin E, and 0.4 mg/kg monensin sodium.

[7] Estimated using NDS (CNCPS Version 6.55, RUM&N).

[8] Presented as the percentage of particles retained on each sieve of the Penn State Particle Separator (>19.0 mm, 8.0–19.0 mm, 1.18–8.0 mm, and <1.18 mm; Kononoff et al., 2003).

Table 5.6.3 Gene names and primer sequences for real-time quantitative PCR analysis (qPCR)

Gene	Forward Primer	Reverse Primer	Source
Claudin 1 (*CLDN1*)	GCGCTGCCCCAGTGGAAAGT	GGATCTGCCCGGTGCTCTGC	Malmuthuge et al. (2013)
Claudin 4 (*CLDN4*)	CATGATCGTGGCCGGCGTG	AGGGCTTGTCGTTGCGGG	Bach et al., 2018
Occludin (*OCLN*)	ATCAACCCCGGTGCCGGAAG	GTGGTCTTGCTCTGCCCGCC	Bach et al., 2018
Zonula occludens 1 (*ZO1*)	CCGAATGAAACCACACACAAA	CCACGCCACTGTCAAACTC	Wang et al., 2021
Toll-like receptor 4 (*TLR4*)	GGT TTC CAC AAA AGC CGT AA	AGG ACG ATG AAG ATG ATG CC	Charavaryamath et al. 2011
Interleukin 10 (*IL10*)	CTTGTCGGAAATGATCCAGTTTT	TTCACGTGCTCCTTGATGTCA	Pang et al., 2009
β-Actin (*ACTB*)	CTA GGC ACC AGG GCG TAA TG	CCA CAC GGA GCT CGT TGT AG	Charavaryamath et al. 2011

Table 5.6.4 Effects of offering hay or no hay for the first 5 d postpartum on plasma energy metabolite and serum inflammatory marker concentrations at 3 DIM and chromium urine output from 3-5 DIM

	Treatment			
Item	Hay	No Hay	SE	*P*-value
Day 3				
Plasma glucose, mg/dL	62.8	64.5	3.02	0.69
Plasma fatty acids, μEq/L	632	632	64.7	0.99
Plasma BHB, mg/dL	18.7	16.6	3.77	0.71
Serum haptoglobin, mg/mL	0.95	1.52	0.22	0.08
Serum amyloid A, μg/mL	538	700	115	0.36
Day 3-5				
Chromium urine output, mg	633	487	103	0.35

Table 5.6.5 Effects of offering hay or no hay for the first 5 d postpartum on colon gene expression at 5 DIM

Gene name	HAY	No Hay	SE	*P*-value
Claudin 1 (*CLDN1*)	4.07	1.47	1.83	0.33
Claudin 4 (*CLDN4*)	7.76	2.42	4.27	0.39
Occludin (*OCLN*)	1.69	2.54	0.90	0.51
Zonula occludens 1 (*ZO1*)	1.08	1.28	0.27	0.62
Toll-like receptor 4 (*TLR4*)	8.70	2.12	4.41	0.30
Interleukin 10 (*IL10*)	4.56	2.26	2.03	0.43

Table 5.6.6 Effects of offering hay or no hay for the first 5 d postpartum on TMR DMI, total DMI, starch intake, and forage NDF intake at 1-2, 1-5, and 6-21 DIM

Item	Treatment		SE	*P*-value		
	Hay	No Hay		Treatment	Day	Treatment × Day
Day 1-2						
TMR intake, kg/d	13.9	15.7	1.02	0.06	0.69	0.65
Hay intake, kg/d	1.3	-	0.19	-	-	-
Total DMI, kg/d[1]	15.1	15.7	0.97	0.64	0.78	0.71
Forage NDF intake, kg/d	3.89	3.45	0.27	0.33	0.99	0.61
Forage NDF intake, % of total DMI	27.5	23.4	0.95	< 0.01	0.37	0.37
Starch, kg/d	3.19	4.16	0.37	< 0.01	0.62	0.95
Starch, % of total DMI	24.8	26.8	0.28	< 0.0001	0.77	0.77
Starch:forage NDF ratio[2]	0.91	1.14	0.03	< 0.0001	0.36	0.33
Day 1-5						
TMR DMI, kg/d	15.0	17.1	0.93	< 0.01	0.01	0.94
Hay intake, kg/d	0.85	-	0.15	-	-	-
Total DMI, kg/d[1]	15.9	17.1	0.87	0.05	0.05	0.81
Forage NDF intake, kg/d	3.93	3.87	0.26	0.87	0.03	0.12
Forage NDF intake, % of total DMI	26.2	23.4	0.60	< 0.001	0.47	0.47
Starch intake, kg/d	3.63	4.48	0.37	0.08	< 0.001	0.26
Starch intake, % of total DMI	25.2	26.8	0.41	< 0.01	< 0.001	< 0.001
Starch:forage NDF ratio[2]	0.98	1.14	0.02	< 0.001	0.08	0.08
Day 6-21						
TMR DMI, kg/d	20.3	20.8	0.69	0.06	<0.0001	0.99

[1] Hay intake + TMR intake.

[2] Starch intake (kg/d)/forage NDF intake (kg/d).

Table 5.6.7 Effects of offering hay or no hay for the first 5 d postpartum on milk yield, feed efficiency, and rumination time at 1-5 and 6-21 DIM, and change in BCS and BW from d 1-21

Item	Treatment		SE	*P*-value		
	Hay	No Hay		Treatment	Day	Treatment × Day
Day 1-5						
Milk yield, kg/d	27.3	28.0	1.32	0.38	< 0.0001	0.57
Feed efficiency[1]	2.36	1.70	0.38	0.21	0.43	0.93
Rumination time, min/d	429	418	19.4	0.57	< 0.0001	0.68
Day 6-21						
Milk yield, kg/d	44.5	44.8	1.39	0.63	< 0.0001	0.95
Feed efficiency[1]	2.23	2.17	0.04	0.06	0.99	0.96
Rumination time, min/d	579	589	16.4	0.19	< 0.01	0.99
Day 1-21						
BCS change, /21d	-0.20	-0.30	0.08	0.34	-	-
BW change, kg/d	-3.49	-4.00	0.49	0.50	-	-

[1] Feed efficiency calculated as kg milk yield/kg DMI.

Figure 5.6.1 Feed provision setup for cows assigned to the free choice hay treatment (HAY). From d 1-5, HAY cows were offered ad libitum free choice timothy hay (blue manger), outside of the TMR. Ad libitum TMR was provided in a separate manager (white manger).

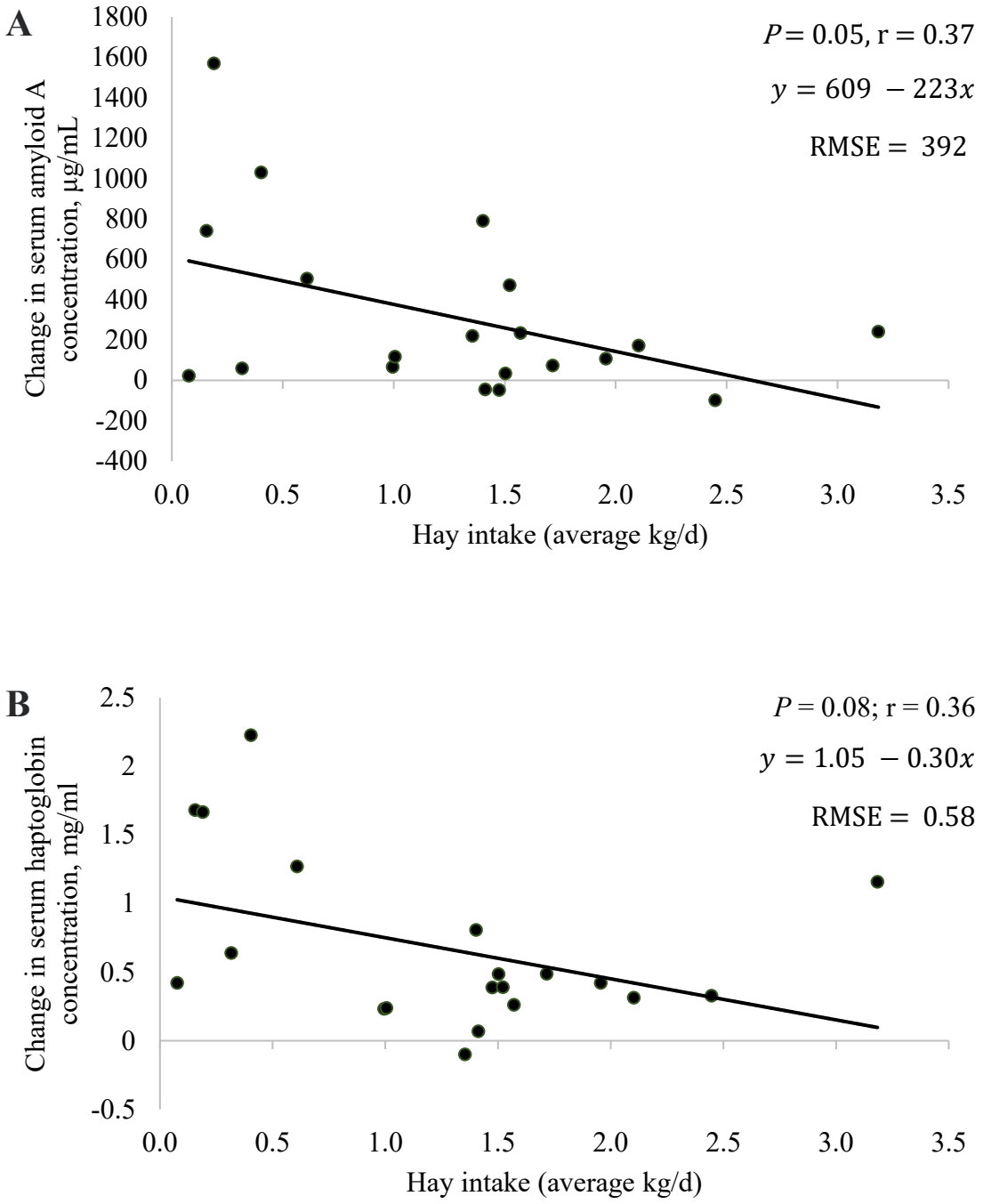

Figure 5.6.2 The relationship between hay intake (average d 1 and 2 kg/d) and change (d 3 concentration – baseline concentration) in serum amyloid A (A) and haptoglobin concentration (B) for cows offered ad libitum free choice hay. Regression equation and root mean square error (RMSE) value are shown for each graph. Baseline plasma and serum samples were collected after calving, but before fresh cow feed and hay were offered to cows.

Chapter 6. Evaluation of calprotectin as a potential marker of gastrointestinal inflammation.

6.1 Background

Gastrointestinal inflammation is common with the onset of acidosis in lactating dairy cattle and presents an array of management and welfare challenges to producers. Inflammation is an energy-expensive process as it activates the immune system, thus when animals are in inflammatory states, less energy can be allocated towards milk production (Loor et al., 2005; Bertoni et al., 2008). Many producers incur economic losses when their cows are experiencing acidosis and its associated GIT inflammation due to reductions in growth, milk fat, milk yield, inflammation and treatment costs of metabolic disorders associated with gastrointestinal inflammation (Abdela, 2016). As such, detection and treatment of GIT inflammation is imperative to promote animal health, welfare, and production efficiency. There are currently no non-invasive detection methods for identifying gastrointestinal-specific inflammation in cattle. Much cattle research relies on systemic inflammatory markers such as serum amyloid A and haptoglobin to make inferences about the extent of acidosis and GIT inflammation (Krause and Oetzel, 2006; Zebeli and Zebeli, 2012; Plaizier et al., 2018). However, these markers may originate from many different sources of inflammation in the cow, such as calving-related events, or other common postpartum inflammatory conditions such as mastitis (Grönlund et al., 2003; Nazif et al., 2010) or metritis (Biswal et al., 2014). Alternatively, some groups have utilized colon biopsies (Bach et al., 2018; van Niekerk, 2018) to collect tissue and assess genes and proteins related to gastrointestinal inflammation. However, this technique is somewhat invasive and not feasible for on-farm assessment of gastrointestinal inflammation. As there is currently no non-invasive marker of gastrointestinal inflammation, we are limited in our ability

to identify when gastrointestinal inflammation is occurring. Further, the evaluation of the efficacy of treatments and management practices intended to reduce gastrointestinal inflammation is limited as such inflammation cannot be easily quantified. Therefore, it is imperative to identify and validate a specific and non-invasive marker of gastrointestinal inflammation in cattle to better evaluate gastrointestinal inflammation treatments, and ultimately reduce gastrointestinal inflammation in dairy cows.

A candidate marker may be fecal calprotectin, a protein released by neutrophils at sites of GIT inflammation (Alibrahim et al., 2015; D'Angelo et al., 2017). Calprotectin concentration in feces reflects the extent of neutrophils migrating from blood circulation to an inflamed portion of the GIT mucosa (Bunn et al., 2001), and is highly correlated with endoscopic and histological scoring systems of gastrointestinal inflammation in human medicine (Konikoff and Denson, 2006). Fecal calprotectin is resistant to heat and proteolytic enzymes, and is stable at room temperature for at least one week (Røseth et al., 1992) or at -20˚C for at least 1.5 years (Caenepeel et al., 2019). As well, calprotectin concentration is consistent throughout the entirety of feces collected at a particular time point; calprotectin concentration within random aliquots of feces, or "spot samples" are highly correlated with calprotectin concentration of a whole fecal sample ($r > 0.90$; Røseth et al., 1992). The consistency of calprotectin concentration throughout a fecal sample at a given time point allows accurate calprotectin assessment in spot samples, reducing the need to collect the entirety of feces, homogenize the feces, and take a representative sample. The stability of calprotectin and its homogenous concentration in feces makes calprotectin a desirable marker for on-farm collection of samples suitable for future analysis. Calprotectin is made up of two calcium-binding subunits encoded by the S100A8 and S100A9 genes, which are also present in the bovine genome (Sayers et al., 2022), and have been reported

in beef cattle rectum tissue (Wang et al., 2016), with increased expression during pathogenic infection of the GIT (Wang et al., 2018a). Thus, calprotectin may also serve as an accurate marker for gastrointestinal inflammation in cattle. However, there is currently no published literature investigating the efficacy of fecal calprotectin in identifying gastrointestinal inflammation in cattle.

By correctly identifying gastrointestinal inflammation when it occurs, we can adjust management practices to reduce inflammation, improving animal welfare and reducing economic losses associated with gastrointestinal inflammation. The overall objective of this project was to adapt existing calprotectin assays for use in cattle, subsequently allowing for determination of the range of fecal calprotectin in dairy cows and assessment of the relationship of fecal calprotectin with performance measures, health status, and inflammatory markers. However, due to an array of technical challenges related to calprotectin extraction and analysis, described later, the outcomes of this project deviated from the original objectives, and instead, extensive troubleshooting was conducted to attempt to improve calprotectin extraction and detection.

6.2 Analytical Procedures

6.2.1 Verification of Commercial Kit

6.2.1.1 Procedures and Rationale

A total of 533 fecal samples from 124 cows at varying stages of the transition period (-7, 4, 10, 21 DIM) were collected in April through September of 2020. Samples were stored at -20˚C until time of analysis. At this stage of the project, samples were intended to verify commercial kit efficacy in detecting fecal calprotectin in dairy cows, thus samples were collected from cows receiving no experimental treatments. Samples underwent extraction and analysis according to the instructions in the MyBioSource bovine ELISA kit (Bovine Calprotectin (CP)

ELISA Kit, MyBioSource), with slight modifications, starting in November, 2021. The protocol called for wet fecal samples (10 mg) to be combined with phosphate buffered saline (PBS; 100 µL), "shaken", and centrifuged at 1000 × g (or 3000 rpm) for approximately 20 minutes. A slight modification in the protocol was made in which a larger volume of feces (100 mg) to improve precision of weighing, and larger volume of PBS (2000 µL) were used to facilitate adequate dissolving and homogenization of feces in PBS. Thus, the final dilution ratio was 1:20, rather than 1:10. Following centrifugation, supernatant was collected and assayed as per ELISA instructions. Multiple ELISAs were conducted from November 2021 through March 2022 using the modified kit instructions.

6.2.1.2 Outcomes and Interpretation

Results from the analysis showed very little variation among samples from different cows, stages of lactation, and parities (Table 6.7.1), ranging from 22,411 to 30,177 ng/g. In humans, fecal calprotectin concentration varies considerably in patients experiencing gastrointestinal inflammation with active Crohn's disease (60,000-10,000,000 ng/g) or active ulcerative colitis (30,000-100,000,000 ng/g), and fecal calprotectin concentration also widely varies in healthy patients (800-500,000 ng/g; Fukunaga et al., 2017). While the fecal calprotectin concentration in cows in the present study were within the "healthy" range for humans, the lower values and lack of variation in concentration amongst samples, regardless of cow or DIM, were surprising. Gastrointestinal tract acidosis, which is known to induce GIT inflammation (Nocek, 1997; Kleen et al., 2003; Steele et al., 2009), variably affects cows (Penner et al., 2009a,b; Zebeli and Metzler-Zebeli, 2012), thus we expected to see variations in the GIT inflammatory marker, calprotectin. However, even if cows in the present study were not experiencing GIT inflammation, and all cows were considered "healthy", the lack of variation amongst cows was

concerning as calprotectin concentration in healthy humans is widely variable due to normal biological variation (Husebye et al., 2001; Li et al., 2015). Additionally, variation was expected between samples collected in the prepartum (7 days before calving) and postpartum period (4, 10, and 21 days after calving) as it is well documented that inflammation increases in the days following calving (Zecconi et al., 2018), even in healthy cows without disease (Bionaz et al., 2007; Graugnard et al., 2012) thus, we expected to see increases in calprotectin concentration in postpartum samples, compared to prepartum samples. The magnitude of the increase in inflammatory markers from pre- to postpartum is also reported to be highly variable amongst cows (Bradford et al., 2015; Trevisi et al., 2015), therefore greater variation in calprotectin concentration at the same DIM among cows was expected. Therefore, extensive troubleshooting was conducted to investigate the cause for the lack of variation in calprotectin concentration between cows.

6.2.2 Troubleshooting Approaches and Outcomes

6.2.2.1 Procedures and Rationale

The kit manufacturer, MyBioSource, was contacted regarding the lack of variation between samples, and technical support suggested the duration of sample storage may have reduced the viability and detection of calprotectin. At the time the first kit was ran, samples were approximately 12-16 months old, and stored at -20˚C. Therefore, fresh fecal samples were collected from five cows at different stages of lactation (fresh, far off dry, closeup dry) and stored at 4˚C. Samples were processed the following day using a 2 × 2 factorial approach; samples were either 1) lyophilized or 2) fresh, and diluted at either 1) 12 mg feces: 1600 µL PBS or 2) 80 mg feces: 1600 µL PBS. Thus, four aliquots of each individual fecal sample underwent four different processing procedures to allow direct comparison of dilution factors and

lyophilization vs. fresh wet samples. One set of samples was lyophilized to reduce variation attributed to variable moisture content in among fresh wet samples as well as increasing calprotectin concentration per gram of analyzed sample. One set of samples remained fresh, and was not lyophilized, for comparison of concentration to lyophilized samples. In theory, dried lyophilized samples should have greater calprotectin concentration compared to fresh wet samples of the same weight as the moisture was removed in lyophilized sample. Additionally, two different dilution factors of feces (12 mg feces: 1600 µL PBS, or 80 mg feces: 1600 µL PBS), either fresh or lyophilized, were assessed. When lyophilizing samples, 80 mg of fresh feces resulted in 12 mg of dry feces following lyophilization.

The recommended kit extraction procedure was utilized initially, however, following centrifugation, the lyophilized samples remained homogenous, and did not separate into supernatant and precipitate fractions, thus an array of centrifugation speeds were assessed in increasing increments of 1000 × g until adequate particle separation was achieved. It was determined that 9000 × g was necessary for adequate separation of lyophilized sample fractions thus both fresh and lyophilized samples underwent centrifugation at this speed. Following supernatant collection from all samples, supernatants were assayed using the MyBioSource bovine kit.

6.2.2.2 Outcomes and Interpretation

Sample readings were again, all very similar regardless of cow, stage of lactation, or processing method. In fact, samples that underwent a greater dilution (12 mg feces: 1600 µL PBS) sometimes had a greater reading than their identical counterparts at a lower dilution (80 mg feces: 1600 µL PBS). Similarly, lyophilized samples that should be greater in calprotectin concentration compared to their fresh counterparts of identical weight (i.e., 80 mg lyophilized

fecal sample vs. 80 mg fresh fecal sample), due to the removal of water during lyophilization, often had a lower concentration compared to fresh samples. Therefore, it was determined that fecal calprotectin concentrations obtained using the MyBioSource bovine kit were inaccurate and not reflective of calprotectin concentration in the feces.

6.2.3 Follow-up Trouble Shooting Approaches and Outcomes

It was suspected that one or more of the following possibilities caused inappropriate calprotectin extraction or detection, and each were investigated:

1. Interference with detection during ELISA assay from an unknown compound in the feces
2. Insufficient protein extraction from feces prior to ELISA analysis
3. Commercial calprotectin kits are unsuitable for bovine fecal calprotectin detection

6.2.3.1 Interference with ELISA Assay from an Unknown Compound in Feces

Due to little variation in calprotectin concentration among samples, as described, it was speculated there may be an interfering compound present in the fecal extracts that may block or neutralize (Tate and Ward, 2004) calprotectin, resulting in underestimated calprotectin values. Various substances present in biological samples, such as proteins, antibodies, or metabolites, may cross-react or non-specifically bind with antibodies in ELISA, interfering with protein detection (Tate and Ward, 2004). To assess this, fecal samples underwent extraction as previously described, and were spiked with a calprotectin standard of a known concentration, provided in the MyBioSource kit. Results showed that calprotectin was detected at the predicted value, thus the lack of variation among samples was not likely due to interference of a compound present in fecal extracts.

6.2.3.2 Insufficient Protein Extraction from Feces prior to ELISA Analysis

It was also considered that the current extraction procedure provided by MyBioSource was inadequate, and insufficient quantities of calprotectin were available for analysis in fecal extracts. Therefore, the protein content of fecal extracts was evaluated, alternative protein extraction protocols were explored, and Western Blot was performed to confirm if calprotectin was in fact present in fecal extracts.

Following the modified extraction protocol from the MyBioSource bovine ELISA kit, a Bradford protein assay (Bradford, 1976), was used to determine total protein content of fecal extracts. The Bradford assay utilizes acidified Coomassie Brilliant Blue G-250 which binds proteins present in samples, initiating a color change (Reisner et al., 1975; Bradford, 1976). The absorbance of each sample was measured at 595 nm with a plate reader (SpectraMax 190; Molecular Devices Crop.) and final protein concentration was calculated using standards of known bovine serum albumin concentration. The Bradford results showed overall protein yield was very low, indicating poor protein extraction from fecal samples, and it was assumed that insufficient protein extraction led to poor calprotectin detection, thus it was determined that protein extraction must be improved.

In May through October 2022, efforts to improve protein extraction from fecal samples were made, prior to conducting further ELISA assays. The original extraction protocol (feces combined with PBS, centrifuged, supernatant assayed), provided in the MyBioSource bovine ELISA, was not used. Instead, an extraction buffer formulation was used from another commercial calprotectin kit protocol (Buffer A; Table 2; Calprotectin ELISA kit, HK382, Hycult Biotech). Additionally, a new extraction protocol was developed; lyophilized fecal sample replicates were combined with the new buffer, vortexed, and left at 4˚C overnight to allow

complete saturation of the samples. The following day, the homogenate was vortexed and centrifuged at 9000 × g. Supernatant was used for a series of dilutions which were then assessed in the Bradford protein assay to determine the efficacy of the new extraction buffer. Undiluted samples had low protein concentrations whereas diluted samples had very low to no protein content. These results suggest that either protein extraction techniques were insufficient, or protein content of the feces was low.

An alternative extraction buffer with a modified procedure (Buffer B; Karl et al., 2008) was assessed. Briefly, extraction buffer was prepared and combined with lyophilized feces and a protease inhibitor (100 mM Phenylmethylsulfonyl fluoride; Thermofisher Scientific). Samples were vortexed continuously for 30 minutes and then incubated at 4˚C overnight. The next day samples were centrifuged at 9000 × g and supernatant were collected and assayed undiluted and at a two-fold dilution. The modified extraction technique and buffer improved protein yield by 30%, as determined by the Bradford assay, compared to the original procedure outlined by MyBioSource. This extraction technique and buffer was used for all additional analysis, and is referred to as "modified extraction technique and buffer B", throughout the remainder of this chapter.

Following the improvement in protein yield, a Western Blot was performed to confirm the presence of Calprotectin in fecal samples. Samples extracted using the previously described protocol underwent Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) using a 12% resolving gel, with 20 µL of protein ladder or 50 µL sample per lane ran, ran at 180 V for 50 minutes. Following completion of SDS-PAGE, transfer occurred using polyvinylidene difluoride membranes (PVDF/Filter Paper Sandwich, 0.2 µm, 8.3 x 7.3 cm; Invitrogen) in Towbin buffer (Bio-Rad) at 80 V for 90 minutes. After transfer, membranes were saturated with

5% non-fat milk powder (Instant skim milk powder; Compliments) in tris-buffered saline (7.4 pH) + 1% tween (TBST) for one hour at room temperature. After incubation, the blot was rinsed 3 times with TBST prior to adding the primary antibody (Mouse calprotectin monoclonal antibody; specified for multi-species use, including bovines; ThermoFisher Scientific), and incubating overnight at 4˚C. The next day the blot was rinsed 3 times with TBST and the secondary antibody (Goat anti-mouse IgG (H+L) Secondary Antibody, HRP) was added and incubated for 1 hour at room temperature. The blot was again rinsed 3 times with TBST and 0.5 ml of each luminol and chemiluminescence reagents (Pierce™ ECL Western Blotting Substrate; ThermoFisher Scientific) were added prior to imaging.

Calprotectin failed to be detected on the Western Blot, and this was suspected to be due to low calprotectin concentration in the sample despite the improvement in protein yield from revised extraction procedures. The use of ion column separation, a technique used to separate biological particles according to their charge, was considered as it has been utilized in previous research to further extract and purify calprotectin (Heilmann et al., 2008; Nilsen et al., 2018; Fagerhol and Rugtveit, 2022). However, the equipment was unavailable to our lab group, thus further extraction and purification were not possible, and it was decided to proceed with further analysis despite this.

6.2.3.3 Commercial Calprotectin Kits are Unsuitable for Bovine Fecal Calprotectin Detection

6.2.3.3.1 Procedures and Rationale

Finally, the efficacy of an array of commercially-available calprotectin kits, in addition to another MyBioSource bovine plate were evaluated. A new MyBioSource bovine plate was ran as well as additional plates validated in humans; MyBioSource human (Human Calprotectin (CP)

ELISA Kit, MyBioSource), Bühlmann (Bühlmann fCAL ELISA, Bühlmann Laboratories AG), Calprest (Calprest NG, Eurospital Diagnostic), and Invitrogen (Human Calprotectin L1/S100-A8/A9 Complex ELISA Kit, Invitrogen). Upper and lower detection limits, and buffer information of each kit are outlined in Table 3.

Buhlmann and Calprest kits contained kit-specific extractions buffers, thus these were used rather than the in-house buffers for each respective plate. The Invitrogen plate did not contain an extraction buffer; therefore, four different extraction buffers were used for sample replicates: 1) In-house buffer A, 2) In-house buffer B, 3) Buhlmann kit extraction buffer, or 4) Calprest kit extraction buffer. For each of the MyBioSource kits, as neither contained an extraction buffer, lyophilized sample replicates were combined with 1 of 5 buffers: 1) PBS, as recommended by the kit, 2) In-house buffer A, 3) In-house buffer B, 4) Buhlmann kit extraction buffer, or 5) Calprest kit extraction buffer. For each plate, the modified extraction technique and buffer B, outlined previously, was followed. Sample replicates were serially diluted; the dilution factor recommended by the kit was assayed in addition to multiple other dilutions of the same sample replicate to evaluate if calprotectin concentration was reduced linearly with increasing dilutions. Plates were each ran according to the respective kit's instructions.

6.2.3.3.2 Outcomes and Interpretation

The Bulhmann kit failed to detect calprotectin in feces; regardless of sample type or dilution factor, all samples were below the standard curve (Table 4). The Invitrogen kit appeared to detect calprotectin in some samples, however the values obtained were infeasible (i.e. sample replicates of greater dilutions often showed higher calprotectin values than identical sample replicates at lower dilutions), thus it was deduced the results were inaccurate and unreliable.

Conversely, both the human and bovine MyBioSource kits, in addition to the Calprest kit, were successful in detecting calprotectin in feces.

The MyBioSource kits were only able to detect calprotectin in samples that underwent extraction using in-house buffers A and B; the Calprest and Buhlmann buffers used with the MyBioSource kits were unsuccessful in calprotectin detection. Similarly, the Buhlmann extraction buffer and Buhlmann kit was unsuccessful in detecting calprotectin as all values were below the kit's detection limit. However, the Calprest extraction buffer and Calprest kit, when used together, successfully detected calprotectin. The Invitrogen kit was unsuccessful in detecting calprotectin in any sample using any extraction buffer as all values were below the detection limit, despite the Invitrogen kit having the lowest detection limit.

Although the objective of this project was to assess calprotectin in bovine samples, human kits were utilized due to the novel nature of this work, and lack of current commercial kits for bovine analysis, other than the MyBioSource bovine kit. The protein subunits of calprotectin, A8 and A9, have percent identities of approximately 70% and 72%, respectively, between bovines and humans. Identity describes the extent of alignment in residues between two amino acid sequences, typically expressed as a percentage (Fassler and Cooper, 2011, BLAST Glossary). In this case, the percent identity describes the percentage of residues that are aligned between bovine and humans in each of the calprotectin subunits. Proteins are typically described as homologous with a percent identity of 30% or greater (Sander and Schneider, 1991), thus, in theory human calprotectin kits should be suitable for bovine calprotectin detection. However, the results of the present study cannot confirm this due to the failure of calprotectin detection by the human Buhlmann and Invitrogen kits, and highly variable calprotectin concentrations detected between kits; amongst kits successful in detecting calprotectin, the concentration of calprotectin

in identical sample replicates varied widely between kits. Therefore, it is unknown at this time which kit, if any, is reliable in detecting fecal calprotectin in cattle. This supports our previous suspicion that the MyBioSource Bovine kit, as well as all other kits assessed, may be unsuitable for fecal calprotectin detection in cows.

6.3 Discussion

The exact mechanism behind the great variation in calprotectin concentrations among kits is unclear. Some variation may be due to the different extraction buffers used; the MyBioSource kits were successful in detecting calprotectin, although the accuracy of these results is unknown. The variation among kits in detection of calprotectin, even when the same extraction buffers were used, may suggest that the different reagents and chemistries of each ELISA kit result in different calprotectin concentration measurements of sample replicates amongst kits. Each kit utilizes a sandwich ELISA method; plates were pre-coated with capture antibodies that allow the calprotectin antigen in fecal extract samples to bind when added to the plate. Following addition of the sample to the well, the secondary antibody was added. The MyBioSource kits, Buhlmann and Calprest kits each used a secondary antibody that was conjugated with horseradish peroxidase (HRP) enzyme, while the Invitrogen kit used a biotinylated secondary antibody and HRP was added in an additional step. Each kit used 3,3',5,5'-Tetramethylbenzidine (TMB) as a chromogen to initiate color change of the HRP, followed by a stop solution to cease the color change reaction. The MyBioSource kit however also included an additional unknown chromogen reagent that was added in the step prior to TMB addition.

It is possible different antibodies were used amongst the different kits, each with differing affinities for the calprotectin antigen in samples, perhaps due to the species each antibody was raised in, and the intended target species; antibodies with lower affinities for calprotectin may

result in underestimated values, and antibodies with affinities for proteins other than calprotectin may result in non-specific binding and "background noise", resulting in overestimated values. Additionally, the epitope of calprotectin targeted may vary between commercial kits depending on the specific antibodies used for each calprotectin ELISA (Walsham and Sherwood, 2016). However, the exact makeup of each reagent as well as the exact antibodies used in each kit is unknown due to the commercial nature of these kits. Therefore, direct comparison between reagents and antibodies is not possible, and it cannot be determined if these discrepancies are responsible for the differences in calprotectin detection in each kit. Further investigation using buffers of known composition and antibodies with specified epitopes may better elucidate why such large variation exists among kits, but due to limited funding and time to pursue this project, further analysis and original objectives were not achieved.

Research assessing various commercial kits and analysis methods for human calprotectin measurements have also reported difference in calprotectin quantification amongst kits (Labaere et al., 2014; Juricic et al., 2019). However, while the absolute value of calprotectin concentration varied greatly amongst kits, Labaere et al. (2014) noted that calprotectin concentrations of sample replicates were significantly correlated between kits, and kit-specific cut off points for diagnosis of GIT inflammation could be determined through statistical modelling, rather than using a universal cut off point which was identical between kits. Using this approach, authors determined a cut off point for GIT inflammation diagnosis unique to each kit, which allowed authors to make similar diagnosis outcomes (i.e. gastrointestinal inflammation present, or not present) of the same samples between kits, which was further confirmed by histological and endoscopic evaluation (Labaere et al., 2014). Future research may assess if this approach is suitable for identification of gastrointestinal inflammation in cattle as well.

The possibility that calprotectin does not represent GIT inflammation in cows, as it does in humans, cannot be excluded. Despite little variation detected between samples following the MyBioSource bovine ELISA, it was still believed calprotectin should be present to some extent as the genes encoding for protein subunits S100 A9 and S100 A8 are found in the bovine genome (Sayers et al., 2022), their gene expression has been reported in rectum tissue of beef cows (Wang et al., 2016), and similar S100 calcium-binding proteins have been identified in bovine in-vitro cell culture work (Tang et al., 1993). In fact, calprotectin protein may have been previously found in bovine neutrophil homogenates, but at the time of this research, it was not identified as calprotectin; bovine neutrophil protein complexes, p7 and p23, were found and confirmed to have large primary structure homologies with human proteins MRP8, and MRP14 (Dianoux et al., 1992) respectively, which are also known as S100 calcium-binding proteins A8 and A9, respectively, the two subunits of calprotectin (Wang et al., 2018b). Furthermore, amino acid sequences of calcium binding proteins were compared amongst multiple mammalian species, and it was revealed that calcium-binding protein sequences are highly similar within mammals, regardless of species (Fullmer and Wassermer, 1975). Thus, it appears calprotectin is also present in bovines, with large homology to human and other mammalian species, and it theoretically should be detected to some extent in fecal samples of the present study, even when using kits intended for humans. The similar homology of calprotectin in cattle and humans is further supported by commercially available antibodies that have been verified to bind to calprotectin in both humans and cows; for example, calprotectin antibody 387 (MAC387; ThermoFisher Scientific) has been validated for use in both human (Gahremanpour et al., 2013) and bovine cells (Lü et al., 2010) via an epitope expressed exclusively on the MRP14 (also known as S100A8) protein complex (Goebeler et al. 1994). However, as mentioned previously,

the various commercial kits used in the present study may have used antibodies that targeted different epitopes of calprotectin. As we do not know the precise antibodies used in each kit, we do not know what epitopes were targeted or if these epitopes are conserved between humans and cows. Depending on the antibody used, the epitope may not be conserved between humans and cows, thus antibodies intended for detection of calprotectin in humans may fail to bind calprotectin in cow samples, or conversely, may non-specifically bind other S100 proteins.

It is possible that fecal calprotectin concentration may not vary significantly amongst cows, regardless of the extent of gastrointestinal inflammation, perhaps due to intrinsic differences in immune cell function between cows and humans. However, as noted, calcium binding proteins are described to be evolutionary conserved amongst mammals (Fullmer and Wasserman, 1975; Donato et al., 2013; Zimmer et al., 2013), thus it is reasonable to predict calcium binding proteins, such as calprotectin, will respond similarly to GIT inflammation in cattle, as they do in humans, but this cannot be confirmed due to the lack of research comparing calcium binding proteins in human and bovines. Previous in vitro work identified the presence of three different calcium-binding proteins in the bovine GIT (Fullmer and Wasserman, 1973), which supports the presence of calprotectin in cows. More recently, gene expression of S100 A9 and S100 A8, the two subunits of calprotectin, was reported in rectum tissue from beef cows (Wang et al., 2016), confirming that calprotectin is present in the GIT of cattle. However, it is possible that calcium-binding proteins other than calprotectin, are more active during GIT inflammation in cattle, and perhaps future research should assess these proteins and their relationship with GIT inflammation in cattle. As well, much of the research investigating the role and presence of calcium binding proteins in cows was conducted more than 40 years ago, thus our understanding on calcium binding proteins and their role in GIT inflammation in cows is limited.

An additional possibility is that there was limited variation in fecal calprotectin concentration amongst cows due to similar genetics, due to a limited genetic pool for Holstein cows (Stachowicz et al., 2011), and similar diets and housing environments for cows used in the present study. Comparatively, humans vary extensively in their genetics, diets, lifestyles, and environments, which may contribute to greater variation in fecal calprotectin concentrations in humans compared to cows. We must also consider that samples collected for calprotectin analysis may have been from cows experiencing no or low-grade GIT inflammation, resulting in similar calprotectin values in all samples. Barbosa et al. (2021) reported when analyzing fecal samples from pigs, human-designated calprotectin ELISA kits could identify pigs experiencing high levels of inflammation, but not low levels of inflammation. The same study suggested human calprotectin ELISA kits are appropriate for veterinary use, but sensitivity may be somewhat reduced due to human antibodies, rather than swine, or bovine in the present study, being used in these kits (Barbosa et al., 2021). This is further confirmed by Fullmer and Wassermer (1975) that reported despite very similar amino acid sequences in calcium-binding proteins between mammals, immunological cross-species reactivity is limited. In other words, detection of bovine calprotectin may be limited when using antibodies designated for human calprotectin detection, and in cases of low-grade inflammation, the reduced sensitivity of human kits may be unable to identify GIT inflammation in bovines.

A very recent paper was published that may confirm the speculation that calprotectin can only be detected in cows experiencing considerable GIT inflammation; Ruiz-González et al. (2023) found that dairy cows subjected to a heat stress challenge had increased fecal calprotectin concentration alongside increases in other markers of gut hyperpermeability and inflammation. Ruiz-González et al. (2023) data suggest that calprotectin is present in dairy cows and may be

reflective of the extent of leaky gut and inflammation, however, this is the first published paper reporting fecal calprotectin in dairy cows, thus, much additional research is needed to further explore if we were unable to accurately quantify fecal calprotectin concentration due to limited GIT inflammation, or if other problems were indeed present in our detection of calprotectin.

During the troubleshooting procedures previously described, Dr. Ruiz-González was contacted to discuss problems experienced in calprotectin detection. The same MyBioSource bovine kit used in the present study was also used in the study by Ruiz-González et al. (2023) to measure fecal calprotectin concentration, thus we expected similar efficacy in detection. Step-by-step procedures were discussed with Dr. Ruiz-González, including lyophilization, extraction, and ELISA analysis, however, the precise reason for the infeasible and inaccurate results produced in the present study could not be determined by our group or Dr. Ruiz-González. Ruiz-González et al. (2023) reported fecal calprotectin concentrations of heat stressed cows ranging from approximately 550-700 ng/ml, which equates to 8,250-10,500 ng/g. Comparatively, when following similar procedures (lyophilization and extraction procedures) to Ruiz-González et al. (2023), and when using the same MyBioSource bovine kit, we reported a mean value of 3,911 ng/g in fresh cows. As heat stressed cows are expected to have high gut inflammation (Koch et al., 2019), it is reasonable to expect higher values in the heat-stressed cows in Ruiz-González et al. (2023). However, due to the substantial variation in calprotectin concentration detected amongst commercial kits, and the infeasible values often detected (e.g. samples with higher dilution factors producing higher calprotectin concentration, compared to less diluted counter-parts), the calprotectin values produced in the present study are still deemed unreliable.

6.4 Conclusion

Little variation was found in fecal calprotectin concentration amongst cows, regardless of stage of lactation. Additionally, calprotectin concentration did not appear to vary from the pre- to postpartum period, contrary to what was expected. Moreover, calprotectin values detected were often infeasible, and therefore were considered inaccurate. It is unknown why calprotectin concentration did not vary amongst cows, or within cows at different stages of lactation, or why inaccurate calprotectin values were detected by commercial kits. Calprotectin concentration of sample replicates between different commercially available kits were highly variable, and we are unable to deduce which kit, if any, produces accurate values. The exact reason for highly variable fecal calprotectin concentration detection between commercial kits is unknown and requires further attention. Overall, the present study was unsuccessful in validating fecal calprotectin as a marker of gastrointestinal-specific inflammation in transition dairy cows using commercially available kits. Further research is needed to assess if fecal calprotectin can be used to accurately identify gastrointestinal-specific inflammation. There are still currently no non-invasive markers of gastrointestinal-specific inflammation in cattle and a critical need still exists for future research to validate such a marker.

6.6 Tables

Table 6.6.1 Average calprotectin concentration in fecal samples from cows at -7, 4, 10, and 21 d relative to calving. Fecal samples were extraction and analyzed following the protocol outlined in the MyBioSource Bovine ELISA kit

Days relative to calving	Average calprotectin concentration (ng/g)	SD
-7	27,252	495
4	27,083	3,093
10	26,225	364
21	23,995	1,584

Table 6.6.2 Composition of in-house buffers for calprotectin extraction from fecal samples

Buffer A[1]	Buffer B[1]
100 mM Tris	100 mM Tris
1000 mM Urea	1000 mM Urea
10 mM CaCl2	10 mM CaCl2
100 mM Citric acid monohydrate	100 mM Citric acid monohydrate
150 mM NaCl	100 mM Phenylmethylsulfonyl fluoride
0.25 mM Thimerosal	
0.1% Tween 20	

[1]Buffer A formulation adapted from Calprotectin ELISA kit, HK382, Hycult Biotech

[2]Buffer B formulation adapted from Karl et al., 2008

Table 6.6.3 Commercially-available calprotectin kits and buffers used for fecal calprotectin analysis of cattle feces

Kit	Lower detection limit	Upper detection limit	Buffer Used
MyBioSource Bovine[1]	6.25 ng/ml	200 ng/ml	Buffer A, Buffer B, Calprest, Buhlmann
MyBioSource Human[1]	6.25 ng/ml	200 ng/ml	Buffer A, Buffer B, Calprest, Buhlmann
Calprest	2.5 ng/ml	150 ng/ml	Calprest
Bulhmann	4 ng/ml	240 ng/ml	Buhlmann
Invitrogen	0.0328 ng/ml	8 ng/ml	Buffer A, Buffer B, Calprest, Buhlmann

Table 6.6.4 Average calprotectin concentration in fecal samples analyzed with different commercially-available kits. Samples analyzed using Calprest and Bulhmann kits were extracted with each kit's unique extraction buffer. MyBioSource and Invitrogen kits did not contain extraction buffer solution, thus in-house buffers A and B were used

Kit	Average concentration, ng/g	SD
MyBioSource Bovine[1]	3,911	203
MyBioSource Human[1]	13,484	2,968
Calprest	1,524	120
Bulhmann	Below detection limit	NA
Invitrogen	Below detection limit	NA

[1] Mean of samples extracted with B1 and B2 buffers as other buffers below detection limit

Chapter 7. General Discussion

It is well established that the calving transition period is a challenging, if not the most challenging, period for dairy cows due to the array of changes, metabolic stresses, and inflammation faced by the cow. Managing this period has historically been difficult for producers, nutritionists, and researchers alike. As emphasized by Drackley (1999) nearly 25 years ago, transition cow management is poorly understood, and improved management of this period is a great area of opportunity for increased gains in productivity and profitability. Research in 25 years since has made great progress in the understanding and management practices of transition cows, however, there are still significant gaps in our knowledge, stressing the need for substantial additional research.

7.1 Thesis research objectives, outcomes, and considerations

The overall objective of this thesis research was investigating inflammation management in dairy cows during the calving transition. This research can be summarized into three main themes:

1) Evaluation of nutritional (Chapters 2, 3, 5) and anti-inflammatory treatments (NSAID; Chapters 2 and 3)
2) Assessment of variation in transition cow physiology and metabolism, and the relationship with inflammation (Chapters 4 and 5)
3) Validation of a marker specific to gastrointestinal inflammation (Chapter 6)

7.1.1 Evaluation of nutritional and anti-inflammatory treatments

This thesis evaluated the effect of nutritional management strategies on inflammation in postpartum dairy cows; butyrate supplementation was not effective in reducing systemic

inflammation (Chapter 2) or uterine inflammation (Chapter 3), however, free choice hay tended to reduce serum Hp concentration in fresh cows (Chapter 5). Chapter 2 and 5 both hypothesized treatments would reduce systemic inflammation, at least partially through increased gut barrier function and reduced GIT inflammation. In our butyrate study, however, we did not evaluate any markers specific to GIT inflammation or permeability, thus we do not know if butyrate impacted these variables or not, therefore this is a limitation of Chapter 2. The lack of an available non-invasive GIT inflammatory marker is what largely motivated the study described in Chapter 6, and will be described in greater detail in section 7.3. The failure of butyrate to reduce systemic inflammatory markers in butyrate-fed cows, compared to the control-fed counterparts, in the fresh period could be due to a lack of treatment effect on GIT inflammation or permeability; however, we cannot conclude if this was the case or if another mechanisms, or lack thereof, is present as we did not measure GIT inflammation or permeability.

Conversely, in our hay study we did evaluate the treatment effects on gut barrier function and GIT inflammation through urinary chromium excretion, and gene expression analysis of colon tissue. However, while cows offered hay tended to have reduced concentration of systemic inflammatory marker, Hp, compared to cows not offered hay, there were no treatment differences for gut permeability or colon gene expression. Therefore, changes in gut permeability or colon gene expression do not appear to explain reduced systemic inflammation in Chapter 5. A major limitation of the hay study was lack of rumen data; the original experimental design included evaluation of rumen pH, rumen tissue, and rumen fluid, however, we were unable to collect this data as batch calving of cannulated cows occurred during research facility closures due to COVID-19. Rumen biopsies would have allowed for analysis of gene expression related to inflammation and barrier function, and rumen fluid would have been used for evaluation of

rumen microbes, VFA profiles, and other substances such as LPS, ethanol, or histamine. Rumen pH data would have also provided further insight on the relationship between rumen pH, gut permeability, and inflammation. When the study was designed, we anticipated free choice hay intake would reduce inflammation, at least in part due to altered rumen dynamics, however, due to COVID-19 restrictions, we were unable to investigate this.

When interpreting results from Chapters 2 and 5, dietary starch content should be considered; research frequently assesses the effects of dietary starch and NDF content, as well as the effects of feed additives on inflammation. Both dietary starch and feed additives appear to produce variable effects throughout literature, and precise rationale has not been determined. One area of research that should be further investigated is the interaction between dietary starch content and feed additives. In Chapter 2, butyrate supplementation had little effect on cow performance or health, perhaps due to moderate starch content in the fresh cow diet (22.1% starch); Fukumori et al. (2020) found that butyrate supplementation may only be effective at higher dietary starch content (27.5%). In Chapter 5, a higher starch ration was fed to fresh cows (26.8%), and cows offered free choice hay tended to have reduced serum Hp concentration. Although supplemental hay is not a feed additive, this may suggest that anti-inflammatory effects of treatments are only evident when starch-induced inflammation is present, thus allowing the treatment to take effect. Therefore, inflammation may be reduced in fresh cows through nutritional strategies, but perhaps only above a certain threshold of dietary starch content.

As previously described, hay treatment in Chapter 5 may have been successful in reducing inflammation as cows were fed higher starch diets while butyrate treatment in Chapter 2 did not reduce inflammation perhaps as cows were fed lower starch diets and there may have been little to no starch-induced inflammation to mitigate. Similarly, NSAID administration is reportedly

effective in improving reproduction in cows with high PMN, but not in cows with low PMN (Priest et al., 2013); in Chapter 3, only 22 cows were classified as "high PMN", and the remainder were classified as "low PMN", meaning NSAID may have only been potentially effective for a small number of cows, which may have lacked statistical power to detect a treatment effect of NSAID. Thus, NSAID treatment may have been ineffective in reducing uterine inflammation in NSAID vs. placebo-treated cows due to a relatively low number of cows experiencing uterine inflammation, meaning there was little inflammation for NSAID to mitigate. Thus, this finding combined with previous findings (Priest et al., 2013), questions the efficacy, and appropriateness, of administering NSAID to all cows, regardless of health status.

7.1.2 Assessment of variation in transition cow physiology and metabolism, and the relationship with inflammation

An additional challenge of nutritional management of inflammation in transition cows is the extensive variation in metabolism and response to dietary changes, even when cows are fed the same ration and are of similar DIM. Chapters 4 and 5 demonstrated inter-cow variation in pre-calving intake, and inflammation and metabolism at calving, each of which are associated with postpartum physiology and feed selection. Perhaps the next frontier of research will involve more individualized cow management, where the varying physiologies of cows is considered, and nutritional strategies accommodate individual cow needs and responses, rather than assuming cows will all respond similarly to a single TMR.

7.1.3 Validation of a marker specific to gastrointestinal inflammation

Detection of inflammation is essential to managing inflammation; if we cannot accurately identify when inflammation is occurring, we cannot effectively manage it. Identifying the source of inflammation is difficult due to clinical signs ubiquitous to many metabolic disorders and

dysfunctions, and due to the general lack of inflammatory markers unique to specific sources of inflammation. Research, including that of Chapters 2, 3, 4, and 5, frequently uses systemic inflammatory markers to assess the extent of inflammation in cows, regardless of the type of inflammation being assessed. In order to adequately assess efficacy of treatments intended to reduce specific sources of inflammation, we must be able to accurately measure inflammation at specific tissues.

In particular, the lack of a non-invasive marker unique to GIT inflammation was highlighted during this thesis research, and is what largely motivated the calprotectin study in Chapter 6. As mentioned, Chapter 2 hypothesized butyrate treatment would reduce systemic inflammation by reducing localized gut inflammation, thus improving gut barrier function, however, no specific measures of gut inflammation were assessed in Chapter 2. Extensive research has been conducted on the onset, consequences, and treatment of GIT inflammation; however, research largely relies on non-specific systemic inflammatory markers as indicators of GIT inflammation. As described in Chapter 6, validation of calprotectin was unsuccessful, despite extensive troubleshooting. Failure of this assay may be due to a multitude of possibilities, however, as only one prior study has assessed calprotectin in cattle, and the precise contents of commercial kits were unknown and were not provided by commercial companies, despite our requests. Thus, interpretation of results is limited. Should future studies attempt to evaluate calprotectin in cattle, it may be worthwhile to develop an in-house ELISA, rather than relying on commercial kits, to ensure appropriate reagents are used, and to allow careful selection of antibodies specific to bovine calprotectin detection. Despite the failure of calprotectin as a GIT inflammatory marker in this thesis research, this research serves as a valuable starting point for

future research to evaluate other proteins that increase in concentration with specific inflammatory events, such as GIT inflammation.

7.2 Key takeaways and future directions

7.2.1 Reconsidering the relationship between starch, acidosis, leaky gut, and inflammation

It is important to consider that the precise mode of action of starch-induced inflammation is not well understood at present. The failure of butyrate to reduce systemic inflammation, and of free choice hay to improve gut permeability and colon gene expression may be due to their inadequacy in improving these response variables, or each treatment may have the potential to improve gut permeability, GIT inflammation, and systemic inflammation, but perhaps not in the experimental conditions used in our studies. It is evident that the scientific community lacks clarity on the link between high starch diets, leaky gut, GIT inflammation, and systemic inflammation (Aschenbach et al., 2019; Abeyta et al., 2023a; Krogstad and Bradford, 2023). This limited understanding hinders our ability to treat inflammation as we do not clearly understand the pathway in which starch may, or may not, induce inflammation; if we do not understand the onset of inflammation, and the associated pathway to inflammation, it is difficult to propose an effective treatment intended to target specific aspects of this pathway. The limited knowledge of the relationship between each of these variables is a major knowledge gap and improved understanding would greatly improve the ability of the research community to propose suitable inflammation treatments.

Much of the existing research assumes acidosis-induced leaky gut is the culprit for inflammation following high starch diet consumption (Plaizier et al., 2018), however, data throughout literature does not consistently support this (van Gastelen et al., 2021a,b; Krogstad and Bradford, 2023), and Chapter 5 reported starch consumption was not associated with

inflammation. Additionally, it must be noted that the majority of research defines acidosis using a rumen pH threshold, which has been criticized due to the weak relationship of rumen pH with clinical outcomes (Bramley et al., 2008; Lean and Golder, 2018, 2019), as other factors likely contribute to the symptoms reported during GIT acidosis (Plaizier et al., 2022). The traditional dogma regarding the relationship between acidosis, leaky gut, and inflammation partially originates from studies assessing the effects of experimentally induced GIT acidosis, through feed restriction and/or high or unlimited grain provision (Emmanuel et al., 2008; Khafipour et al., 2009a; Steele et al., 2011a,b). These studies provide valuable information regarding the potential effects of high starch diets on gut permeability and systemic inflammation. However, the application of these results to management of dairy cows in industry should be considered with caution as these experimental conditions are extreme and are not representative of current industry practices. Thus, when treatments and prevention strategies are targeting starch-induced inflammation, their application and efficacy may be less pronounced, or absent, under typical industry conditions. This may partially explain the lack of treatment effects in Chapters 2 and 5, when acidosis was not intentionally induced through feed restriction or unlimited grain provision. In order to determine suitable treatments to manage inflammation in fresh cows, further exploration of the relationship between dietary starch content (under typical conditions, not acidosis challenges), acidosis, gut inflammation and permeability, and systemic inflammation is necessary.

Future research should critically revalue the impacts of GIT acidosis as research has clearly demonstrated reduced GIT pH alone is not necessarily related to inflammation or compromised production. It appears that cows can handle large extents of grain fermentation throughout the GIT without experiencing negative consequences. This highlights that GIT

acidosis alone may not be a problem, as we have previously believed. Perhaps in the coming years, we will focus less on preventing acidosis itself, as defined by pH, and instead, we will shift our focus to other factors that may interact with high starch intake to cause negative health outcomes; negative consequences of high starch intake appear to be contingent on leaky gut.

Previously it was believed high starch intake could induce leaky gut, which would then allow absorption of toxins into the bloodstream resulting in systemic inflammation. However, through a series of controlled experiments (van Gastelen et al., 2021a,b; Abeyta et al., 2023a; Krogstad and Bradford, 2023), it has been demonstrated that high starch intake alone does not result in leaky gut. Instead, cows already experiencing leaky gut, perhaps due to metabolic disorders, infection, or reduced intake, may be vulnerable to high starch intake as fermentation products may then be absorbed, inducing systemic inflammation. Thus, while GIT acidosis alone may not be problematic, it appears that if leaky gut precedes GIT acidosis, cows may experience health consequences. This is of particular concern in the transition period where metabolic disorders and reduced intake are common, thus high starch intake should be limited in the transition period, whereas cows in peak lactation may be fed greater allocations of starch as they are less vulnerable to leaky gut.

In addition to the interaction of high starch intake with leaky gut, other factors may influence the health outcomes of high starch consumption such as: stress or previous immune activation (Trevisi and Minuti, 2018; Horst et al., 2021), the presence of harmful substances in the GIT (Abeyta et al., 2023b), sudden dietary changes (Gott et al., 2015; Plaizier et al., 2017; Krogstad and Bradford, 2023) the extent of rumen and hindgut fermentation (Khafipour et al., 2009a,b; Gressley et al., 2011; Sanz-Fernandez et al., 2020), and the influence of rumen dynamics on the hindgut (Abeyta et al., 2023b), thus, each require further investigation.

7.2.2 Reconsidering the role of inflammation

Research has historically investigated methods of preventing, reducing, or treating inflammation due to compromised performance and health of cows outlined in Chapter 1. However, as also highlighted in Chapter 1, inflammation itself is not necessarily a bad thing as it appears to be closely intertwined with many metabolic and physiological pathways (Farney et al., 2013b; Bradford et al., 2015), thus increased inflammation may also simply be related to modulation of particular pathways. In Chapter 2, we reported higher serum Hp concentration in the prepartum period in butyrate-fed cows. Butyrate has been reported to upregulate various inflammatory genes (Andoh et al., 1999; Hýžd'alová et al., 2008; Xiao et al., 2018) as a protective mechanism to maintain GIT homeostasis (Xiao et al., 2018), thus increased inflammation in this scenario may indicate butyrate is protecting the cow's GIT, rather than hindering it. This raises the point that we should not always attempt to reduce or prevent inflammation, particularly in fresh cows, as it may be of benefit to the animal's health.

As inflammation appears to positively impact cow performance in some scenarios, attempting to reduce inflammation may also interfere with other physiological processes, such as resumption of cyclicity after calving. Inflammation promotes uterine involution, microbial clearance, and tissue repair, as discussed in Chapter 1, each of which is necessary to allow cows to return to reproductive cyclicity. In Chapter 3, cows treated with NSAID tended to have a delayed return to ovulation compared to placebo-treated cows, perhaps due to the inhibition of pathways related to inflammation, thus hindering return to cyclicity.

Future research should evaluate more precisely how inflammation interacts with cow adaption to lactation. In a general sense, we understand that inflammation contributes, at least to

some extent, to an array of metabolic pathways following calving. However, the specific metabolites and physiological changes influenced by inflammation are not well outlined in literature. Our understanding of the role of inflammation would be improved by studies intentionally limiting inflammation, and evaluating gene expression and metabolites, in addition to the commonly assessed phenotypic outcomes in cows. Most studies assessing anti-inflammatory treatments focus on reproductive and milk production outcomes, but data is lacking regarding how physiologic and metabolic pathways are affected. Dairy cattle researchers may benefit from assessing research in other mammalian species, particularly in studies where inflammatory genes were knocked out in mice. Inflammation is generally well-conserved across mammals, thus this research serves as a valuable starting point to improve our understanding of the role of inflammation in dairy cows. However, as many aspects of dairy cattle management are unique, additional research in dairy cows is needed.

7.2.3 The shift to inflammation *management*

As highlighted, many efforts have been made to prevent, reduce, or treat inflammation amongst all cows. However, cows experience different degrees of inflammation, thus not all cows require inflammation intervention. Additionally, this thesis research and others have outlined that inflammation may not always be harmful in all cows, and instead, inflammation assists adaptation to lactation (Farney et al., 2013b; Bradford et al., 2015). Thus, treating every cow with anti-inflammatory drugs, regardless of individual health status, may interfere with natural inflammatory processes, impeding cow performance, as demonstrated in Chapter 3 and by others (Duffield et al., 2009; Newby et al., 2013). Therefore, future research should assess how inflammation can be *managed*, rather than attempting to offset inflammation in all cows entirely.

An essential component to inflammation *management* is identifying individual cows to be treated, rather than treating all cows with anti-inflammatories, regardless of health status. There is currently little evidence to support the use of NSAID in transition cows, aside from extreme cases. Conversely, when transition cows are all administered anti-inflammatories as a "blanket" treatment, there is an increased risk of retained fetal membranes (Duffield et al., 2009; Newby et al., 2013) and metritis (Duffield et al., 2009). Thus instead of providing all cows with NSAID in the fresh period, cows should be evaluated on a case-by-case basis; for example, Barrier et al. (2014) reported NSAID improved recovery in beef cows that underwent a caesarean section. Additionally, anti-inflammatory treatments have value in improving cow welfare in cases of dystocia, injury, infection, or surgery as they provide pain relief (Faulkner and Weary, 2000; Barrier er al., 2014). Thus in atypical scenarios, NSAID administration is justified, however, the majority of transition cows may not benefit from NSAID administration.

It has also been suggested that anti-inflammatory treatments may be helpful in instances of dysregulated or excessive inflammation (Trimboli et al., 2020), beyond what is necessary for tissue repair and microbial clearance (Bradford et al., 2015). This being said, we currently do not know how to establish the precise point at which inflammation goes beyond what is necessary. It is likely impossible to define a specific threshold of "healthy" inflammation, as this would vary extensively amongst cows due to intrinsic variation between cows, as demonstrated in Chapters 4 and 5. In a review by Bradford et al. (2015) that explored the impacts and contribution of inflammation in transition cows, it was suggested that reducing the duration of inflammation, rather than preventing it entirely, may benefit cow health and performance. For example, with respect to uterine inflammation, inflammation in the first three weeks is generally considered normal as it is associated with uterine remodeling, thus it is inappropriate to treat uterine

inflammation before 21 DIM (Gilbert and Santos, 2016). As well, due to the series of rapid physiological and hormonal changes during the transition period, and the crucial role inflammation appears to play in these changes, limiting inflammation through NSAID may not benefit cows. Priest et al. (2013) that reported cows with subclinical endometritis had improved pregnancy rates when treated with NSAID between 21-31 DIM, however, Jeremejeva et al. (2012) reported that NSAID administration between 3-6 DIM did not reduce uterine inflammation or reproductive variables, thus it appears NSAID treatment within the fresh period may not be efficacious.

Additional research is warranted in assessing which fresh cows may benefit from NSAID administration, such as those undergoing caesarean section or other extreme scenarios. As well, assessing the adequate duration of inflammation may provide better insight of when it is appropriate and beneficial to treat inflammation, improving overall management of fresh cow inflammation. Overall, anti-inflammatory treatments, such as NSAID administration, are not recommended for transition cows under "typical" calving conditions.

7.2.4 Improving inflammation detection

In order to manage inflammation, we must be able to measure the extent of inflammation to identify when it is occurring and to evaluate the efficacy of treatments. Research is currently limited in assessing the specific impacts of inflammation due to a lack of markers unique to specific sources of inflammation. As previously described, we cannot manage what we cannot measure, thus identifying more specific inflammatory markers would vastly improve management of inflammation during the fresh period. In regards to GIT inflammation, proteins used in human medicine, other than calprotectin, may be evaluated as markers of GIT-specific inflammation in cattle, such as lactoferrin (Walker et al., 2007) and IgA (Macpherson et al.,

2018), however, extensive additional research is required to understand their role in GIT inflammation in cattle. Beyond GIT inflammation, other markers should be explored as markers of inflammation specific to other tissues. Proteomics is a growing field of study which has begun to explore alternative proteins that may allow more specific inflammation identification through differential protein expression during different types of inflammatory events. Research has already identified proteins with differential expression during mastitis (Turk et al., 2012), and perhaps similar techniques may be used in future studies to identify proteins with differential expression during specific inflammatory events. Additionally, isoforms of SAA and Hp may have potential in identifying specific sources of inflammation as different isoforms increasing to greater extents according to the specific tissue that is inflamed (Upadhyaya et al., 2016; Murata et al., 2020), thus allowing specific identification of inflamed tissues.

Overall, it is imperative that future research considers evaluating inflammation more precisely by identifying specific tissues that are inflamed to allow specific treatment of tissues and prevent the onset of systemic or dysregulated inflammation. By accurately identifying the source of inflammation, our ability to manage inflammation will be substantially improved. If inflammatory markers specific to inflammation type are identified, commercial laboratory kits may be developed, allowing improved accuracy in evaluating anti-inflammatory treatments, which would have been largely helpful in this thesis research. As well, there is potential for the development of on-farm kits that could identify specific inflammatory events, allowing producers to treat cows for specific inflammatory conditions.

7.2.5 Accommodating inter-cow variation

Feeding systems designed to accommodate individual animal requirements and responses may be a large area of opportunity for future research. As described, Chapter 4 reported variation

in feed selection according to individual metabolism and inflammation; lower prepartum DMI and greater concentration of plasma ketones and serum Hp at calving was associated with greater postpartum free choice hay consumption. As well, Chapter 5 reported variable metabolism and physiology in cows offered the same diet; lower prepartum DMI and greater postpartum serum Hp concentration tended to be associated with greater postpartum gut permeability. Providing cows with some choice of feed type consumed may accommodate inter-cow variation to some extent, as feed selection is at least partially regulated by internal metabolism and requirements (Chapters 1, 4, and 5).

The present research evaluated how free choice hay may accommodate individual variation and preference, however, future research may evaluate a variety of other feedstuffs, such as other forage sources, mineral mixes, protein sources, and energy sources, to assess how individual variation is associated with individual feed selection. Perhaps future research will move away from TMR feeding for fresh cows, and instead allow more "cow-directed" feed consumption. The transition period is clearly challenging for all cows, thus it may be beneficial to allow cows to consume feed based on individual preference during this period, rather than feeding all cows a single ration intended for maximum milk yield. Perhaps our focus should be on promoting cow health in the fresh period, even if that means allowing cows to voluntarily reduce energy intake in the short term. Transition disorders can compromise milk production and reproductive performance for the entirety of lactation, even after disorders have resolved. Thus, by improving cow health and avoiding transition disorders, cow performance may be improved throughout the entire lactation, despite a brief reduction in intake during the fresh period. This being said, in Chapter 5, cows that increased free choice hay intake did not experience reduced

milk production, so it is possible additional forage intake in the short term does not offset milk production, but this requires further evaluation.

Additional research is also required to determine to what extent individual feed selection matches individual needs; in other words, to what extent can cows individually balance feed selection to meet energy and metabolic requirements? While Chapter 4 reported that inflammation at calving was associated with increased postpartum hay intake, and Chapter 5 reported that cows offered free choice hay had lower inflammation, additional data are needed to confirm if cows experiencing inflammation are motivated to consume hay in order to mitigate inflammation. As well, further research is needed to understand the extent metabolic feedback regulates feed intake, compared to individual preference based on palatability or previous feed exposure. Further work is also needed to explore what specific metabolic pathways regulate feed preference, as current literature broadly describes "metabolic feedback" or "metabolic needs" as influencing feed preference, but the precise feedback loops are not thoroughly described. Thus, we do not know if cows are capable of self-regulating feed intake according to nutrient and metabolic demands, and if there is a direct improvement in cow health following adjusted feed intake. Improved understanding may allow us to better manage inflammation, and improve overall management of fresh cows.

7.3 Conclusion

Based on the data in this thesis, butyrate supplementation and NSAID administration are not effective in reducing inflammation and are not recommended for use in transition dairy cows (Chapters 2 and 3). However, offering free choice hay to fresh cows may be effective in mitigating postpartum inflammation (Chapter 5). Data also demonstrate that periparturient factors influence postpartum free choice hay consumption (Chapter 4). Thus, producers may

offer free choice hay to cows in the fresh period to accommodate metabolic needs of individual cows and associated feed preference, and to mitigate postpartum inflammation, but milk production may not increase due to reduced DMI. Finally, this research highlights the remaining need for validation of a marker specific to gastrointestinal inflammation in dairy cows, which would vastly improve our ability to manage such inflammation.

Bibliography

Abdela, N. 2016. Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: a review of past and recent research at global prospective. Achiev. Life Sci. 10:187–196. https://doi.org/10.1016/j.als.2016.11.006.

Abeni, F., and G. Bertoni. 2009. Main causes of poor welfare in intensively reared dairy cows. Ital. J. Anim. Sci. 8(Suppl. 1):45–66. https://doi.org/10.4081/ijas.2009.s1.45.

Abeyta, M. A., B. M. Goetz, E. J. Mayorga, S. Rodriguez-Jimenez, J. Opgenorth, A. D. Freestone, J. M. Lourenco, T. R. Callaway, and L. H. Baumgard. 2023b. Effects of abomasally infused rumen fluid from corn-challenged donor cows on production, metabolism, and inflammatory biomarkers in healthy recipient cows. J. Dairy Sci. https://doi.org/10.3168/jds.2022-22809.

Abeyta, M. A., E. A. Horst, E. J. Mayorga, B. M. Goetz, M. Al-Qaisi, C. S. McCarthy, M. R. O'Neil, B. C. Dooley, P. Piantoni, G. F. Schroeder, and L. H. Baumgard. 2023a. Effects of hindgut acidosis on metabolism, inflammation, and production in dairy cows consuming a standard lactation diet. J. Dairy Sci. 106:1429–1440. https://doi.org/10.3168/jds.2022-22303.

Agarwal, U., Q. Hu, R. L. Baldwin, and B. J. Bequette. 2015. Role of rumen butyrate in regulation of nitrogen utilization and urea nitrogen kinetics in growing sheep. J. Anim. Sci. 93:2382–2390. https://doi.org/10.2527/jas.2014-8738.

Aiello, R. J., L. E. Armentano, S. J. Bertics, and A. T. Murphy. 1989. Volatile fatty acid uptake and propionate metabolism in ruminant hepatocytes. J. Dairy Sci. 72:942–949. https://doi.org/10.3168/jds.S0022-0302(89)79187-6.

Albornoz, R. I., K. J. Harvatine, and M. S. Allen. 2019. Diet starch concentration and starch fermentability affect energy intake and energy balance of cows in the early postpartum period. J. Dairy Sci. 102:5161–5171. https://doi.org/10.3168/jds.2018-15634.

Albornoz, R. I., L. M. Sordillo, G. A. Contreras, R. Nelli, L. K. Mamedova, B. J. Bradford, and M. S. Allen. 2020. Diet starch concentration and starch fermentability affect markers of inflammatory response and oxidant status in dairy cows during the early postpartum period. J. Dairy Sci. 103:352–367. https://doi.org/10.3168/jds.2019-16398.

Alibrahim, B., M. I. Aljasser, and B. Salh. 2015. Fecal calprotectin use in inflammatory bowel disease and beyond: a mini-review. Can. J. Gastroenterol. Hepatol. 29:157–163. https://doi.org/10.1155/2015/950286.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2.

Allen, M. S., and P. Piantoini. 2013. Metabolic control of feed intake. Implications for metabolic disease of fresh cows. Vet. Clin. Food Anim. 29:279–297. http://dx.doi.org/10.1016/j.cvfa.2013.04.001.

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board Invited Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. https://doi.org/10.2527/jas.2009-1779.

Alnakip, M. E., M. Quintela-Baluja, K. Böhme, I. Fernández-No, S. Caamaño-Antelo, P. Calo-Mata, and J. Barros-Velázquez. 2014. The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions. J. Vet. Med. https://doi.org/10.1155/2014/659801.

Amiridis, G. S., L. Leontides, E. Tassos, P. Kostoulas, and G. C. Fthenakis. 2001. Flunixin meglumine accelerates uterine involution and shortens the calving-to-first-oestrus interval in cows with puerperal metritis. J. Vet. Pharmacol. Ther. 24:365–367. https://doi.org/10.1046/j.1365-2885.2001.00358.x.

Andersen, J. B., J., Sehested, and K. L. Ingvartsen. 1999. Effect of dry cow feeding strategy on rumen pH, concentration of volatile fatty acids and rumen epithelium development. Acta Agric. Scand. 49: 149–155 https://doi.org/10.1080/090647099424051.

Anderson, B. H., D. L. Watson, and I. G. Colditz. 1999. The effect of dexamethasone on some immunological parameters in cattle. Vet. Res. Commun. 23:399–413.

Andoh, A., Y. Fujiyama, K. Hata, A. Araki, H. Takaya, M. Shimada, and T. Bamba. 1999. Counter-regulatory effect of sodium butyrate on tumour necrosis factor-alpha (TNF-alpha)-induced complement C3 and factor B biosynthesis in human intestinal epithelial cells. Clin. Exp. Immunol. 118:23–29. https://doi.org/10.1046/j.1365-2249.1999.01038.x.

AOAC Int. AOAC International. 2002. Official Methods of Analysis. 17th ed. 1st revision.

AOAC Int. AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC Int.

AOAC International. 2000. Official Methods of Analysis. 17th ed.

Argenzio R. A., and M. Southworth. 1975. Sites of organic acid production and absorption in gastrointestinal tract of the pig. Am. J. Physiol. 228:454-460. https://doi.org/10.1152/ajplegacy.1975.228.2.454.

Arrieta M. C., L. Bistritz, and J. B. Meddings. 2006. Alterations in intestinal permeability. Gut. 55:1512–1520. https://doi.org/10.1136/gut.2005.085373.

Aschenbach, J. R., G. B. Penner, F. Stumpff, and G. Gabel. 2011. Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. J. Anim. Sci. 89:1092–1107. https://doi.org/10.2527/jas.2010-3301.

Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon, and G. B. Penner. 2010. Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. IUBMB Life 62:869–877. https://doi.org/10.1002/iub.400.

Aschenbach, J. R., Q. Zebeli, A. K. Patra, G. Greco, S. Amasheh, and G. B. Penner. 2019. Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. J. Dairy Sci. 102:1866–1882. https://doi.org/10.3168/jds.2018-15243.

Aschenbach, J. R., S. Bilk, G. Tadesse, F. Stumpff, and G. Gäbel. 2009. Bicarbonate-dependent and bicarbonate-independent mechanisms contribute to nondiffusive uptake of acetate in the ruminal epithelium of sheep. Am. J. Physiol. Gastrointest. Liver Physiol. 296:G1098–G1107. https://doi.org/10.1152/ajpgi.90442.2008.

Atwood, S. B., F. D. Provenza, R. D. Wiedmeier, and R. E. Banner. 2001. Influence of free-choice vs. mixed-ration diets on food intake and performance of fattening calves. J. Anim. Sci. 79:3034–3040. https://doi.org/10.2527/2001.79123034x.

Bach, A. 1978. Oxaloacetate deficiency in MCT-induced ketogenesis. Arch. Int. Physiol. Biochim. 86:1133–1142. https://doi.org/10.3109/13813457809055968.

Bach, A., I. Guasch, G. Elcoso, F. Chaucheyras-Durand, M. Castex, F. Fàbregas, E. Garcia-Fruitos, and A. Aris. 2018. Changes in gene expression in the rumen and colon epithelia during the dry period through lactation of dairy cows and effects of live yeast supplementation. J. Dairy Sci. 101:2631–2640. https://doi.org/10.3168/jds.2017-13212.

Baird, G. D., R. J. Heitzman, and K. G. Hibbitt. 1972. Effects of starvation on intermediary metabolism in the lactating cow. Biochem. J. 128:1311–1318. https://doi.org/10.1042/bj1281311.

Baldwin R. L. 1998 Use of isolated ruminal epithelial cells in the study of rumen metabolism. J. Nutr. 128:293S–296S. https://doi.org/10.1093/jn/128.2.293S.

Baldwin R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87:E55–E65. https://doi.org/10.3168/jds.S0022-0302(04)70061-2.

Ballard, S. T., J. H. Hunter, and A. E. Taylor. 1995. Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. Annu. Rev. Nutr. 15:35–55. https://doi.org/10.1146/annurev.nu.15.070195.000343.

Ballou, M. A. 2012. Growth and development symposium: inflammation: role in the etiology and pathophysiology of clinical mastitis in dairy cows. J. Anim. Sci. 90:1466–1478. https://doi.org/10.2527/jas.2011-4663

Ballou, M. A. 2012. Growth and development symposium: inflammation: role in the etiology and pathophysiology of clinical mastitis in dairy cows. J. Anim. Sci. 90:1466–1478. https://doi.org/10.2527/jas.2011-4663

Bannink, A., J. France, S. Lopez, W. J. J. Gerrits, E. Kebreab, S. Tamminga, and J. Dijkstra. 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Anim. Feed Sci. Technol. 143:3–26. https://doi.org/10.1016/j.anifeedsci.2007.05.002.

Bannink, A., W. J. J. Gerrits, J. France, and J. Dijkstra. 2012. Variation in rumen fermentation and the rumen wall during the transition period in dairy cows. Anim. Feed Sci. Technol. 172:80–94. http://dx.doi.org/10.1016/j.anifeedsci.2011.12.010.

Barbosa, J. A., L. A. Rodrigues, D. A. Columbus, J. C. Aguirre, J. C. Harding, V. S. Cantarelli and M. de O. Costa. 2021 Experimental infectious challenge in pigs leads to elevated fecal calprotectin levels following colitis, but not enteritis. Porc. Health Manag. 7:48. https://doi.org/10.1186/s40813-021-00228-9.

Barrier, A. C., T. M. Coombs, C. M. Dwyer, M. J. Haskell, and L. Goby. 2014. Administration of a NSAID (meloxicam) affects lying behaviour after caesarean section in beef cows. Appl. Anim. Behav. Sci. 155:28–33. https://doi.org/10.1016/j.applanim.2014.02.015.

Bayley, H. S. 1978. Comparative physiology of the hindgut and its nutritional significance. J. Anim. Sci. 46:1800–1802. https://doi.org/10.2527/jas1978.4661800x.

Beam S. W., and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56:133–142. https://doi.org/10.1095/biolreprod56.1.133.

Beauchemin, K., and G. Penner. 2009. New developments in understanding ruminal acidosis in dairy cattle. In Proceedings of the 18th Annual Tri-State Dairy Nutr. Conf. Grand Wayne Convention Center, Fort Wayne, Indiana, USA.

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. Neoplasia 2:265–278. https://doi.org/10.1023/A:1026336505343.

Berczi I., L. Bertok, and T. Bereznai. 1966. Comparative studies on the toxicity of Escherichia coli lipopolysaccharide endotoxin in various animal species. Can. J. Microbiol. 12:1070–1071. https://doi.org/10.1139/m66-143.

Beretta, C., G. Garavaglia, and M. Cavalli. 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: An in vitro analysis. Pharmacol. Res. 52:302–306. https://doi.org/10.1016/j.phrs.2005.04.004.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567– 590. https://doi.org/10.1152/physrev.1990.70.2.567.

Berndtson, W. E. 1991. A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. J. Anim. Sci. 69:67–76. https://doi.org/10.2527/1991.69167x.

Bertoni, G., Trevisi E., Calamari L., Bionaz M. 2006. The inflammation could have a role in the liver lipidosis occurrence in dairy cows. Pages 157–158 in Production Diseases in Farm Animals (originally presented in 2004, in 12th International Production Diseases in Farm Animals Conference, East Lansing, Michigan, USA). Wageningen Academic Publ., Wageningen, The Netherlands.

Bertoni, G., E. Trevisi, and F. Piccioli-Cappelli. 2004b. Effects of acetyl-salicylate used in post-calving of dairy cows. Vet. Res. Commun. 28:217–219. https://doi.org/10.1023/b:verc.0000045410.86004.03.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300– 3310. https://doi.org/10.3168/jds.2008-0995.

Bertoni, G., Minuti, A., Trevisi, E., 2015. Immune system, inflammation and nutrition. Anim. Prod. Sci. 55:354–360. https://doi.org/10.1071/AN14863.

Bikker, J. P., H. van Laar, P. Rump, J. Doorenbos, K. van Meurs, G. M. Griffioen, J. Dijkstra. 2014. Technical note: Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. J. Dairy Sci. 97:2974-2979. https://doi.org/10.3168/jds.2013-7560.

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90:1740–1750. https://doi.org/10.3168/jds.2006-445.

Bissell, H. A., and M. B. Hall. 2010. Cattle differ in ability to adapt to small intestinal digestion of starch. J. Dairy Sci. 93(E-Suppl.1):845. (Abstr.)

Biswal, S. S., S Das, S. Balasubramanian, and D. N. Mohanty. 2014. Serum amyloid A and haptoglobin levels in crossbred cows with endometritis following different therapy. Vet. World 7:1066–1070. Https://doi.org/10.14202/vetworld.2014.1066-1070.

Bjarnason, I. 2017. The use of fecal calprotectin in inflammatory bowel disease. J. Gastroenterol. and Hepatol. 13:53–56.

Blikslager A. T., A. J. Moeser, J. L. Gookin, S. L. Jones, and J. Odle. 2007. Restoration of barrier function in injured intestinal mucosa. Physiol. Rev. 87:545–64. https://doi.org/10.1152/physrev.00012.2006. PMID: 17429041.

Blottière, H. M., B. Buecher, J. P. Galmiche, and C. Cherbut. 2003. Molecular analysis on the effect of short chain fatty acids on intestinal proliferation. Proc. Nutr. Soc. 62:101–106. https://doi.org/10.1079/PNS2002215.

Bobe G., J. W. Young, and D. C. Beitz. 2004. Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87:3105–3124. https://doi.org/10.3168/jds.S0022-302(04)73446-3.

Boerman, J. P., S. B. Potts, M. J. VandeHaar, M. S. Allen, and A. L. Lock. 2015. Milk production responses to a change in dietary starch concentration vary by production level in dairy cattle. J. Dairy Sci. 98:4698–4706. https://doi.org/10.3168/jds.2014-8999.

Boosman, R., C. W. Mutsaers, and A. Klarenbeek. 1991. The role of endotoxin in the pathogenesis of acute bovine laminitis. Vet. Q. 13:155–162. https://doi.org/10.1080/01652176.1991.9694301.

Bordin, M., F. D'Atri, L. Guillemot, and S. Citi. 2004. Histone deacetylase inhibitors up-regulate the expression of tight junction proteins. Mol. Cancer Res. 2:692–701.

Boro, P., A. Kumaresan, A. K. Singh, D. Gupta, S. Kumar, A. Manimaran, A. K. Mohanty, T. K. Mohanty, R. Pathak, N. M. Attupuram, R. K. Baithalu, and S. Prasad. 2014. Expression of short chain fatty acid receptors and pro-inflammatory cytokines in utero-placental tissues is altered in cows developing retention of fetal membranes. Placenta 35:455–460. https://doi.org/10.1016/j.placenta.2014.04.009.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova and A. J. Carpenter. 2015. Invited review: inflammation during the transition to lactation: new adventures with an old flame. J. Dairy Sci. 98:6631–6650. https://doi.org/doi:10.3168/jds.2015-9683.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Bramley, E., I. J. Lean, W. J. Fulkerson, M. A. Stevenson, A. R. Rabiee, and N. D. Costa. 2008. The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. J. Dairy Sci. 91:308–321. https://doi.org/10.3168/jds.2006-601.

Breed, M. D., and J. Moore. 2016. Foraging and Optimality Theory. Pages 293-292 in Animal Behavior, 2nd edition. Academic Press, Cambridge, Massachusetts, USA. https://doi.org/10.1016/B978-0-12-801532-2.00009-X.

Brøchner A. C., and P. Toft. 2009. Pathophysiology of the systemic inflammatory response after major accidental trauma. Scand. J. Trauma Resusc. Emerg. Med. 15:17–43. https://doi.org/10.1186/1757-7241-17-43.

Bröer, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. Physiol. Rev. 88:249–286. https://doi.org10.1152/physrev.00018.2006.

Brown, M. S., C. H. Ponce, and R. Pulikanti. 2006. Adaptation of beef cattle to high-concentrate diets: Performance and ruminal metabolism. J. Anim. Sci. 84:E25–E33. https://doi.org/10.2527/2006.8413_supple25x.

Brown, M. S., C. R. Krehbiel, M. L. Galyean, M. D. Remmenga, J. P. Peters, B. Hibbard, J. Robinson, and W. M. Moseley. 2000. Evaluation of models of acute and subacute acidosis on dry matter intake, ruminal fermentation, blood chemistry, and endocrine profiles of beef steers. J. Anim. Sci. 78:3155–3168. https://doi.org/10.2527/2000.78123155x.

Brubaker, P. L. 2018. Glucagon-like peptide-2 and the regulation of intestinal growth and function. Compr. Physiol. 8:1185–1210. https://doi.org/10.1002/cphy.c170055.

Bruewer, M, and A. Nusrat. 2000-2013. Regulation of paracellular transport across tight junctions by the actin cytoskeleton. In: Madame Curie Bioscience Database. Austin, Texas, USA. Landes Bioscience. https://www.ncbi.nlm.nih.gov/books/NBK6487/

Bunn, S. K., W. M. Bisset, M. J. Main, E. S. Gray, S. Olson, and B. E. Golden. 2001. Fecal calprotectin: validation as a noninvasive measure of bowel inflammation in childhood inflammatory bowel disease. J. Pediatr. Gastroenterol. Nutr. 33:14–22. https://doi.org/10.1097/00005176-200107000-00002.

Burke, C. R., J. K. Kay, C. V. C. Phyn, S. Meier, J. M. Lee, and J. R. Roche. 2010a. Short communication: Effects of dietary non-structural carbohydrates prepartum and postpartum on reproduction of grazing dairy cows. J. Dairy Sci. 93:4292–4296. https://doi.org/10.3168/jds.2009-2869

Burke, C. R., S. Meier, S. McDougall, C. Compton, M. Mitchell, and J. R. Roche. 2010b. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J. Dairy Sci. 93:5363–5373. https://doi.org/10.3168/jds.2010-3356.

Burton, L., M. E. Kehrli, S. Kapil, and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. J. Leukoc. Biol. 57:317–325. https://doi.org/10.1002/jlb.57.2.317.

Butler, S. T., S. H. Pelton, and W. R. Butler. 2006. Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol. J. Dairy Sci. 89:2938–2951. https://doi.org/10.3168/jds.S0022-0302(06)72566-8.

Cabezas-Garcia, E.H., S. J. Krizsan, K. J. Shingfield, and P. Huhtanen. 2017. Between-cow variation in digestion and rumen fermentation variables associated with methane production. J. Dairy Sci. 100:4409–4424, https://doi.org/10.3168/jds.2016-12206.

Caenepeel C., K. Machiels, S. Vieira-Silva, N. Ardeshir Davani, M. Ferrante, and S. Vermeire. 2019. The impact of storage time and freeze–thaw cycles on faecal calprotectin concentration in inflammatory bowel disease patients and controls. J. Crohns Colitis 13:S223. https://doi.org/10.1093/ecco-jcc/jjy222.377.

Calamari L., S. Care', A. Ferrari, G. Bertoni. 2008. Preliminary study to develop a reference tool to validate the models of animal welfare assessment in dairy farms. Book of abstracts. 4th international workshop on the assessment of animal welfare at farm and group level. P. Koene. Ponsen and Looijen: Wageningen, The Netherlands.

Camilleri, M., B. J. Lyle, K. L. Madsen, J. Sonnenburg, K. Verbeke, and G. D. Wu. 2019. Role for diet in normal gut barrier function: developing guidance within the framework of food-labeling regulations. Am. J. Physiol. Gastrointest. Liver Physiol. 317:G17–G39. https://doi.org/10.1152/ajpgi.00063.2019.

Canani, R. B., M. Di Costanzo, L. Leone, M. Pedata, R. Meli, and A. Calignano. 2011. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J. Gastroenterol. 17:1519–1528. https://doi.org/10.3748/wjg.v17.i12.1519.

Cappellozza, B. I., R. F. Cooke, C. Trevisanuto, V. D. Tabacow, F. N. T. Cooke, and D. W. Bohnert. 2011. Feed and water restriction elicits an acute-phase protein response in beef cattle. J. Anim. Sci. 89:269. (Abstr.)

Carpenter, A. J., C. M. Ylioja, C. F. Vargas, L. K. Mamedova, L. G. Mendonça, J. F. Coetzee, L. C. Hollis, R. Gehring, and B. J. Bradford. 2016. Hot topic: Early postpartum treatment of

commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. J. Dairy Sci. 99:672–679. https://doi.org/10.3168/jds.2015-10048.

Carpenter, A. J., C. M. Ylioja, L. K. Mamedova, K. E. Olagaray, and B. J. Bradford. 2018. Effects of early postpartum sodium salicylate treatment on long-term milk, intake, and blood parameters of dairy cows. J. Dairy Sci. 101:1437–1447.

Cavallini, D., L. M. E. Mammi, M. Fustini, A. Palmonari, A. J. Heinrichs, and A. Formigoni. 2018. Effects of ad libitum or restricted access to total mixed ration with supplemental long hay on production, intake, and rumination. J. Dairy Sci. 101:10922–10928. https://doi.org/10.3168/jds.2018-14770.

Ceciliani F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. J Proteomics 75:4207–4231. https://doi.org/10.1016/j.jprot.2012.04.004.

Ceron, J. J., P. D. Eckersall, and S. Martýnez-Subiela. 2005. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet. Clin. Pathol. 34:85-99. https://doi.org/10.1111/j.1939-165x.2005.tb00019.x.

Chan, J. P., C. Chang, W. Hsu, W. Liu, and T. Chen. 2010. Association of increased serum acute-phase protein concentrations with reproductive performance in dairy cows with postpartum metritis. Vet. Clin. Pathol. 39:72–78. https://doi.org/10.1111/j.1939-165X.2009.00182.x.

Chang, G., J. Yan, N. Ma, X. Liu, H. Dai, M. S. Bilal, and X. Shen. 2018. Dietary sodium butyrate supplementation reduces high concentrate diet feeding-induced apoptosis in mammary cells in dairy goats. J. Agric. Food Chem. 66:2101–2107. https://doi.org/10.1021/acs.jafc.7b05882.

Charavaryamath, C., P. Fries, S. Gomis, C. Bell, K. Doig, L. L. Guan, A. Potter, S. Napper, and P. J. Griebel. 2011. Mucosal changes in a long-term bovine intestinal segment model following removal of ingesta and microflora. Gut Microbes 2:134–144. https://doi.org/10.4161/gmic.2.3.16483.

Chen, L., H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, and L. Zhao. 2017. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 9:7204–7218. https://doi.org/10.18632/oncotarget.23208.

Chen, L., H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, and L. Zhao. 2018. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9:7204. https://doi.org/10.18632/oncotarget.23208.

Chen, Y., R. Arsenault, S. Napper, and P. Griebel. 2015. Models and methods to investigate acute stress responses in cattle. Animals (Basel). 5:1268-1295. https://doi.org/10.3390/ani5040411.

Cheng, W. N. and S. G. Han. 2020. Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments—A review. Asian-Australasian J. Anim. Sci. 33:1699. https://doi.org/10.5713/ajas.20.0156.

Cheong S. H., O. G. Sá Filho, V. A. Absalon-Medina, A. Schneider, W. R. Butler, and R. O. Gilbert. 2017. Uterine and systemic inflammation influences ovarian follicular function in postpartum dairy cows. PLoS One 12:e0177356. https://doi.org/10.1371/journal.pone.0177356.

Cheong, S. H., O. G. Sa Filho, V. A. Absalon-Medina, S. H. Pelton, W. R. Butler, and R. O. Gilbert. 2015. Metabolic and endocrine differences between dairy cows that do or do not

ovulate first postpartum dominant follicles. Biol. Reprod. 94:18. https://doi.org/10.1095/biolreprod.114.127076 PMID: 26632612

Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Anal. Biochem. 162:156–159. https://doi.org/10.1006/abio.1987.9999.

Chung, H., S. J. Pamp, J. A. Hill, N. K. Surana, S. M. Edelman, E. B. Troy, N. C. Reading, E. J. Villablanca, S. Wang, J. R. Mora, Y. Umesaki, D. Mathis, C. Benoist, D. A. Relman, and D. L. Kasper. 2012. Gut immune maturation depends on colonization with a host-specific microbiota. Cell. 149:1578–1593. https://doi.org/10.1016/j.cell.2012.04.037.

Coetzee, J. F., B. KuKanich, R. Mosher, and P. S. Allen. 2009. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. Vet Ther. 10:E1–E8.

Colucci, P. E., L. E. Chase, and P. J. Van Soest. 1982. Level of feed intake and diet digestibility in dairy cattle. J. Dairy Sci. 65:1445–1456. https://doi.org/10.3168/jds.S0022-0302(82)82367-9

Contreras, G. A., C. Strieder-Barboza, and J. D. Koster. 2018. Symposium review: Modulating adipose tissue lipolysis and remodeling to improve immune function during the transition period and early lactation of dairy cows. J. Dairy Sci. 101:2737–2752. https://doi.org/10.3168/jds.2017-13340.

Contreras, G. A., N. J. O'Boyle, T. H. Herdt, and L. M. Sordillo. 2010. Lipomobilization in periparturient dairy cows influences the composition of plasma nonesterified fatty acids and leukocyte phospholipid fatty acids. J. Dairy Sci. 93:2508–2516. https://doi.org/10.3168/jds.2009-2876

Cooper, S. D. B., I. Kyriazakis, J. V. Nolan, and D. H. Anderson. 1995. Diet selection in sheep: the role of the rumen environment in the selection of a diet from two feeds that differ in their energy density. Br. J. Nut. 74:39–54. https://doi.org/10.1079/BJN19950105.

Coppock, C. E. 1985. Energy nutrition and metabolism of the lactating dairy cow. J. Dairy Sci. 68:3403–3410. https://doi.org/10.3168/jds.S0022-0302(85)81253-4.

Costa, J. H. C., N. A. Adderley, D. M. Weary, and M. A. G. von Keyserlingk. 2016. Short communication: Effect of diet changes on sorting behavior of weaned dairy calves. J. Dairy Sci. 99:5635–5639. https://doi.org/10.3168/jds.2015-10052.

Counotte, G. H., A. T. van't Klooster, J. van der Kuilen, and R. A. Prins. 1979. An analysis of the buffer system in the rumen of dairy cattle. J. Anim. Sci. 49:1536–1544. https://doi.org/10.2527/jas1979.4961536x.

Cox, M. A., J. Jackson, M. Stanton, A. Rojas-Triana, L. Bober, M. Laverty, X. Yang, F. Zhu, J. Liu, S. Wang, F. Monsma, G. Vassileva, M. Maguire, E. Gustafson, M. Bayne, C. C. Chou, D. Lundell, and C. H. Jenh. 2009. Short-chain fatty acids act as anti-inflammatory mediators by regulating prostaglandin E2 and cytokines. World J. Gastroenterol. 15:5549–5557. https://doi.org/10.3748/wjg.15.5549.

Cryer, B., and M. Feldman. 1998. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. Am. J. Med. 104:413–421. https://doi.org/10.1016/S0002-9343(98)00091-6.

D'Angelo, F., C. Felley, and J. L. Frossard. 2017. Calprotectin in daily practice: where do we stand in 2017? Digestion. 95:293–301. https:// doi.org/10.1159/000476062.

Dai, H., X. Liu, J. Yan, Z. Aabdin, M. S. Bilal, and X. Shen. 2017. Sodium butyrate ameliorates high-concentrate diet-induced inflammation in the rumen epithelium of dairy goats. J. Agric. Food Chem. 65:596–604. https://doi.org/10.1021/acs.jafc.6b04447.

D'Angelo, F., C. Felley, and J. L. Frossard. 2017. Calprotectin in daily practice: where do we stand in 2017?. Digestion 95:293–301. https://doi.org/10.1159/000476062.

Dann, H. M. and B. H. Nelson. 2011. Early lactation diets for dairy cattle – focus on starch. Pages 46–56 in Proc. Cornell Nutr. Conf. Feed Manuf., East Syracuse, New York, USA.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563–3577. https://doi.org/10.3168/jds.S0022-0302(06)72396-7.

De Koster J. D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 2013 29:299–322. https://doi.org/10.1016/j.cvfa.2013.04.002.

Devaraj, S., U. Singh, I. Jialal. 2009. The evolving role of C-reactive protein in atherothrombosis. Clin. Chem.55:229–238. https://doi.org/10.1373/clinchem.2008.108886.

DeVries, T. J., F. Dohme, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. J. Dairy Sci. 91:3958–3967. https://doi.org/10.3168/jds.2008-1347.

DeVries, T. J., T. Schwaiger, K. A. Beauchemin, and G. B. Penner. 2014a. The duration of time that beef cattle are fed a high-grain diet affects feed sorting behavior, both prior to and after acute ruminal acidosis. J. Anim. Sci. 92:1728–1737. https://doi.org/10.2527/jas.2013-7252.

DeVries, T. J., T. Schwaiger, K. A. Beauchemin, and G. B. Penner. 2014b. Impact of severity of ruminal acidosis on feed sorting behaviour of beef cattle. Anim. Prod. Sci. 54:1238–1242. https://doi.org/10.1071/AN14227

Dhondt, G., C. Burvenich, G. Peeters. 1977. Mammary blood flow during experimental Escherichia coli endotoxin induced mastitis in goats and cows. J. Dairy Res. 44:433–440. https://doi.org/10.1017/s0022029900020392

DiAngelo, J. R., M. L. Bland, S. Bambina, S. Cherry, and M. J. Birnbaum. 2009. The immune response attenuates growth and nutrient storage in Drosophila by reducing insulin signaling. Proc. Natl. Acad. Sci. 106:20853–20858. https://doi.org/10.1073/pnas.0906749106.

Dianoux, A. C. M. J. Stasia, J. Garin, J. Gagnon, and P. V. Vignais. 1992. The 23-kilodalton protein, a substrate of protein kinase C, in bovine neutrophil cytosol is a member of the S100 family. Biochemistry 31:5898–5905. https://doi.org/10.1021/bi00140a028.

Dieho, K., A. Bannink, I. Geurts, J. Schonewille, G. Gort, and J. Dijkstra. 2016a. Morphological adaptation of rumen papillae during the dry period and early lactation as affected by rate of increase of concentrate allowance. J. Dairy Sci. 99:2339–2352. https://doi.org/10.3168/jds.2015-9837.

Dieho, K., J. Dijkstra, J. T. Schonewille, and A. Bannink. 2016b. Changes in ruminal volatile fatty acid production and absorption rate during the dry period and early lactation as affected by rate of increase of concentrate allowance. J. Dairy Sci. 99:5370–5384. https://doi.org/10.3168/jds.2015-10819.

Dionissopoulos, L., A. H. Laarman, O. AlZahal, S. L. Greenwood, M. A. Steele, J. C. Plaizier, J. C. Matthews, and B. W. McBride. 2013. Butyrate-mediated genomic changes involved in

non-specific host defenses, matrix remodeling and the immune response in the rumen epithelium of cows afflicted with subacute ruminal acidosis. Am. J. Anim. Vet. Sci. 8:8–27.

Dirksen, G. U., H. G. Liebich, and E. Mayer. 1985. Adaptive changes of the ruminal mucosa and their functional and clinical significance. Bovine Pract. 20:116–120.

Djoković, R., V. Kurćubić, Z. Ilić, M. Cincović, M. Lalović, B. Jašović, and J. Bojkovski. 2017. Correlation between blood biochemical metabolites milk yield, dry matter intake and energy balance in dairy cows during early and mid lactation. Advances in Diabetes and Metabolism 5:26–30. https://doi.org/10.13189/adm.2017.050202

Doepel, L., D. Pacheco, J. J. Kennelly, M. D. Hanigan, I. F. Lopez and H. Lapierre. 2004. Milk protein synthesis as a function of amino acid supply. J. Dairy Sci. 87:1279–1287. https://doi.org/10.3168/jds.S0022-0302(04)73278-6.

Dohme, F., T. J. DeVries, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for acidosis: Ruminal pH. J. Dairy Sci. 91:3554–3567. https://doi.org/10.3168/jds.2008-1264.

Donato, R., B. R. Cannon, G. Sorci, F. Riuzzi, K. Hsu, D. J. Weber, and C. L. Geczy. 2013. Functions of S100 proteins. Curr. Mol. Med. 13:24–57.

Dosogne H., C. Burvenich, A. E. Freeman, M. E. Kehrli, J. C. Detilleux, J. Sulon, J. F. Beckers, and D. Hoeben. 1999. Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. Vet. Immunol. Immunopathol. 67:47–54. https://doi.org/10.1016/s0165-2427(98)00215-3.

Dosogne, H., A. V. Capuco, M. J. Paape, E. Roets, C. Burvenich, and B. Fenwick. 1998. Reduction of acyloxyacyl hydrolase activity in circulating neutrophils from cows after

parturition. J. Dairy Sci. 81:672–677. https://doi.org/10.3168/jds.S0022-0302(98)75622-X.

Douglas, G. N., T. R. Overton, H. G. Bateman, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J. Dairy Sci. 89:2141–2157. https://doi.org/10.3168/jds.S0022-0302(06)72285-8

Dourey, A., M. G. Colazo, P. P. Barajas, and D. J. Ambrose. 2011. Research relationships between endometrial cytology and interval to first ovulation, and pregnancy in postpartum dairy cows in a single herd. Res. Vet. Sci. 91:e149–e153. https://doi.org/10.1016/j.rvsc.2010.11.011.

Drackley J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84:100–112. https://doi.org/10.3168/jds.S0022-0302(01)70204-4.

Drackley, J. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259–2273. https://doi.org/10.3168/jds.S0022-0302(99)75474-3.

Drillich, M., D. Voigt, D. Forderung, and W. Heuwieser. 2007. Treatment of acute puerperal metritis with flunixin meglumine in addition to antibiotic treatment. J. Dairy Sci. 90:3758–3763. https://doi.org/10.3168/jds.2007-0052.

Duchateau, F. L., P. Boutet, P. Lekeux, P. Rainard, M. J. Paape, and C. Burvenich. 2005. Effect of carprofen treatment following experimentally induced Escherichia coli mastitis in primiparous cows. J. Dairy Sci. 88:2361–2376. https://doi.org/10.3168/jds.S0022-0302(05)72914-3.

Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. Vet. Clin. N. Am. Food Anim. Pract. 16:231–253. https://doi.org/10.1016/s0749-0720(15)30103-1.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571–580. https://doi.org/10.3168/jds.2008-1507.

Eckel, E. F. and B. N. Ametaj. 2016. Invited review: Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. J. Dairy Sci. 99:5967–5990. https://doi.org/https://doi.org/10.3168/jds.2015-10727

Eckersall P. D., F. J. Young, C. McComb, C. J. Hogarth, S. Safi, A. Weber, T. McDonald, A. M. Nolan and J. L. Fitzpatrick. 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet. Rec. 148:35–41. https://doi.org/10.1136/vr.148.2.35.

Eckersall, P. D., and R. Bell. 2010. Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Vet. J. 185:23–27. https://doi.org/10.1016/j.tvjl.2010.04.009.

Ehrhardt R. A., A. Foskolos, S. L. Giesy, S. R. Wesolowski, C. S. Krumm, W. R. Butler, S. M. Quirk, M. R. Waldron, and Y. R. Boisclair. 2016. Increased plasma leptin attenuates adaptive metabolism in early lactating dairy cows. J. Endocrinol. 229:145–157. https://doi.org/10.1530/JOE-16-0031.

El Ghmati, S. M., E. M. Van Hoeyveld, J. G. Van Strijp, J. L. Ceuppens, and E. A. Stevens. 1996. Identification of haptoglobin as an alternative ligand for CD11b/CD18. J. Immunol. 156:2542–2552.

Ellis, W. C., J. H. Matis, T. M. Hill, and M. R. Murphy. 1994. Methodology for estimating digestion and passage kinetics of forages. Pages 682–756 in Forage Quality, Evaluation,

and Utilization. American Society of Agronomy, Madison, WI, USA. https://doi.org/10.2134/1994.foragequality.c17.

Elsden, S. R., M. W. S. Hitchcock, R. A. Marshall, and A. T. Phillipson. 1946. Volatile acid in the digesta of ruminants and other animals. J. Exp. Biol. 22:191–202. https://doi.org/10.1242/jeb.22.3-4.191.

Emery, R. S., J. S. Liesman and T. H. Herdt. 1992. Metabolism of long chain fatty acids by ruminant liver. J. Nutr. 122:832–837. https://doi.org/10.1093/jn/122.suppl_3.832.

Emmanuel, D. G. V., S. M. Dunn, and B. N. Ametaj. 2008. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. J. Dairy Sci. 91:606–614. https://doi.org/10 .3168/jds.2007-0256.

Emmanuel, D. G., K. L. Madsen, T. A. Churchill, S. M. Dunn, and B. N. Ametaj. 2007. Acidosis and lipopolysaccharide from Escherichia coli B:055 cause hyperpermeability of rumen and colon tissues. J. Dairy Sci. 90:5552–5557. https://doi.org/10.3168/jds.2007-0257.

Engelking, L. E., D. J. Ambrose, and M. Oba. 2022. Effects of dietary butyrate supplementation and oral non-steroidal anti-inflammatory drug administration on serum inflammatory markers and productivity of dairy cows during the calving transition. J. Dairy Sci. 105:4144–4155. https://doi.org/10.3168/jds.2021-21553.

Erdman, R. A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71:3246–3266. https://doi.org/10.3168/jds.S0022-0302(88)79930-0.

Esposito, G., Irons, P.C., Webb, E.C. & Chapwanya, A., 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci.144: 60–71. https://doi.org/10.1016/j.anireprosci.2013.11.007.

Etherton, T. D.. and D. E. Bauman. 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiol. Rev. 78:745–761. https://doi.org/10.1152/physrev.1998.78.3.745.

Fagerhol M. K., and J. Rugtveit. 2022. Heterogeneity of fecal calprotectin reflecting generation of neutrophil extracellular traps (nets) in the gut: new immunoassays are available. J. Mol. Path. 3:38–51. https://doi.org/10.3390/jmp3010004.

Farney, J. K., L. K. Mamedova, J. F. Coetzee, B. KuKanich, L. M. Sordillo, S. K. Stoakes, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013b. Antiinflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 305:R110–R117. https://doi.org/10.1152/ajpregu.00152.2013.

Farney, J. K., L. K. Mamedova, J. F. Coetzee, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013a. Sodium salicylate treatment in early lactation increases whole-lactation milk and milk fat yield in mature dairy cows. J. Dairy Sci. 96:7709–7718. http://dx.doi.org/ 10.3168/jds.2013-7088.

Fassler J., and P. Cooper. 2011. BLAST Glossary. BLAST® Help. National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/books/NBK62051/

Faulkner, P. M., and D. M. Weary. 2000. Reducing pain after dehorning in dairy calves. J. Dairy Sci. 83:2037–2041. https://doi.org/10.3168/jds.S0022-0302(00)75084-3.

Fernando, S. C., H. T. Purvis, F. Z. Najar, L. O. Sukharnikov, C. R. Krehbiel, and T. G. Nagaraja. 2010. Rumen microbial population dynamics during adaptation to a high grain diet. Appl. Environ. Microbiol. 76:7482–7490. https://doi.org/10.1128/AEM.00388-10.

Ferraris R. P., and J. Diamond J. 1997. Regulation of intestinal sugar transport. Physiol. Rev. 77:257–302. https://doi.org/10.1152/physrev.1997.77.1.257.

Fleck, A., 1989. Clinical and nutritional aspects of changes in acute phase proteins during inflammation. Proc. Nutr. Soc. 48:347–354. https://doi.org/10.1079/pns19890050.

Flint H. J. and E. A. Bayer. 2008. Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. Annals of the New York Academy of Sciences 1125:280–288. https://doi.org/10.1196/annals.1419.022.

Forbes J. M., and F. D. Provenza. 2000. Integration of learning and metabolic signals into a theory of dietary choice and food intake. Pages 3–19 in Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. CAB. International, Wallingford, UK.

Forbes, J. M. 2003. The multifactorial nature of food intake control. J. An. Sci. 81:E139–E144. https://doi.org/10.2527/2003.8114_suppl_2E139x.

Forbes, J. M., and I. Kyriazakis. 1995. Food preferences in farm animals: Why don't they always choose wisely? Proc. Nutr. Soc. 54:429–440. https://doi.org/10.1079/PNS19950012.

Fruth B., N. B. Ikombe, G. K. Matshimba, S. Metzger, D. M. Muganza, R. Mundry, and A. Fowler. 2014. New evidence for self-medication inbonobos:Manniophyton fulvumleaf- and stemstrip-swallowing from LuiKotale, Salonga National Park, DR Congo. Am. J. Primatol. 76:146–158. https://doi.org/10.1002/ajp.22217.

Fukumori, R., M. Oba, K. Izumi, M. Otsuka, K. Suzuki, S. Gondaira, H. Higuchi, and S. Oikawa. 2020. Effects of butyrate supplementation on blood glucagon-like peptide-2 concentration and gastrointestinal functions of lactating dairy cows fed diets differing in starch content. J. Dairy Sci. 103:3656–3667. https://doi.org/10.3168/jds.2019-17677.

Fukunaga, S., K. Kuwaki, K. Mitsuyama, H. Takedatsu, S. Yoshioka, H. Yamasaki, R. Yamauchi, A. Mori, T. Kakuma, O. Tsuruta, and T. Torimura. 2018. Detection of calprotectin in inflammatory bowel disease: Fecal and serum levels and

immunohistochemical localization. Intl. J. Mol. Med. 41:107–118. https://doi.org/10.3892/ijmm.2017.3244.

Fullmer, C. S., and R. H. Wasserman. 1973. Bovine intestinal calcium-binding proteins and purification and some properties. Biochimica et Biophysica Acta Protein Structure. 317:172–186. https://doi.org/10.1016/0005-2795(73)90210-9

Gabary, C., and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. 340:448–454. https://doi.org/0.1056/NEJM199902113400607.

Gäbel G., M. Bestmann, H. Martens. 1991. Influences of diet, short-chain fatty acids, lactate and chloride on bicarbonate movement across the reticulo-rumen wall of sheep. Zentralbl Veterinarmed A. 38:523–529. https://doi.org/10.1111/j.1439-0442.1991.tb01043.x.

Gahremanpour, A., D. Vela, Y. Zheng, G. V. Silva, W. Fodor, C. O. Cardoso, F. Baimbridge, M. R. Fernandes, L. M. Buja, and E.C. Perin. 2013. Xenotransplantation of human unrestricted somatic stem cells in a pig model of acute myocardial infarction. Xenotransplantation 20:110–122. https://doi.org/10.1111/xen.12026.

Gallo, R. L., and L. V. Hooper. 2012. Epithelial antimicrobial defence of the skin and intestine. Nat. Rev. Immunol. 12:503–516.

Gao, X., and M. Oba. 2014. Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high grain diet. J. Dairy Sci. 97:3006–3016. https://doi.org/10.3168/jds.2013-7472.

García-Roche, M., G. Cañibe, A. Casal, D. A. Mattiauda, M. Ceriani, A. Jasinsky, A. Cassina, C. Quijano, and M. Carriquiry. 2021. Glucose and fatty acid metabolism of dairy cows in a

total mixed ration or pasture-based system during lactation. Front. Anim. Sci. 2:622500. https://doi.org/10.3389/fanim.2021.622500.

Gaytán, M., C. Morales, C. Bellido, J. E. Sanchez-Criado, and F. Gaytan. 2006. Non-steroidal anti-inflammatory drugs (NSAIDs) and ovulation: Lessons from morphology. Histol. Histopathol. 21:541–556. https://doi.org/10.14670/HH-21.541.

Geboes, K., K. Geboes, and A. Jouret-Mourin. 2013. Endoscopy and histopathology. Pages 3–32 in Endoscopy. https://doi.org/10.5772/52739.

Giannetto, C., F. Fazio, S. Casella, S. Marafioti, E. Giudice, G. Piccione. 2011. Acute phase protein response during road transportation and lairage at a slaughterhouse in feedlot beef cattle. J. Vet. Med. Sci. 73:1531–1534. https://doi.org/10.1292/jvms.11-0157.

Gilbert R. O., S. T. Shin, C. L. Guard, H. N. Erb, and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64:1879–1888. https://doi.org/10.1016/j.theriogenology.2005.04.022.

Gilbert, R. O., and N. R. Santos. 2016. Dynamics of postpartum endometrial cytology and bacteriology and their relationship to fertility in dairy cows. Theriogenology 85:1367–1374. http://dx.doi.org/10.1016/j.theriogenology.2015.10.045.

Ginane, C., M. Bonnet, M. R. Baumont, and D. K. Revell. 2015. Feeding behaviour in ruminants: a consequence of interactions between a reward system and the regulation of metabolic homeostasis. Anim. Prod. Sci. 55:247–260. https://doi.org/10.1071/AN14481.

Giri S. N., P. Emau, J. S. Cullor, G. H. Stabenfeldt, M. L. Bruss, R. H. Bondurant, and B. I. Osburn. 1990. Effects of endotoxin infusion on circulating levels of eicosanoids, progesterone, cortisol, glucose and lactic acid, and abortion in pregnant cows. Vet. Microbiol. 21:211–231. https://doi.org/10.1016/0378-1135(90)90033-r.

Goebeler, M., J. Roth, S. Teigelkamp, and C. Sorg. 1994. The monoclonal antibody MAC387 detects an epitope on the calcium-binding protein MRP14. J. Leuk. Biol. 55:259–261. https://doi.org/10.1002/jlb.55.2.259.

Goff, J. P., Horst, R. L. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260–1268. https://doi.org/10.3168/jds.S0022-0302(97)76055-7.

Goodlad R. A. 1981. Some effects of diet on the mitotic index and the cell cycle of the ruminal epithelium of sheep. Q. J. Exp. Physiol. 66:487–499. https://doi.org/10.1113/expphysiol.1981.sp002590.

Górka, P., Z. M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, J. J. Holst, R. Guilloteau, and R. Zabielski. 2011a. Effect of method of delivery of sodium butyrate on rumen development in newborn calves. J. Dairy Sci. 94:5578–5588. https://doi.org/10.3168/jds.2011-4166.

Górka, P., Z. M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, and R. Zabielski. 2011b. Is rumen development in newborn calves affected by different liquid feeds and small intestine development? J. Dairy Sci. 94:3002–3013. https://doi.org/10.3168/jds.2010-3499.

Górka, P., Z. M. Kowalski, R. Zabielski, and P. Guilloteau. 2018. Invited review: Use of butyrate to promote gastrointestinal tract development in calves. J. Dairy Sci. 101:4785–4800. https://doi.org/10.3168/jds.2017-14086.

Gott, P. N., J. S. Hogan, and W. P. Weiss. 2015. Effects of various starch feeding regimens on responses of dairy cows to intramammary lipopolysaccharide infusion. J. Dairy Sci. 98:1786–1796. https://doi.org/10.3168/jds.2014-8638.

Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2006. Rumen lipopolysaccharide and inflammation during grain adaptation and subacute ruminal acidosis in steers. J. Dairy Sci. 89:4404–4413. https://doi.org/10.3168/jds.S0022-0302(06)72487-0.

Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2007. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. J. Dairy Sci. 90:856–866. https://doi.org/10.3168/jds.S0022-0302(07)71569-2.

Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci. 88:1399–1403. https://doi.org/10.3168/jds.S0022-0302(05)72807-1.

Graham C., and N. L. Simmons. 2005. Functional organization of the bovine rumen epithelium. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288:R173–R18. https://doi.org/10.1152/ajpregu.00425.2004.

Graugnard, D. E., K. M. Moyes, E. Trevisi, M. J. Khan, D. Keisler, J. K. Drackley, G. Bertoni, and J. J. Loor. 2013. Liver lipid content and inflammometabolic indices in peripartal dairy cows are altered in response to prepartal energy intake and postpartal intramammary inflammatory challenge. J. Dairy Sci. 96:918–935. https//doi.org/10.3168/jds.2012-5676.

Graugnard, D. E., M. Bionaz, E. Trevisi, K. M. Moyes, J. L. Salak-Johnson, R. L. Wallace, J. K. Drackley, G. Bertoni, and J. J. Loor. 2012. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. J. Dairy Sci. 95:1749–1758. https://doi.org/10.3168/jds.2011-4579.

Green, K.J., and C. L. Simpson. 2007. Desmosomes: new perspectives on a classic. J. Invest. Dermatol. 127:2499–2515. https://doi.org/10.1038/sj.jid.5701015.

Green, M. P., A. M. Ledgard, S. E. Beaumont, M. C. Berg, K. P. McNatty, A. J. Peterson, and P. J. Back. 2011. Long-term alteration of follicular steroid concentrations in relation to subclinical endometritis in postpartum dairy cows. J. Anim. Sci. 89:3551–3560. https://doi.org/10.2527/jas.2011-3958.

Gregorini, P., J. J. Villalba, F. D. Provenza, P. C. Beukes, and J. M. Forbes. 2015. Modelling preference and diet selection patterns by grazing ruminants: a development in a mechanistic model of a grazing dairy cow, MINDY. Anim. Prod. Sci. 55: 360–375. https://doi.org/10.1071/AN14472.

Gressley, T. F., M. B. Hall, and L. E. Armentano. 2011. Ruminant nutrition symposium: Productivity, digestion, and health responses to hindgut acidosis in ruminants. J. Anim. Sci. 89:1120–1130. https://doi.org/10.2527/jas.2010-3460.

Greter, A. M., T. J. DeVries, and M. A. G. von Keyserlingk. 2008. Nutrient intake and feeding behavior of growing dairy heifers: Effects of dietary dilution. J. Dairy Sci. 91:2786–2795. https://doi.org/10.3168/jds.2008-1052.

Grilli, D. J., K. Fliegerova, J. Kopecný, S. P. Lama, V. Egea, N. Sohaefer, C. Pereyra, M. S. Ruiz, M. A. Sosa, G. N. Arenas, and J. Mrazek. 2016. Analysis of the rumen bacterial diversity of goats during shift from forage to concentrate diet. Anaerobe 42:17–26. http://doi.org/10.1016/j.anaerobe.2016.07.002.

Grönlund, U., C. H. Sandgren, and K. P. Waller. 2005. Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. Vet. Res. 36:191–198. https://doi.org/10.1051/vetres:2004063

Grönlund, U., C. Hultén, P. Eckersall, C. Hogarth, and K. Waller. 2003. Haptoglobin and serum amyloid A in milk and serum during acute and chronic experimentally induced Staphylococcus aureus mastitis. J. Dairy Res. 70:379–386. https://doi.org/10.1017/S0022029903006484.

Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850– 1864. https://doi.org/10.3168/jds.S0022-0302(96)76553-0.

Grummer R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J Dairy Sci. 76:3882–96. https://doi.org10.3168/jds.S0022-0302(93)77729-2.

Grummer, R. R. 2008. Nutritional and management strategies for the prevention of fatty liver in dairy cattle. Vet. J. 176:10–20. https://doi.org/10.1016/j.tvjl.2007.12.03.

Gruys, E., M. J. M. Toussaint, T. A. Niewold, S. J. Koopmans. 2005. Acute phase reaction and acute phase proteins. 6:1045–1056. https://doi.org/10.1631/jzus.2005.B1045.

Guilloteau, P., L. Martin, V. Eeckhaut, R. Ducatelle, R. Zabielski, and F. van Immerseel. 2010. From the gut to the peripheral tissues: The multiple effects of butyrate. Nutr. Res. Rev. 23:366–384. https://doi.org/10.1017/S0954422410000247.

Guilloteau, P., R. Zabielski, J. C. David, J. W. Blum, J. A. Morisset, M. Biernat, J. Woliński, D. Laubitz, and Y. Hamon. 2009. Sodium-butyrate as a growth promoter in milk replacer formula for young calves. J. Dairy Sci. 92:1038–1049. https://doi.org/10.3168/jds.2008-1213.

Guinguina, A., T. Yan, A. R. Bayat, P. Lund, and P. Huhtanen. 2020a. The effects of energy metabolism variables on feed efficiency in respiration chamber studies with lactating dairy cows. J. Dairy Sci. 103:7983–7997. https://doi.org/10.3168/jds.2020-18259.

Guinguina, T., P. Yan, P. Lund, A. R. Bayat, A. L. F. Hellwing, and P. Huhtanen. 2020b. Between-cow variation in the components of feed efficiency. J. Dairy Sci. 103:7968–7982. https://doi.org/10.3168/jds.2020-18257.

Guliński, P. 2021. Ketone bodies - causes and effects of their increased presence in cows' body fluids: A review. Vet World 14:1492–1503. https://doi.org/10.14202/vetworld.2021.1492-1503.

Guo J., G. Chang, K. Zhang, L. Xu, D. Jin, M. S. Bilal, and X. Shen. 2017. Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. Oncotarget 8:46769–46780. https://doi.org/10.18632/oncotarget.18151.

Habel, R. E. 1963. Carbohydrates, phosphatases and esterases in the mucosa of the ruminant forestomach during postnatal development. Amer. J. Vet. Res. 24:199–211.

Haisan, J., Y. Inabu, W. Shi, and M. Oba. 2021. Effects of pre- and postpartum dietary starch content on productivity, plasma energy metabolites, and serum inflammation indicators of dairy cows. J. Dairy Sci. 104:4362–4374. https://doi.org/10.3168/jds.2020-19611.

Hakansson, A., and G. Molin. 2011. Gut microbiota and inflammation. Nutrients 3:637–82. https://doi.org/10.3390/nu3060637.

Halfen, J., C. Faccio Demarco, J. P. Soares Falson, A. Amaral Barbosa, J. de Oliveira Feijó, V. Rohrig Babassa, E. Schmitt, M. Nunes Corrêa, C. Cassal Brauner, and F. A. Burkert Del Pino. 2021. Sodium butyrate supplementation and the effect on glucose levels and lipid metabolism of dairy cows. Arch. Zootec. 70:28–32. https://doi.org/10.21071/az.v70i269.5415.

Hall, D. M., G. R. Buettner, L.W. Oberley, L. Xu, R. D. Matthes, and C.V. Gisolfi. 2001. Mechanism of circulatory and intestinal barrier dysfunction during whole body

hyperthermia. Am. J. Physiol. Heart Circ. Physiol. 280:H509–H521. https://doi.org/10.1152/ajpheart.2001.280.2.H509.

Hall, M. B. 2009. Analysis of starch, including malt oligosaccharides in animal feeds: A comparison of methods and a recommended method for AOAC collaborative study. J. AOAC Int. 92:42–49. https://doi.org/10.1093/jaoac/92.1.42.

Hall, M. B. and M.L. Eastridge. 2014. Invited review: Carbohydrate and fat: Considerations for energy and more. Prof. Anim. Sci. 30:140–149. https://doi.org/10.15232/S1080-7446(15)30101-7.

Hall, M. B., and P. J. Van Soest. 2019. Liquid digesta markers: A method for synthesis of crystallized chromium-EDTA and comparison of its degree of complexation with an uncrystallized preparation. Anim. Feed Sci. Technol. 253:32–38. https://doi.org/10.1016/j.anifeedsci.2019.05.002.

Hamer, H. M., D. Jonkers, K. Venema, S. Vanhoutvin, F. J. Troost, and R. J. Brummer. 2008. Review article: The role of butyrate on colonic function. Aliment. Pharmacol. Ther. 27:104–119. https://doi.org/10.1111/j.1365-2036.2007.03562.x.

Hammon D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. Immunopathol. 113:21–29. https://doi.org/10.1016/j.vetimm.2006.03.022.

Han, I. K., and I. H. Kim. 2005. Risk factors for retained placenta and the effect of retained placenta on the occurrence of postpartum diseases and subsequent reproductive performance in dairy cows. J. Vet. Sci. 6:53–59.

Hansen, P. J. 2013. Physiology and endocrinology symposium: Maternal immunological adjustments to pregnancy and parturition in ruminants and possible implications for

postpartum uterine health: Is there a prepartum–postpartum nexus? J. Anim. Sci. 91:1639–1649. https://doi.org/10.2527/jas.2012-5934.

Harmon, D. L. 1992. Impact of nutrition on pancreatic exocrine and endocrine secretion in ruminants: A review. J. Anim. Sci. 70:1290–1301. https://doi.org/10.2527/1992.7041290x.

Hartnell, G. F., and L. H. Satter. 1979. Determination of rumen fill, retention time and ruminal turnover rates of ingesta at different stages of lactation in dairy cows. J. Anim. Sci. 48:381–392. https://doi.org/10.2527/jas1979.482381x.

Havekes, C.D., T. F. Duffield, A. J. Carpenter, T. J. DeVries. 2020. Effects of wheat straw chop length in high-straw dry cow diets on intake, health, and performance of dairy cows across the transition period. J. Dairy Sci. 103:254-271. https://doi.org/10.3168/jds.2019-17033.

Hayirli, A., D. H. Keisler, L. Doepel, and H. Petit. 2011. Peripartum responses of dairy cows to prepartal feeding level and dietary fatty acid source. J. Dairy Sci. 94:917–930. https://doi.org/10.3168/jds.2010-3674

Hayirli, A., and R. R. Grummer. 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. Can. J. Anim. Sci. 84:337–347. https://doi.org/10.4141/A03-122.

Heilmann, R. M., J. S. Suchodolski, and J. M. Steiner. 2008. Purification and partial characterization of canine calprotectin. Biochimie. 90:306–1315. https://doi.org/10.1016/j.biochi.2008.03.008.

Hendel S. K., L. Kellermann, A. Hausmann, N. Bindslev, K. B. Jensen, and O. H. Nielsen. 2022. Tuft cells and their role in intestinal diseases. Front. Immunol. 13. https://doi.org/10.3389/fimmu.2022.822867.

Herr, M., H. Bostedt, and K. Failing. 2011 IgG and IgM levels in dairy cows during the periparturient period. Theriogenology. 75:377–385. https://doi.org/10.1016/j.theriogenology.2010.09.009.

Herrick, K. H., A. R. Hippen, K. F. Kalscheur, D. J. Schingoethe, D. P. Casper, S. C. Moreland, and J. E. van Eys. 2017. Single dose infusion of sodium butyrate, but not lactose, increases plasma β-hydroxybutyrate and insulin in lactating dairy cows. J. Dairy Sci. 100:757–768. https://doi.org/10.3168/jds.2016-11634

Herrick, K. J., A. R. Hippen, K. F. Kalscheur, D. J. Schingoethe, S. D. Ranathunga, J. L. Anderson, S. C. Moreland, and J. E. van Eys. 2018. Infusion of butyrate affects plasma glucose, butyrate, and beta-hydroxybutyrate but not plasma insulin in lactating dairy cows. J. Dairy Sci. 101:3524–3536. https://doi.org/10.3168/jds.2017-13842.

Hillman, R., and R. O. Gilber. 2008. Reproductive Diseases in Rebhun's Diseases of Dairy Cattle (Second Edition).

Hoeben D., R. Heyneman, and C. Burvenich. 1997. Elevated levels of β-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. Vet. Immunol. Immunopathol. 58:165–170. https://doi.org/10.1016/s0165-2427(97)00031-7.

Hogeveen, H., K. Huijps, and T. J. G. M. Lam. 2011. Economic aspects of mastitis: New developments. N. Z. Vet. J. 59:16–23. https://doi.org/10.1080/00480169.2011.547165.

Holschbach, C. L., and S. F. Peek. 2018. Salmonella in dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 34:133–154. https://doi.org/10.1016/j.cvfa.2017.10.005.

Holtenius, K., S. Agenäs, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J. Dairy Sci. 86:883–891. https://doi.org/ 10.3168/jds.S0022-0302(03)73671-6.

Holtenius, P., and K. Holtenius. 1996. New aspects of ketone bodies in energy metabolism of dairy cows: A review. J. Vet. Med. A Physiol. Pathol. Clin. Med. 43:579–587. https://doi.org/10.1111/j.1439-0442.1996.tb00491.x

Hooper, A. P., and J. G. Welch. 1985. Effects of particle size and forage composition on functional specific gravity. J. Dairy Sci. 68:1181–1188. https://doi.org/10.3168/jds.S0022-0302(85)80945-0.

Horst, E. A., E. J. Mayorga, M. Al-Qaisi, S. Rodriguez-Jimenez, B. M. Goetz, M. A. Abeyta, P. J. Gorden, S. K. Kvidera, and L. H. Baumgard. 2020. Evaluating effects of zinc hydroxychloride on biomarkers of inflammation and intestinal integrity during feed restriction. J. Dairy Sci. 103:11911–11929. https://doi.org/10.3168/jds.2020-18860.

Horst, E. A., S. K. Kvidera, and L. H. Baumgard. 2021. Invited review: The influence of immune activation on transition cow health and performance—A critical evaluation of traditional dogmas. J. Dairy Sci. 104:8380–8410. https://doi.org/10.3168/jds.2021-20330.

Horst, E. A., S. K. Kvidera, M. Al-Qaisi, M. A. Abeyta, R. P. Rhoads, and L. H. Baumgard. 2018. Energy cost of inflammation in dairy cows. Pages 65–80 in Tri State Dairy Nutr. Conf., Fort Wayne, Indiana, USA.

Horta, A. E. M. 1984. Efeitos das prostaglandinas E2 e F2 alpha sobre a retencao placentaria induzida pelo acetilsalicilato de lisina em vacas leiteiras; estudo da motilidade uterina. Zootechnia 33:39–47.

Hoyumpa A. M., H. L. Greene, G. D. Dunn, and S. Schenker. 1975. Fatty liver: biochemical and clinical considerations. Am. J. Dig. Dis. 20:1142–70. https://doi.org/10.1007/BF01070758.

Hu, X., S. Li, R. Mu, J. Guo, C. Zhao, Y. Cao, N. Zhang, and Y. Fu. 2022. The rumen microbiota contributes to the development of mastitis in dairy cows. Microbiol. Spectr. 10:e0251221. https://doi.org/10.1128/spectrum.02512-21.

Huhtanen, P., H. Miettinen, and M. Ylinen. 1993. Effect of increasing ruminal butyrate on milk yield and blood constituents in dairy cows fed a grass silage-based diet. J. Dairy Sci. 76:1114–1124. https://doi.org/10.3168/jds.S0022-0302(93)77440-8.

Huhtanen, P., S. Ahvenjärvi, M. R. Weisbjerg, and P. Nørgaard. 2006. Digestion and passage of fibre in ruminants. Pages 87–135 in Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition in Gene Impression, Immunology and Stress. Wageningen Academic Publishers, Wageningen, the Netherlands.

Humblet M. F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J. M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clin. Pathol. 35:188–193. https://doi.org/10.1111/j.1939-165x.2006.tb00112.x.

Hume, I. D. 1997. Fermentation in the hindgut of mammals. Pages 84–115 in Gastrointestinal Microbiology. Gastrointestinal Ecosystems and Fermentations, New York, NY, USA.

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84 Suppl:E14–24. https://doi.org/10.2527/2006.8413_supplE14x.

Hurley, J. C. 1995. Endotoxemia: Methods of detection and clinical correlates. Clin. Microbiol. Rev. 8:268–292. https://doi.org/10.1128/CMR.8.2.268.

Husebye, E., H. Ton, and B. Johne. 2001. Biological variability of fecal calprotectin in patients referred for colonoscopy without colonic inflammation or neoplasm. Am. J. Gastroenterol. 96: 2683–2687. https://doi.org/10.1111/j.1572-0241.2001.04126.x.

Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2011. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. J. Dairy Sci. 94:5878–5889. https://doi.org/10.3168/jds.2010-3391.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92:621–625. https://doi.org/10.3168/jds.2008-152.

Hýzd'alová, M., J. Hofmanová, J. Pacherník, A. Vaculová, and A. Kozubík. 2008. The interaction of butyrate with TNF-alpha during differentiation and apoptosis of colon epithelial cells: Role of NFkappaB activation. Cytokine 44:33–43. https://doi.org/10.1016/j.cyto.2008.06.003.

Ilievska, K., B. Atanasov, T. Dovenski, O. Smolac, B. Stojanov, and P. Trojachanec. 2019. Acute phase proteins – as indicators of claw diseases in dairy cattle. *Macedonian Vet. Rev.* 42:95–100. https://doi.org/10.2478/macvetrev-2019-0011.

Immig, I. 1996. The rumen and hindgut as source of ruminant methanogenesis. Environ. Monit. Assess. 42:57–72. https://doi.org/10.1007/BF00394042.

Itle, A., J. Huzzey, D. Weary, and M. Von Keyserlingk. 2015. Clinical ketosis and standing behavior in transition cows. J. Dairy Sci. 98:128–134. https://doi.org/10.3168/jds.2014-7932.

Iwasaki, A. and R. Medzhitov. 2004. Toll-like receptor control of the adaptive immune responses. Nature Immunology 5:987–995. https://doi.org/10.1038/ni1112.

Izumi, K., R. Fukumori, S. Oikawa, and M. Oba. 2019. Short communication: Effects of butyrate supplementation on productivity of lactating dairy cows fed diets differing in starch content. J. Dairy Sci. 102:11051–11056. https://doi.org/10.3168/jds.2019-17113.

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows J. Dairy Sci. 94:1385–1400. https://doi.org/10.3168/jds.2010-3303.

Jeremejeva, J., T. Orro, A. Waldmann, and K. Kask. 2012. Treatment of dairy cows with PGF2α or NSAID, in combination with antibiotics, in cases of postpartum uterine inflammation. Acta. Vet. Scand. 54:45. https://doi.org/10.1186/1751-0147-54-45.

Johansson, M. E. V., M. Phillipson, J. Petersson, L. Holm, A. Velcich, and G. C. Hansson. 2008. The inner of the two Muc2 mucin dependent mucus layers in colon is devoid of bacteria. Proc. Natl. Acad. Sci. 105:15064–15069. https://doi.org/10.1073/pnas.0803124105.

Jorritsma R., H. Jorritsma, Y. H. Schukken, P. C. Bartlett, T. Wensing, and G. H. Wentink. 2001. Prevalence and indicators of post partum fatty infiltration of the liver in nine commercial

dairy herds in The Netherlands. Livest. Prod. Sci. 68:53–60. https://doi.org/10.1016/S0301-6226(00)00208-6.

Kabara, J. J., D. M. Swieczkowski, A. J. Conley, and J. P. Truant. 1972. Fatty acids and derivatives as antimicrobial agents. Antimicrob. Agents Chemother.2:23–28. https://doi.org/10.1128/aac.2.1.23.

Kammes, K. L., and M. S. Allen. 2012. Nutrient demand interacts with grass particle length to affect digestion responses and chewing activity in dairy cows. J. Dairy Sci. 95:807–823. https://doi.org/10.3168/jds.2011-4588.

Karademir, U., H. Erdogan, M. Boyacioglu, C. Kum, S. Sekkin, and M. Bilgen. 2016. Pharmacokinetics of meloxicam in adult goats: a comparative study of subcutaneous, oral and intravenous administration. N. Z. Vet. J. 64:165–168. https://doi.org/10.1080/00480169.2015.1124811.

Karl, J., N. Wild, M. Tacke, H. Andres, U. Garczarek, W. Rollinger, and W. Zolg. 2008. Improved diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers. Clin. Gastroenterol. Hepatol. 6:1122–1128. https://doi.org/ 10.1016/j.cgh.2008.04.021.

Kasimanickam, R., T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, and W. H. Johnson. 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62:9–23. https://doi.org/10.1016/j.theriogenology.2003.03.001.

Katoh, N., and H. Nakagawa. 1999. Detection of haptoglobin in the high-density lipoprotein and the very high-density lipoprotein fractions from sera of calves with experimental

pneumonia and cows with naturally occurring fatty liver. J. Vet. Med. Sci. 61:119–124. https://doi.org/10.1292/ jvms.61.119.

Kaufmann, T. B., M. Drillich, B. A. Tenhagen, D. Forderung, and W. Heuwieser. 2009. Prevalence of bovine subclinical endometritis 4 h after insemination and its effects on first service conception rate. Theriogenology 71:385–391. https://doi.org/10.1016/j.theriogenology.2008.08.005.

Kauppinen, K. 1983. Correlation of whole blood concentrations of acetoacetate, B-hydroxybutyrate, glucose and milk yield. Acta Vet. Scand. 24:337–348. https://doi.org/10.1186/BF03546708.

Kaya, S., O. Merhan, C. Kacar, A. Colak and K. Bozukluhan. 2016. Determination of ceruloplasmin, some other acute phase proteins, and biochemical parameters in cows with endometritis. Vet. World 9:1056–1062. https:/doi.org/10.14202/vetworld.2016.1056-1062.

Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. J. Dairy Sci. 81:2502–2509. https://doi.org/10.3168/jds.S0022-0302(98)70142-0.

Kerslake, J. I., P. R. Amer, P. L. O'Neill, S. L. Wong, J. R. Roche, and C. V. C. Phyn. 2018. Economic costs of recorded reasons for cow mortality and culling in a pasture-based dairy industry. J. Dairy Sci. 101:1795–1803. https://doi.org/10.3168/jds.2017-13124.

Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. J. Dairy Sci. 85:3304–3313. https://doi.org/10.3168/jds.S0022-0302(02)74419-6.

Khalil, A., A. Batool, and S. Arif. 2022. Healthy cattle microbiome and dysbiosis in diseased phenotypes. Ruminants 2:134–156. https://doi.org/10.3390/ruminants2010009.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009a. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060–1070. https://doi.org/10.3168/jds.2008-1389.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009b. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. J. Dairy Sci. 92:1712–1724. https://doi.org/10.3168/jds.2008-1656.

Khan, M. Z., and A. Khan. 2006. Basic facts of mastitis in dairy animals: A review. Pakistan Vet. J. 26:204.

Kim, Y. S., S. B. and Ho. 2010. Intestinal goblet cells and mucins in health and disease: Recent insights and progress. Curr. Gastroenterol. Rep. 12:319–330. https://doi.org/10.1007/s11894-010-0131-2.

Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): a review. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 50:406–414. https://doi.org/10.1046/j.1439-0442.2003.00569.x.

Kleppe, B. B., R. J. Aiello, R. R. Grummer, and L. E. Armentano. 1988. Triglyceride accumulation and very low-density lipoprotein secretion by rat and goat hepatocytes in vitro. J. Dairy Sci. 71:1813–1822. https://doi.org/10.3168/jds.S0022-0302(88)79750-7.

Kluciński, W., S. P. Targowski, E. Miernik-Degorska, and A. Winnicka. 1990. The phagocytic activity of polymorphonuclear leukocytes isolated from normal uterus and that with experimentally induced inflammation in cows. J. Vet. Med. A Physiol. Pathol. Clin. Med. 37:506–512. https://doi.org/10.1111/j.1439-0442.1990.tb00937.x.

Kmicikewycz, A. D., and A. J. Heinrichs. 2014. Feeding lactating dairy cattle long hay separate from the total mixed ration can maintain dry matter intake during incidents of low rumen pH. J. Dairy Sci. 97:7175–7184. https://doi.org/10.3168/jds.2014-8412.

Knop, R., and H. Cernescu. 2009. Effects of negative energy balance on reproduction in 4 dairy cows. Lucrări Stiinłifice Medicină Veterinară 42:198–205.

Koch, F., U. Thom, E. Albrecht, R. Weikard, W. Nolte, B. Kuhla, and C. Kuehn. 2019. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. Proc. Natl. Acad. Sci. 116:10333–10338. https://doi.org/10.1073/pnas.1820130116.

Königsson, K., H. Gustafsson, A. Gunnarsson, and H. Kindahl. 2001. Clinical and bacteriological aspects on the use of oxytetracycline and flunixin in primiparous cows with induced retained placenta and post-partal endometritis. Reprod. Domest. Anim. 36:247–256. https://doi.org/10.1046/j.1439-0531.2001.00289.x.

Konikoff, M. R. and L. A. Denson. 2006. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. Inflamm. Bowel Dis. 12:524–534. https://doi.org/10.1097/00054725-200606000-00013.

Kononoff, P. J., A. J. Heinrichs, and D. A. Buckmaster. 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. J. Dairy Sci. 86:1858–1863. https://doi.org/10.3168/jds.S0022-0302(03)73773-4.

Kowalski, Z. M., P. Górka, J. Flaga, A. Barteczko, K. Burakowska, J. Oprzadek, and R. Zabielski. 2015. Effect of microencapsulated sodium butyrate in the close-up diet on

performance of dairy cows in the early lactation period. J. Dairy Sci. 8:3284–3291. https://doi.org/10.3168/jds.2014-8688

Krause, K. M., and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Technol. 126:215–236. https://doi.org/10.1016/j.anifeedsci.2005.08.004

Krehbiel, C. R., D. L. Harmon, and J. E. Schneider. 1992. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. J. Anim. Sci. 70:904–914. https://doi.org/10.2527/1992.703904x.

Krizsan, S. J., S. Ahvenjärvi, and P. Huhtanen. 2010. A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. J. Dairy Sci. 93:5890–901. https://doi.org/10.3168/jds.2010-3457.

Krnjaić, S., M. Cincović, R. Djoković, B. Belić, J. Ježek, and J. Starič. 2022. The influence of energy balance, lipolysis and ketogenesis on metabolic adaptation in cows milked twice and three times daily. Metabolites 12:1090. https://doi.org/10.3390/metabo12111090.

Krogstad, K. C. and B. J. Bradford. 2023. Does feeding starch contribute to the risk of systemic inflammation in dairy cattle? JDS Communications 4:14–18. https://doi.org/10.3168/jdsc.2022-0303.

Kuhla, B. 2020. Review: Pro-inflammatory cytokines and hypothalamic inflammation: Implications for insufficient feed intake of transition dairy cows. Animal 14:S65–S77. https://doi.org/10.1017/S1751731119003124.

Kunz, P. L., J. W. Blum, I. C. Hart, J. Bickel, and J. Landis. 1985. Effects of different energy intakes before and after calving on food intake, performance and blood hormones and

metabolites in dairy cows. Anim. Prod. 40:219–231. https://doi.org/10.3929/ethz-b-000422479

Kunzelmann K., and M. Mall. 2002. Electrolyte transport in the mammalian colon: mechanisms and implications for disease. Physiol. Rev. 82:245–289. https://doi.org/ 10.1152/physrev.00026.2001.

Kushibiki, S., K. Hodate, H. Shingu, Y. Obara, E. Touno, M. Shinoda, and Y. Yokomizo. 2003. Metabolic and lactational responses during recombinant bovine tumor necrosis factor-α treatment in lactating cows. J. Dairy Sci. 86:819–827. https://doi.org/10.3168/jds.S0022-0302(03)73664-9.

Kvidera, S. K. E. A. Horst, M. V. Sanz Fernandez, M. Abuajamieh, S. Ganesan, P. J. Gorden, H. B. Green, K. M. Schoenberg, W. E. Trout, A. F. Keating, L. H. Baumgard. 2017b. Characterizing effects of feed restriction and glucagon-like peptide 2 administration on biomarkers of inflammation and intestinal morphology. J. Dairy Sci. 100:9402–9417. https://doi.org/10.3168/jds.2017-13229.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2017c. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci. 100:2360–2374. https://doi.org/10.3168/jds.2016-12001.

Kvidera, S. K., M. J. Dickson, M. Abuajamieh, D. B. Snider, M. V. Sanz Fernandez, J. S. Johnson, A. F. Keating, P. J. Gorden, H. B. Green, K. M. Schoenberg, and L. H. Baumgard. 2017a. Intentionally induced intestinal barrier dysfunction causes inflammation, affects metabolism, and reduces productivity in lactating Holstein cows. J. Dairy Sci.100:4113–4127. https://doi.org/10.3168/jds.2016-12349.

Kyriazakis, I., B. Tolkamp, and G. Emmans. 1999. Diet selection and animal state: An integrative framework. Proc. Nutr. Soc. 58:765-772. https://doi.org/10.1017/S0029665199001044

Lager, K. and E. Jordan. 2012. The metabolic profile for the modern transition dairy cow. Pages 9–16 in Mid-South Ruminant Nutrition Conf., Grapevine, TX, USA.

Launchbaugh, K. L., and L. D. Howery. 2005. Understanding landscape use patterns of livestock as a consequence of foraging behavior. Rangel. Ecol. Manag. 58:99–108. https://doi.org/10.2111/03-146.1

Laven, R., P. Chambers, and K. Stafford. 2012. Using non-steroidal antiinflammatory drugs around calving: Maximizing comfort, productivity and fertility. Vet. J. 192:8–12. https://doi.org/10.1016/j.tvjl.2011.10.023.

Laven, R.A., A. R. Peters. 1996. Bovine retained placenta: aetiology, pathogenesis and economic loss. Vet. Rec. 139:465–471. https://doi.org/10.1136/vr.139.19.465.

Lavker R. M. 1969a. Fine structure of mucus granules in rumen epithelium. J. Cell Biol. 41:657–60. https://doi.org/10.1083/jcb.41.2.657.

Lavker, R. M., and A. G. Matoltsy. 1970. The fate of cell organelles and differentiation products in ruminal epithelium. J. Cell Biol. 44:501–512. https://doi.org/10.1083/jcb.44.3.501.

Lavker, R., W. Chalupa, and P. Opliger. 1969b. Histochemical observations on rumen mucosa structure and composition. J. Dairy Sci. 52:266–269. https://doi.org/10.3168/jds.S0022-302(69)86544-6.

Lean, I. J. and H. M. Golder. 2018. Ruminal acidosis–much more than pH. Pages 123–142 in Florida Rum. Nutr. 29th Symposium, University of Florida, Gainesville, FL, USA.

Lean, I., and H. Golder. 2019. Ruminal acidosis: Beyond pH and rumen. Pages 127–149 in Proc. Cornell Nutr. Conf. Feed Manuf., East Syracuse, NY, USA. Cornell Univ., Ithaca, NY, USA.

LeBlanc, S. J. 2006. Monitoring programs for transition dairy cows. Pages 460–472 in Proceedings of the 26th World Buiatrics Congress, Nice, France.

LeBlanc, S. J. 2012. Interactions of metabolism, inflammation, and reproductive tract health in the postpartum period in dairy cattle. Reprod. Domest. Anim. 47:18–30. https://doi.org/10.1111/j.1439-0531.2012.02109.x.

Lechartier, C. and J. L. Peyraud. 2010. The effects of forage proportion and rapidly degradable dry matter from concentrate on ruminal digestion in dairy cows fed corn silage–based diets with fixed neutral detergent fiber and starch contents. J. Dairy Sci. 93:666–681. https://doi.org/10.3168/jds.2009-2349.

Lees, P., M. F. Landoni, J. Giraudel, and P. L. Toutain. 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest J. J. Vet. Pharmacol. Ther. 27:479–490. https://doi.org/10.1111/j.1365-2885.2004.00617.x.

Legen, I. and A. Kristl. 2002. Ketoprofen-induced intestinal permeability changes studied in side-by-side diffusion cells. J. Pharm. Pharmacol. 54:1419–1422. https://doi.org/10.1211/002235702760345527.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557–564. https://doi.org/ 10.3168/jds.S0022-0302(03)73634-0

Lescourret, F., and J. B. Coulon. 1994. Modeling the impact of mastitis on milk production by dairy cows. J. Dairy Sci. 77:2289–2301.https://doi.org/10.3168/jds.S0022-0302(94)77172-1.

Lewis, K., F. Lutgendorff, V. Phan, J. D. Soderholm, P. M. Sherman, and D. M. McKay. 2010. Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. Inflamm. Bowel Dis. 16:1138–1148. https://doi.org/10.1002/ibd.21177

Li, F., J. Ma, S. Geng, J. Wang, J. Liu, J. Zhang, and X. Sheng. 2015. Fecal calprotectin concentrations in healthy children aged 1-18 months. PLoS One 10:e0119574. https://doi.org/10.1371/journal.pone.0119574

Li, R. W., S. Wu, R. L. Baldwin 6th, W. Li, and C. Li. 2012b. Perturbation dynamics of the rumen microbiota in response to exogenous butyrate. PLoS One 7:e29392. https://doi.org/10.1371/journal.pone.0029392.

Li, S., E. Khafipour, D. O. Krause, A. Kroeker, J. C. Rodriguez-Lecompte, G. N. Gozho, and J. C. Plaizier. 2012a. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. J. Dairy Sci. 95:294–303. https://doi.org/10.3168/jds.2011-4447

Liu, J. H., T. T. Xu, Y. J. Liu, W. Y. Zhu, and S. Y. Mao. 2013. A high-grain diet causes massive disruption of ruminal epithelial tight junctions in goats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 305:R232–R241. https://doi.org/10.1152/ajpregu.00068.2013.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262.

Lomax, M. A., and D. G. Baird. 1983. Blood flow and nutrient exchange across the liver and gut of the dairy cow. Effects of lactation and fasting. Br. J. Nutr. 49:481–496. https://doi.org/10.1079/bjn19830057.

Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. Guretzky, S. L. Rodriguez-Zas, H.A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23:217–226. https://doi.org/10.1152/physiolgenomics.00132.2005.

Lü, W. D., M. Zhang, Z. S. Wu, T. H. Hu, Z. J. Xu, W. Liu, and Y. R. Hu. 2010. The performance of photooxidatively crosslinked acellular bovine jugular vein conduits in the reconstruction of connections between pulmonary arteries and right ventricles. Biomaterials 31:2934–2943. https://doi.org/10.1016/j.biomaterials.2009.12.046.

Lucy, M. C., G. A. Verkerk, B. E. Whyte, K. A. Macdonald, L. Burton, R. T. Cursons, J. R. Roche, and C. W. Holmes. 2009. Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in a pasture system. J. Dairy Sci. 92:526–539. https://doi.org/10.3168/jds.2008-1421.

Ma, L., M. Zhao, L. S. Zhao, J. C. Xu, J. J. Loor, and D. P. Bu. 2017. Effects of dietary neutral detergent fiber and starch ratio on rumen epithelial cell morphological structure and gene expression in dairy cows. J. Dairy Sci. 100:3705–3712. https://doi.org/10.3168/jds.2016-11772.

Machado, V. S., and T. H. Silva. 2002. Adaptive immunity in the postpartum uterus: potential use of vaccines to control metritis. Theriogenology 150:201-209. https://doi.org/10.1016/j.theriogenology.2020.01.040.

Macpherson A. J., M. B. Geuking, E. Slack, S. Hapfelmeier, and K. D. McCoy. 2012. The habitat, double life, citizenship, and forgetfulness of IgA. Immunol. Rev. 245:132–146. https://doi.org/10.1111/j.1600-065X.2011.01072.x.

Macpherson, A. J., B. Yilmaz, J. P. Limenitakis, and S. C. Ganal-Vonarburg. 2018. IgA function in relation to the intestinal microbiota. Annu. Rev. Immunol. 36:359–381. https://doi.org/10.1146/annurev-immunol042617-053238.

Macpherson, A. J., K. D. McCoy, F. E. Johansen, and P. Brandtzaeg. 2008. The immune geography of IgA induction and function. Mucosal Immunol. 1:11–22. https://doi.org/10.1038/mi.2007.6.

Mainau, E., A. Cuevas, J. L. Ruiz-de-la-Torre, E. Abbeloos, and X. Manteca. 2014. Effect of meloxicam administration after calving on milk production, acute phase proteins, and behavior in dairy cows. J. Vet. Behav. 9:357–363. https://doi.org/10.1016/j.jveb.2014.07.007.

Mallikarjunappa, S., L. F. Brito, S. D. Pant, F. S. Schenkel, K. G. Meade, and N. A. Karrow. 2021. Johne's Disease in dairy cattle: An immunogenetic perspective. Front. Vet. Sci. 8:718987. https://doi.org/10.3389/fvets.2021.718987.

Malmuthuge N., M. Li, L. A. Goonewardene, M. Oba, and L. L. Guan. 2013. Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. J. Dairy Sci. 96:3189–200. http://doi.org/10.3168/jds.2012-6200.

Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. 2012. Growth and Development Symposium: Endotoxin, inflammation, and intestinal function in livestock. J. Anim. Sci. 90:1452–65. https://doi.org/10.2527/jas.2011-4627.

Mann, S. O., and E. R. Ørskov. 1973. The effect of rumen and post-rumen feeding of carbohydrates on the caecal microflora of sheep. J. Appl. Bacteriol. 36:475–484. https://doi.org/10.1111/j.1365-2672.1973.tb04130.x.

Mann, S., F. A. Leal Yepes, T. R. Overton, J. J. Wakshlag, A. L. Lock, C. M. Ryan, and D. V. Nydam. 2015. Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. J. Dairy Sci. 98:3366–3382. https://doi.org/10.3168/jds.2014-9024.

Manteca, X., J. J. Villalba, S. B. Atwood, L. Dziba, and F. D. Provenza. 2008. Is dietary choice important to animal welfare?. J. Vet. Behav. 3:229–239. https://doi.org/10.1016/j.jveb.2008.05.005.

Mao, S., M. Zhang, J. Liu, and W. Zhu. 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. Sci. Rep. 5:16116 https://doi.org/10.1038/srep16116.

Marques, R. S., R. F. Cooke, C. L. Francisco, and D. W. Bohnert. 2012. Effects of twenty-four hour transport or twenty-four hour feed and water deprivation on physiologic and performance responses of feeder cattle. J. Anim. Sci. 90:5040–5046. https://doi.org/10.2527/jas.2012-5425.

Martin, P. H. W. Barkema, L. F. Brito, S. G. Narayana, and F. Miglior. 2018. Symposium review: Novel strategies to genetically improve mastitis resistance in dairy cattle. J. Dairy Sci. 101:2724–2736. https://doi.org/10.3168/jds.2017-13554.

Martins, L. F., D. E. Wasson, and A. N. Hristov. 2022. Feeding dairy cows for improved metabolism and health. Anim. Front. 12:29–36. https://doi.org/10.1093/af/vfac059.

Maulfair, D. D., K. K. McIntyre, and A. J. Heinrichs. 2013. Subacute rumen acidosis and total mixed ration preference in lactating dairy cows. J. Dairy Sci. 96:6610–6620. https://doi.org/ https://doi.org/10.3168/jds.2013-6771.

McCarthy, M. M., S. Piepenbrink, and T. R. Overton. 2015a. Associations between hepatic metabolism of propionate and palmitate in liver slices from transition dairy cows. J. Dairy Sci. 98:7015–7024. https://doi.org/10.3168/jds.2015-9695.

McCarthy, M. M., T. Yasui, C. M. Ryan, G.D. Mechor, and T. R. Overton. 2015b. Performance of early-lactation dairy cows as affected by dietary starch and monensin supplementation. J. Dairy Sci. 98:3335-3350. https://doi.org/10.3168/jds.2014-8820.

McCarthy, M. M., T. Yasui, C. M. Ryan, S. H. Pelton, G. D. Mechor, and T. R. Overton. 2015c. Metabolism of early-lactation dairy cows as affected by dietary starch and monensin supplementation. J. Dairy Sci. 98:3351-3365. https://doi.org/10.3168/jds.2014-8821.

McCarthy, R. D., T. H. Klusmeyer, J. L. Vicini, J. H. Clark, and D. R. Nelson. 1989. Effect of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72: 2002–2016. https://doi.org/10.3168/jds.S0022-0302(89)79324-3.

McDougall, S., H. Hussein, D. Aberdein, K. Buckle, S. Morgan, J. Roche, C. Burke, M. Mitchell, S. Meier, and C. Compton. 2009. Diagnosis and consequences of endometritis. Pages 231–241 in Proc. Soc. Dairy Cattle Vet. N. Z. Vet. Assoc., Rotorua, New Zealand.

McNeil, N. I. 1988. Nutritional implications of human and mammalian large intestinal function. World Rev. Nutr. Diet. 56:1–42. https://doi.org/10.1159/000416223.

Medzhitov, R., 2008. Origin and physiological roles of inflammation. Nature 454:428–435. https://doi.org/10.1038/nature07201.

Meier, H. E. M., H. C. Andersson, B. Arheimer, C. Donnelly, K. Eilola, B. G. Gustafsson, L. Kotwicki, T. S. Neset, S. Niiranen, J. Piwowarczyk, O. P. Savchuk, F. Schenk, J. M. Węsławski, and E. Zorita. 2014a. Ensemble modeling of the Baltic Sea ecosystem to provide scenarios for management. AMBIO 43:37–48. https://doi.org/10.1007/s13280-013-0475-6.

Meier, S., N. V. Priest, C. R. Burke, J. K. Kay, S. McDougall, M. D. Mitchell, C. G. Walker, A. Heiser, J. J. Loor, and J. R. Roche. 2014b. Treatment with a nonsteroidal antiinflammatory drug after calving did not improve milk production, health, or reproduction parameters in pasture-grazed dairy cows. J. Dairy Sci. 97:2932– 2943. https://doi.org/10.3168/jds.2013-7838.

Meissner, S., F. Hagen, C. Deiner, D. Gunzel, G. Greco, Z. M. Shen, and J. R. Aschenbach. 2017. Key role of short-chain fatty acids in epithelial barrier failure during ruminal acidosis. J. Dairy Sci. 100:6662–6675. https://doi.org/10.3168/jds.2016-12262.

Melo, L. Q., S. F. Costa, F. Lopes, M. C. Guerreiro, L. E. Armentano, and M. N. Pereira. 2013. Rumen morphometrics and the effect of digesta pH and volume on volatile fatty acid absorption J. Anim. Sci. 91:1775–1783. http://dx.doi.org/10.2527/jas.2011-4999.

Ménard S., N. Cerf-Bensussan, and M. Heyman. 2010. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol. 3:247–59. https://doi.org/10.1038/mi.2010.5.

Meneses, A. M. C., K. Schneeberger, H. S. Kruitwagen, L. C. Penning, F. G. van Steenbeek, I. A. Burgener, and B. Spee. 2016. Intestinal Organoids-Current and Future Applications. Vet Sci. 3:31. https://doi.org/10.3390/vetsci3040031.

Merchen, N. R., J. C. Elizalde, and J. K. Drackley. 1997. Current perspective on assessing site of digestion in ruminants. J. Anim. Sci. 75:2223–2234. https://doi.org/10.2527/1997.7582223x.

Mertens, D.R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80: 1463–1481. https://doi.org/10.3168/jds.S0022-0302(97)76075-2.

Metz, S. H. M., and S. G. d. Bergh. 1972. Effects of volatile fatty acids, ketone bodies, glucose, and insulin on lipolysis in bovine adipose tissue. FEBS Lett. 21:203–206. https://doi.org/10.1016/0014-5793(72)80137-6.

Mezzetti, M., M. Bionaz, and E. Trevisi. 2020. Interaction between inflammation and metabolism in periparturient dairy cows. J. Anim. Sci. 98:S155–S174. https://doi.org/10.1093/jas/skaa134.

Mielenz, M. 2017. Invited review: Nutrient-sensing receptors for free fatty acids and hydroxycarboxylic acids in farm animals. Animal 11:1008–1016. https://doi.org/10.1017/S175173111600238X.

Mikuła, R., E. Pruszyńska-Oszmałek, M. Pszczola, J. Rząsińska, M. Sassek, K. W. Nowak, L. Nogowski, and P. A. Kołodziejski. 2021. Changes in metabolic and hormonal profiles during transition period in dairy cattle–the role of spexin. BMC Vet. Res. 17:359. https://doi.org/10.1186/s12917-021-03069-4.

Miller-Cushon, E. K., and T. J. DeVries. 2011. Effect of early feed type exposure on diet-selection behavior of dairy calves. J. Dairy Sci. 94:342–350. https://doi.org/10.3168/jds.2010-3382.

Miller-Cushon, E. K., and T. J. DeVries. 2017. Feed sorting in dairy cattle: Causes, consequences, and management. J. Dairy Sci.100:4172–4183. https: //doi.org/10 .3168/jds.2016 -11983.

Miller-Cushon, E. K., C. Montoro, A. Bach, and T. J. Devries. 2013a. Effect of early exposure to mixed rations differing in forage particle size on feed sorting of dairy calves. J. Dairy Sci. 96:3257–3264. https://doi.org/10.3168/jds.2012-6415.

Miller-Cushon, E. K., R. Bergeron, K. E. Leslie, G. J. Mason, and T. J. Devries. 2013b. Effect of early exposure to different feed presentations on feed sorting of dairy calves. J. Dairy Sci. 96:4624–4633. https://doi.org/10.3168/jds.2013-6670.

Minuti, A., S. Ahmed, E. Trevisi, F. Piccioli-Cappelli, G. Bertoni, N. Jahan, and P. Bani. 2014. Experimental acute rumen acidosis in sheep: Consequences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry. J. Anim. Sci. 92:3966–3977. https://doi.org/10.2527/jas.2014-7594.

Mireles, A. J., S. M. Kim, and K. C. Klasing. 2005. An acute inflammatory response alters bone homeostasis, body composition, and the humoral immune response of broiler chickens. Poult. Sci.84:553–560. https://doi.org/10.1093/ps/84.4.553.

Mitani, T., T. Kubota, H. Mizuguchi, S. Kushibiki, and K. Ueda. 2020. Effects of grazing adaptation on intake, ruminal fermentation, blood metabolites, and body weight change in dairy cows after turning out to pasture in early spring. Anim. Sci. J. 91:e13481. https://doi.org/10.1111/asj.13481.

Moore, S. M., and T. J. DeVries. 2020. Effect of diet-induced negative energy balance on the feeding behavior of dairy cows. J. Dairy Sci. 103:7288–7301. https://doi.org/10.3168/jds.2019-17705.

Morin D. E., and P. D. Constable. 1998. Characteristics of dairy cows during episodes of bacteriologically negative clinical mastitis or mastitis caused by Corynebacterium spp. J. Am. Vet. Med. Assoc. 213:855–861.

Moyo, M., and I. V. Ignatius Nsahlai. 2017. Rate of passage of digesta in ruminants; are goats different? Pages 39–74 in Goat Science, London, UK. https://doi.org/10.5772/intechopen.69745.

Mulligan, F. J. and M. L. Doherty. 2008. Production diseases of the transition cow. Vet. J. 176:3–9. https://doi.org/10.1016/j.tvjl.2007.12.018.

Mullins, C. R., L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner, and B. J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. J. Dairy Sci. 95:1323–1336. https://doi.org/10.3168/jds.2011-4744.

Murata E., S. Kozaki, T. Murakami, K. Shimizu, A. Okada, N. Ishiguro, and Y. Inoshima. 2020. Differential expression of serum amyloid A1 and A3 in bovine epithelia. J. Vet. Med. Sci. 82:764–770. https://doi.org/10.1292/jvms.19-0473.

Murata, H., N. Schimada, and M. Yoshioka. 2004. Current research on acute proteins in veterinary diagnosis: an overview. Vet. J. 168:28e40. https://doi.org/10.1016/S1090-0233(03)00119-9.

Nagaraja, T. G. 2016. Microbiology of the rumen. Pages 39–61 in Rumenology. Springer International Publishing, Cham, Switzerland. https://doi.org/10.1007/978-3-319-30533-2_2.

NASEM. 2021. National Academies of Sciences, Engineering, and Medicine. Nutrient Requirements of Dairy Cattle: 8th rev. ed. Natl. Acad. Press, Washington, DC, USA. https://doi.org/10.17226/25806.

Nazif, S., S. M. Razavi, M. Reiszadeh, Z. Esmailnezhad, and M. Ansari-Lari. 2010. Diagnostic values of acute phase proteins in Iranian indigenous cattle infected with Theileria annulata. Vet. Arhiv, 80: 205–214.

Nazifi, S., M. Haghkhah, Z. Asadi, M. Ansari-Lari, M. R. Tabandeh, Z. Esmailnezhad, and M. Aghamiri. 2011. Evaluation of sialic acid and acute phase proteins (haptoglobin and serum amyloid A) in clinical and subclinical bovine mastitis. Pak. Vet. J. 31:55–59.

NCBI. 2021. National Library of Medicine, Bethesda, Maryland, USA. National Center for Biotechnology Information; [1988] – [cited 2021]. Available from: https://www.ncbi.nlm.nih.gov/.

Neubauer, V., R. M. Petri, E. Humer, I. Kröger, N. Reisinger, W. Baumgartner, M. Wagner, and Q. Zebeli. 2020. Starch-rich diet induced rumen acidosis and hindgut dysbiosis in dairy cows of different lactations. Animals (Basel) 10:1727. https://doi.org/10.3390/ani10101727.

Newby, N. C., D. L. Pearl, S. J. LeBlanc, K. E. Leslie, M. A. G. von Keyserlingk, and T. F. Duffield. 2013. Effects of meloxicam on milk production, behavior, and feed intake in dairy cows following assisted calving. J. Dairy Sci. 96:3682–3688. https://doi.org/10.3168/jds.2012-6214.

Newby, N. C., K. E. Leslie, H. D. P. Dingwell, D. F. Kelton, D. M. Weary, L. Neuder, S. T. Millman, and T. F. Duffield. 2017. The effects of periparturient administration of flunixin

meglumine on the health and production of dairy cattle. J. Dairy Sci. 100:582–587. https://doi.org/10.3168/jds.2016-11747.

Nielsen, N. I., and K. L. Ingvartsen. 2004. Propylene glycol for dairy cows – a review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and the risk of ketosis. Anim. Feed Sci. Technol. 115:191–213. https://doi.org/10.1016/j.anifeedsci.2004.03.008

Nilsen, T., S. H. Haugen, and A. Larsson. 2018. Extraction, isolation, and concentration of calprotectin antigen (S100A8/S100A9) from granulocytes. Health Sci Rep. 5:e35. https://doi.org/10.1002/hsr2.35.

Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80:1005–1028. https://doi.org/10.3168/jds.S0022-0302(97)76026-0.

Nogalski, Z., M. Wroński, M. Sobczuk-Szul, M. Mochol, and P. Pogorzelska. 2012. The effect of body energy reserve mobilization on the fatty acid profile of milk in high-yielding cows. Asian-Australas. J. Anim. Sci. 25:1712–1720. https://doi.org/10.5713/ajas.2012.12279.

Nordlund, K. V., E. F. Garrett, and G. R. Oetzel. 1995. Herd-based rumenocentesis: A clinical approach to the diagnosis of subacute rumen acidosis. Compendium on Continuing Education for the Practicing Veterinarian. 17:S48.

Nozière P., I. Ortigues-Marty, C. Loncke, and D. Sauvant. 2010. Carbohydrate quantitative digestion and absorption in ruminants: from feed starch and fibre to nutrients available for tissues. Animal 4:1057–1074. https://doi.org/10.1017/S1751731110000844

NRC. 2001. Nutrient Requirements for Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC, USA. https://doi.org/10.17226/9825.

Oba, M., and K. Kammes-Main. 2023. Symposium review: Effects of carbohydrate digestion on feed intake and fuel supply. J. Dairy Sci. 106:P2153–2160. https://doi.org/10.3168/jds.2022-22420.

Odegaard, J. I., and A. Chawla. 2012. Leukocyte set points in metabolic disease. F1000 Biol. Rep. 4:13. https://doi.org/10.3410/B4-13.

Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. N. Amer. Food Anim. 20:651–674. https://doi.org/10.1016/j.cvfa.2004.06.006.

Oetzel, G. R. 2013. Understanding the impact of subclinical ketosis. Pages 15–26, University of Wisconsin, Madison, USA.

Ohira, H., Y. Fujioka, C. Katagiri, R. Mamoto, M. Aoyama-Ishikawa, K. Amako, Y. Izumi, S. Nishiumi, M. Yoshida, M. Usami, and M. Ikeda. 2013. Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages. J. Atheroscler. Thromb. 20:425–442. https://doi.org/10.5551/jat.15065.

Ohtsuka, H., M. Koiwa, A. Hatsugaya, K. Kudo, F. Hoshi, N. Itoh, H. Yokota, H. Okada, and S. Kawamura. 2001. Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. J. Vet. Med. Sci. 63, 1021–1025. https://doi.org/10.1292/jvms.63.1021.

Olde Riekerink, R. G. M., H. W. Barkema, D. F. Kelton, and D. T. Scholl. 2008. Incidence rate of clinical mastitis on Canadian dairy farms. J. Dairy Sci. 91:1366–1377. https://doi.org/10.3168/jds.2007-0757.

Ørskov, E. R., C. Fraser, V. C. Mason, and S. O. Mann. 1970. Influence of starch digestion in the large intestine of sheep on caecal fermentation, caecal microflora and faecal nitrogen excretion. Br. J. Nutr. 24:671–682. https://doi.org/10.1079/bjn19700068.

Ospina, P. A., D. V. Nydam, T. Stokol, T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: critical thresholds for prediction of clinical diseases J. Dairy Sci., 93:546–554. https://doi.org/10.3168/jds.2009-2277.

Ouellette, A. J. 2010. Paneth cells and innate mucosal immunity. Curr. Opin. Gastroenterol. 26:547–553. https://doi.org/10.1097/mog.0b013e32833dccde.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: A review. J. Anim. Sci. 76:275–286. https://doi.org/10.2527/1998.761275x.

Owens, F., and M. Basalan. 2016. Ruminal Fermentation. Pages 63–102 in Rumenology. Springer International Publishing, Cham, Switzerland. https://doi.org/10.1007/978-3-319-30533-2_3.

Pabst, O. 2012. New concepts in the generation and functions of IgA. Nat. Rev. Immunol. 12:821–832. https://doi.org/10.1038/nri3322.

Pang, W., B. Earley, T. Sweeney, V. Gath, and M. A. Crowe. 2009. Temporal patterns of inflammatory gene expression in local tissues after banding or burdizzo castration in cattle. BMC Vet. Res. 5:36. https://doi.org/10.1186/1746-6148-5-36.

Parrott, M. E., and B. E., Thrall. 1978. Functional properties of various fibers: physical properties. J. Food Sci. 43:759–763. https://doi.org/10.1111/j.1365-2621.1978.tb02412.x.

Pascottini, O. B., J. Spricigo, S. J. Van Schyndel, B. Mion, J. Rousseau, J. S. Weese, and S. J. LeBlanc. 2021. Effects of parity, blood progesterone, and non-steroidal anti-inflammatory treatment on the dynamics of the uterine microbiota of healthy postpartum dairy cows. PLoS One 16:e0233943. https://doi.org/10.1371/journal.pone.0233943.

Pascottini, O. B., S. J. Van Schyndel, J. F. W. Spricigo, M. R. Carvalho, B. Mion, E. S. Ribeiro, and S. J. LeBlanc. 2020. Effect of anti-inflammatory treatment on systemic inflammation, immune function, and endometrial health in postpartum dairy cows. Sci. Rep. 10:5236. https://doi.org/10.1038/s41598-020-62103-x.

Pearce, S. C., V. Mani, T. E. Weber, R. P. Rhoads, J. F. Patience, L. H. Baumgard, and N. K. Gabler. 2013. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J. Anim. Sci. 91:5183–5193. https://doi.org/10.2527/jas.2013-6759.

Peng, L., Z. R. Li, R. S. Green, I. R. Holzman, and J. Lin. 2009. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J. Nutr. 139:1619–1625. https://doi.org/10.3945/jn.109.104638.

Penner, G. B., J. R. Aschenbach, G. Gäbel, and M. Oba. 2009a. Epithelial capacity for the apical uptake of short-chain fatty acids is a key determinant for intra-ruminal pH and the susceptibility to sub-acute ruminal acidosis in sheep. J. Nutr. 139:1714–1720. https://doi.org/10.3945/jn.109.108506.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2007. Severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. J. Dairy Sci. 90:365–375. https://doi.org/10.3168/jds.S0022-0302(07)72638-3.

Penner, G. B., M. Oba, G. Gabel, and J. R. Aschenbach. 2010. A single mild episode of subacute ruminal acidosis does not affect ruminal barrier function in the short term. J. Dairy Sci. 93:4838–4845. https://doi.org/10.3168/jds.2010-3406.

Penner, G. B., M. Taniguchi, L. L. Guan, K. A. Beauchemin, and M. Oba. 2009b. Effect of dietary forage to concentrate ratio on volatile fatty acid absorption and the expression of

genes related to volatile fatty acid absorption and metabolism in ruminal tissue. J. Dairy Sci. 92:2767–2781. https://doi.org/10.3168/jds.2008-1716.

Pérez-Báez, J., T. V. Silva, C. A. Risco, R. C. Chebel, F. Cunha, A. De Vries, J. E. P. Santos, F. S. Lima, P. Pinedo, G. M. Schuenemann, and R. C. Bicalho. 2021. The economic cost of metritis in dairy herds. J. Dairy Sci. 104:3158–3168. https://doi.org/10.3168/jds.2020-19125.

Peter, A. T., J. E. Simon, C. W. Luker, and W. T. K. Bosu. 1990. Site of action for endotoxin-induced cortisol release in the suppression of preovulatory luteinizing hormone surges. Theriogenology 33:637–643. https://doi.org/10.1016/0093-691X(90)90540-A.

Petersen H. H., J. P. Nielsen, P. M. Heegaard. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35:163–187. https://doi.org/10.1051/vetres:2004002.

Peterson, L. W., and D. Artis. 2014. Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. Nat. Rev. Immunol. 14:141–153. https://doi.org/10.1038/nri3608.

Petersson, J. O. Schreiber, G. C. Hansson, S. J. Gendler, A. Velcich, J. O. Lundberg, S. Roos, L. Holm, and M. Phillipson. 2011. Importance and regulation of the colonic mucus barrier in a mouse model of colitis. Am. J. Physiol. Gastrointest. Liver Physiol. 300:G327–G333. https://doi.org/10.1152/ajpgi.00422.2010.

Petri, R. M., M. T. Kleefisch, B. U. Metzler-Zebeli, Q. Zebeli, and F. Klevenhusen. 2018. Changes in the rumen epithelial microbiota of cattle and host gene expression in response to alterations in dietary carbohydrate composition. Appl. Environ. Microbiol. 83:e00384–18. https://doi.org/10.1128/AEM.00384-18.

Piantoni, P., A. L. Lock, and M. S. Allen. 2015. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. J. Dairy Sci. 98:3309–3322. https://doi.org/10.3168/jds.2014-8798.

Pires, J. A. A., C. Delavaud, Y. Faulconnier, D. Pomies, and Y. Chilliard. 2013. Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein- Friesian cows. J. Dairy Sci. 96:6423–6439. https://doi.org/10.3168/jds.2013-6801.

Pires, J. A. A., J. B. Pescara, and R. R. Grummer. 2007. Reduction of plasma NEFA concentration by nicotinic acid enhances the response to insulin in feed-restricted Holstein cows. J. Dairy Sci. 90:4635–4642. https://doi.org/10.3168/jds.2007-0146.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176:21–31. https://doi.org/10.1016/j.tvjl.2007.12.016.

Plaizier, J. C., E. Khafipour, S. Li, G. N. Gozho, and D. O. Krause. 2012. Subacute ruminal acidosis (SARA), endotoxins and health consequences. Anim. Feed Sci. Technol. 172:9–21. https://doi.org/10.1016/j.anifeedsci.2011.12.004.

Plaizier, J. C., M. Danesh Mesgaran, H. Derakhshani, H. Golder, E. Khafipour, J. L. Kleen, Lean, J. Loor, G. Penner and Q. Zebeli. 2018. Review: Enhancing gastrointestinal health in dairy cows. Animal 12:s399–s418. https://doi.org/10.1017/S1751731118001921.

Plaizier, J. C., S. Li, H. M. Tun, and E. Khafipour. 2017. Nutritional models of experimentally-induced subacute ruminal acidosis (SARA) differ in their impact on rumen and hindgut

bacterial communities in dairy cows. Front. Microbiol. 7:2128. https://doi.org/10.3389/fmicb.2016.02128.

Plaizier, J. C., F. J. Mulligan, E. W. Neville, L. L. Guan, M. A. Steele, and G. B. Penner. 2022. Invited review: Effect of subacute ruminal acidosis on gut health of dairy cows. J. Dairy Sci. 105:7141–7160. https://doi.org/10.3168/jds.2022-21960.

Pleticha, S., M. Drillich, and W. Heuwieser. 2009. Evaluation of the Metricheck device and the gloved hand for the diagnosis of clinical endometritis in dairy cows. J. Dairy Sci. 92:5429–5435. https://doi.org/10.3168/jds.2009-2117.

Pocius, P. A., and J. H. Herbein. 1986. Effects of in vivo administration of growth hormone on milk production and in vitro hepatic metabolism in dairy cattle. J. Dairy Sci. 69:713–720. https://doi.org/10.3168/jds.S0022-0302(86)80460-X.

Podolsky D. K. 1999. Mucosal immunity and inflammation. V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense. Am. J. Physiol. Gastrointest. 277:G495–G499. https://doi.org/10.1152/ajpgi.1999.277.3.G495.

Pohl, A., O. Burfeind, and W. Heuwieser. 2015. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. J. Dairy Sci. 98:4544–4551. https://doi.org/10.3168/jds.2014-9181.

Potter, T. J., J. Guitian, J. Fishwick, P. J. Gordon, and I. M. Sheldon. 2010. Risk factors for clinical endometritis in postpartum dairy cattle. Theriogenology 74:127–134. https://doi.org/10.1016/j.theriogenology.2010.01.023.

Powanda, M. C. 1980. Host metabolic alterations during inflammatory stress as related to nutritional status. Am. J. Vet. Res. 41:1905–1911.

Priest, N. V., S. McDougall, C. R. Burke, J. R. Roche, M. Mitchell, K. L. McLeod, S. L. Greenwood, and S. Meier. 2013. The responsiveness of subclinical endometritis to a nonsteroidal anti-inflammatory drug in pasture grazed dairy cows. J. Dairy Sci. 96:4323–4332. https://doi.org/10.3168/jds.2012 -6266.

Probo, M., O. B. Pascottini, S. LeBlanc, G. Opsomer, and M. Hostens. 2018. Association between metabolic diseases and the culling risk of high-yielding dairy cows in a transition management facility using survival and decision tree analysis. J. Dairy Sci. 101:9419–9429. https://doi.org/10.3168/jds.2018-14422.

Provenza, F. D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J. Range Manage. 48:2–17. https://doi.org/doi:10.2307/4002498.

Provenza, F. D., J. J. Villalba, L. Dziba, S. B. Atwood, and R. E. Banner. 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Rumin. Res. 49:257–274. https://doi.org/10.1016/S0921-4488(03)00143-3.

Pullen, D., J. Liesman and R. Emery. 1990. A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acids in media. J. Anim. Sci. 68:1395–1399. https://doi.org/10.2527/1990.6851395x.

Puppel, K., and Kuczyńska B. 2016. Metabolic profiles of cow's blood; a review. J. Sci. Food Agric. 96:4321–4328. https://doi.org/10.1002/jsfa.7779.

Qu, Y., A. N. Fadden, M. G. Traber, and G. Bobe. 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. J. Dairy Sci. 97:4151–4165. https://doi.org/ 10.3168/jds.2013-7154.

Quesnell, R. R., S. Klaessig, J. L. Watts, and Y. H. Schukken. 2012. Bovine intramammary Escherichia coli challenge infections in late gestation demonstrate a dominant

antiinflammatory immunological response. J. Dairy Sci. 95:117–126. https://doi.org/10.3168/jds.2011-4289.

Rabelo, E., R. L. Rezende, L., S. J. Bertics, and R. R. Grummer. 2003. Effects of transition diets varying in dietary energy density on lactation performance and ruminal parameters of dairy cows. J. Dairy Sci. 86:916–925. https://doi.org/10.3168/jds.S0022-0302(03)73674-1.

Rabelo, E., Rezende, R.L., Bertics, S.J. & Grummer, 2005. Effects of prepartum and postfresh transition diets varying in dietary energy density on metabolic status of periparturient dairy cows. J. Dairy Sci. 88, 4375–4383. https://doi.org/10.3168/jds.S0022-0302(05)73124-6.

Rakoff-Nahoum S., J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov. 2004. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 118:229–241. https://doi.org/10.1016/j.cell.2004.07.002.

Reisner, A, H., P. Nemes, and C. Bucholtz. 1974. The use of Coomassie Brilliant Blue G250 perchloric acid solution for staining in electrophoresis and isoelectric focusing on polyacrylamide gels. Anal. Biochem. 64:509–516. https://doi.org/10.1016/0003-2697(75)90461-3

Reyher, K. K., S. Dufour, H. W. Barkema, L. Des Côteaux, T. J. Devries, I. R. Dohoo, G. P. Keefe, J. P. Roy, and D. T. Scholl. 2011. The National Cohort of Dairy Farms—A data collection platform for mastitis research in Canada. J. Dairy Sci. 94:1616–1626. https://doi.org/10.3168/jds.2010-3180

Reynolds, C. K., B. Durst, B. Lupoli, D. J. Humphries, and D. E. Beever. 2004. Visceral tissue mass and rumen volume in dairy cows during the transition period from late gestation to

early lactation. J. Dairy Sci. 87:961–971. https://doi.org/10.3168/jds.S0022-0302(04)73240-3.

Ricci, S., C. Pacífico, E. Castillo-Lopez, R. Rivera-Chacon, H. E. Schwartz-Zimmermann, N. Reisinger, F. Berthiller, Q. Zebeli, and R. M. Petri. 2022. Progressive microbial adaptation of the bovine rumen and hindgut in response to a step-wise increase in dietary starch and the influence of phytogenic supplementation. Front. Microbiol. 13:920427. https://doi.org/10.3389/fmicb.2022.920427.

Rinaldi, M., P. Moroni, M. J. Paape, and D. D. Bannerman. 2008. Differential alterations in the ability of bovine neutrophils to generate extracellular and intracellular reactive oxygen species during the periparturient period. Vet. J. 178:208–213. https://doi.org/10.1016/j.tvjl.2007.07.030.

Roberts T., N. Chapinal, S. J. Leblanc, D. F. Kelton, J. Dubuc, and T. F. Duffield. 2012. Metabolic parameters in transition cows as indicators for early-lactation culling risk. J. Dairy Sci. 95:3057–3063. https://doi.org/10.3168/jds.2011-4937.

Roche, J. R., K. A. Macdonald, K. E. Schutz, L. R. Matthews, G. A. Verkerk, S. Meier, J. J. Loor, A. R. Rogers, J. McGowan, S. R. Morgan, S. Taukiri, and J. R. Webster. 2013. Calving body condition score affects indicators of health in grazing dairy cows. J. Dairy Sci. 96:5811–5825. https://doi.org/10.3168/jds.2013-6600.

Rodiño-Janeiro, B. K., C. Alonso-Cotoner, M. Pigrau, B. Lobo, M. Vicario, and J. Santos. 2015. Role of corticotropin-releasing factor in gastrointestinal permeability. J. Neurogastroenterol. Motil. 21:33-50. https://doi.org/10.5056/jnm14084.

Rollwagen, F. M., S. Madhavan, A. Singh, Y. Y. Li, K. Wolcott, and R. Maheshwari. 2006. IL-6 protects enterocytes from hypoxia-induced apoptosis by induction of bcl-2 mRNA and

reduction of fas mRNA. Biochem. Biophys. Res. Commun. 347:1094-1098. https://doi.org/10.1016/j.bbrc.2006.07.016.

Rosales, Eduardo Barahona, and Burim N. Ametaj. 2021. Reproductive tract infections in dairy cows: can probiotics curb down the incidence rate?. Dairy 2:40–64. https://doi.org/10.3390/dairy2010004.

Røseth, A. G., M. K. Fagerhol, E. Aadland, and H. Schjønsby. 1992. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand. J. Gastroenterol. 27:793–798. https://doi.org/10.3109/00365529209011186.

Rothkotter, H. J. and R. Pabst. 1989. Lymphocyte subsets in jejunal and ileal Peyer's patches of normal and gnotobiotic minipigs. Immunology. 67:103–108.

Ruiz-González, A., W. Suissi, L. H. Baumgard, Y. Martel-Kennes, P. Y. Chouinard, R. Gervais, and D. E. Rico. 2023. Increased dietary vitamin D3 and calcium partially alleviate heat stress symptoms and inflammation in lactating Holstein cows independent of dietary concentrations of vitamin E and selenium. J. Dairy Sci. 106:3984–4001. https://doi.org/10.3168/jds.2022-22345.

Rumphorst, T., T. Scheu, C. Koch, and A. Sundrum. 2022. Inter-and intra-individual variation in the behavior of feed intake on nutrient availability in early lactating dairy cows. Animals 12:37. https://doi.org/10.3390/ani12010037.

Ruppin, H., S. Bar-Meir, K. H. Soergel, C. M. Wood, and M. G. Schmitt. 1980. Absorption of short-chain fatty acids by the colon. Gastroenterology. 78:1500–1507. https://doi.org/10.1016/S0016-5085(19)30508-6.

Sakata, T., and H. Tamate. 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. J. Dairy Sci. 61:1109–1113. https://doi.org/10.3168/jds.S0022-0302(78)83694-7.

Sander C., and R. Schneider. 1991. Database of homology-derived protein structures and the structural meaning of sequence alignment. Protein 9:56–68. https://doi.org/10.1002/prot.340090107.

Sansonetti, P. 2006. The innate signaling of dangers and the dangers of innate signaling. Nat. Immunol. 7:1237–1242. https://doi.org/10.1038/ni1420.

Sanz-Fernandez, M. V., J.-B. Daniel, D. J. Seymour, S. K. Kvidera, Z. Bester, J. Doelman, and J. Martín-Tereso. 2020. Targeting the hindgut to improve health and performance in cattle. Animals 10:1817. https://doi.org/10.3390/ani10101817.

Sato, K., Y. Kashiwaya, C. A. Keon, N. Tsuchiya, M. T. King, G. K. Radda, B. Chance, K. Clarke, and R. L. Veech. 1995. Insulin, ketone bodies, and mitochondrial energy transduction. FASEB J. 9:651–658. https://doi.org/10.1096/fasebj.9.8.7768357.

Sauer, J., K. K. Richter, and B. L. Pool-Zobel. 2007. Physiological concentrations of butyrate favorably modulate genes of oxidative and metabolic stress in primary human colon cells. J. Nutr. Biochem. 18:736–745. https://doi.org/10.1016/j.jnutbio.2006.12.012.

Sayers E. W., E. E. Bolton, J. R. Brister, K. Canese, J. Chan, D. C. Comeau, R. Connor, K. Funk, C. Kelly, S. Kim, T. Madej, A. Marchler-Bauer, C. Lanczycki, S. Lathrop, Z. Lu, F. Thibaud-Nissen, T. Murphy, L. Phan, Y. Skripchenko, T. Tse, J. Wang, R. Williams, B. W. Trawick, K. D. Pruitt, and S. T. Sherry. 2022. Database resources of the national center for biotechnology information. Nucleic Acids Res. 50:D20–D26. https//doi.org/10.1093/nar/gkab1112.

Schmitt, R., L. Pieper, L. Gonzalez-Grajales, J. Swinkels, C. Gelfert, and R. Staufenbiel. 2021. Evaluation of different acute-phase proteins for herd health diagnostics in early postpartum Holstein Friesian dairy cows. J. Dairy Res. 88:33–37. https://doi.org/10.1017/S0022029921000078.

Schroedl, W., B. Fuerll, P. Reinhold, M. Krueger, and C. Schuett. 2001. A novel acute phase marker in cattle: Lipopolysaccharide binding protein (LBP). J. Endotoxin Res. 7:49–52.

Schwartz, G., K. L. Hill, M. J. VanBaale, and L. H. Baumgard. 2009. Effects of flunixin meglumine on pyrexia and bioenergetics variables in postparturient dairy cows. J. Dairy Sci. 92:1963–1970. https://doi.org/10.3168/jds.2008-1581.

Sehested J., L. Diernaes, P. D. Moller, and E. Skadhauge. 1999. Transport of butyrate across the isolated bovine rumen epithelium: interaction with short-chain fatty acids, chloride and bicarbonate. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 123:399–408. https://doi.org/10.1016/S1095-6433(99)00082-3.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516–1530. https://doi.org/10.1016/j.theriogenology.2005.08.021.

Sheldon, I. M., A. N. Rycroft, B. Dogan, M. Craven, J. J. Bromfield, A. Chandler, M. H. Roberts, S. B. Price, R. O. Gilbert, and K. W. Simpson. 2010. Specific strains of Escherichia coli are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease in cattle and mice. PLoS One 5:e9192. https://doi.org/10.1371/journal.pone.0009192.

Sheldon, I. M., and S. E. Owens. 2017. Postpartum uterine infection and endometritis in dairy cattle. Anim. Reprod. 14:622–629. https://doi.org/10.21451/1984-3143-AR1006.

Sheldon, I. M., D. E. Noakes, A. N. Rycroft, D. U. Pfeiffer, and H. Dobson. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 123:837–845. https://doi.org/10.1530/rep.0.1230837.

Sheldon, I. M., J. G. Cronin, and J. J. Bromfield. 2019. Tolerance and innate immunity shape the development of postpartum uterine disease and the impact of endometritis in dairy cattle. Annu. Rev. Anim. Biosci. 7:361–384. https://doi.org/10.1146/annurev-animal-020518-115227.

Shen, Z., H. M. Seyfert, B. Löhrke, F. Schneider, R. Zitnan, A. Chudy, S. Kuhla, H. A. Hammon, J. W. Blum, H. Martens, H. Hagemeister, and J. Voigt. 2004. An energy-rich diet causes rumen papillae proliferation associated with more IGF type 1 receptors and increased plasma IGF-1 concentrations in young goats. J. Nutr. 134:11–17. https://doi.org/10.1093/jn/134.1.11.

Shi, W., C. E. Knoblock, K. V. Murphy, T. C. Bruinje, I. Yoon, D. J. Ambrose, and M. Oba. 2019. Effects of supplementing a Saccharomyces cerevisiae fermentation product during the periparturient period on performance of dairy cows fed fresh diets differing in starch content. J. Dairy Sci. 102:3082–3096. https://doi.org/10.3168/jds.2018-15307.

Shi, W., J. Haisan, Y. Inabu, T. Sugino, and M. Oba. 2020. Effects of starch content of close-up diets on responses of dairy cows to grain challenges after calving. J. Dairy Sci. 103: 3082–3096. https://doi.org/10.3168/jds.2018-15307.

Shock, D. A., D. L. Renaud, S. M. Roche, R. Poliquin, R. Thomson, and M. E. Olson. 2018. Evaluating the impact of meloxicam oral suspension administered at parturition on

subsequent production, health, and culling in dairy cows: A randomized clinical field trial. PLoS One 13:e0209236. https://doi.org/10.1371/journal.pone.0209236.

Shock, D., S. Roche, and M. Olson. 2019. A comparative pharmacokinetic analysis of oral and subcutaneous meloxicam administered to postpartum dairy cows. Vet. Sci. 6:73. https://doi.org/10.3390/vetsci6030073.

Shurkin, J. 2014. News Feature: Animals that self-medicate. PNAS. 111:17339–17341. https://doi.org/10.1073/pnas.1419966111.

Shwartz, G., K. L. Hill, M. J. VanBaale, and L. H. Baumgard. 2009. Effects of flunixin meglumine on pyrexia and bioenergetics variables in postparturient dairy cows. J. Dairy Sci. 92:1963–1970. https://doi.org/10.3168/jds.2008-1581.

Simon, L. S. 1999. Role and regulation of cyclooxygenase-2 during inflammation. Am. J. Med. 106(5B):37S–42S. https://doi.org/10.1016/S0002-9343(99)00115-1.

Sirois, J., K. Sayasith, K. A. Brown, A. E. Stock, N. Bouchard, and M. Doré. 2004. Cyclooxygenase-2 and its role in ovulation: A 2004 account. Hum. Reprod. Update 10:373–385. https://doi.org/10.1093/humupd/dmh032.

Smith, Billy I., and C. A. Risco. 2002. Predisposing factors and potential causes of postpartum metritis in dairy cattle. Compendium. 24:S74–80.

Sordillo, L. M. 2016. Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci. 99:4967–4982. http://dx.doi.org/10.3168/jds.2015-10354

Sordillo, L. M., and W. Raphael. 2013. Significance of metabolis stress, lipid mobilization, and inflammation on transition cow disorders. Vet. Clin. North Am. Food Anim. Pract. 29:267–278. https://doi.org/10.1016/j.cvfa.2013.03.002.

Sordillo, L. M., G. M. Pighetti, and M. R. Davis. 1995. Enhanced production of bovine tumor necrosis factor-alpha during the periparturient period. Vet. Immunol. Immunopathol. 49:263-270. https://doi.org/ 10.1016/0165-2427(95)05465-0.

Stachowicz, K., M. Sargolzaei, F. Miglior, and F. S. Schenkel. 2011. Rates of inbreeding and genetic diversity in Canadian Holstein and Jersey cattle. J. Dairy Sci. 94:5160–5175. https://doi.org/10.3168/jds.2010-3308.

Steele, M. A., G. Vandervoort, O. AlZahal, S. E. Hook, J. C. Matthews, B. W. McBride. 2011b. Rumen epithelial adaptation to high-grain diets involves the coordinated regulation of genes involved in cholesterol homeostasis. Physiol. Genomics 43:308–316. https://doi.org/10.1152/physiolgenomics.00117.2010.

Steele, M. A., O. AlZahal, S. E. Hook, J. Croom, and B. W. McBride. 2009. Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. Acta Vet. Scand. 51:39. https://doi.org/10.1186/1751-0147-51-39.

Steele, M. A., C. Schiestel, O. AlZahal, L. Dionissopoulos, A. Laarman, J. C. Matthews, and B. W. McBride. 2015. The periparturient period is associated with structural and transcriptomic adaptations of rumen papillae in dairy cattle. J. Dairy Sci. 98:2583–2595. https://doi.org/10.3168/jds.2014-8640.

Steele, M. A., G. B. Penner, F. Chaucheyras-Durand, and L. L. Guan. 2016. Development and physiology of the rumen and the lower gut: Targets for improving health. J. Dairy Sci. 99:4955–4966. https://doi.org/10.3168/jds.2015-10351.

Steele, M. A., J. Croom, M. Kahler, O. AlZahal, S. E. Hook, K. Plaizier, and B. W. McBride. 2011a. Bovine rumen epithelium undergoes rapid structural adaptations during grain-

induced subacute ruminal acidosis. Am. J. Physiol. Regul. Integr. Comp. Physiol. 300:R1515-23. https://doi.org/10.1152/ajpregu.00120.2010.

Stengärde, L., M. Tråvén, U. Emanuelson, K. Holtenius, J. Hultgren, and R. Niskanen. 2008. Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. Acta Vet. Scand. 50:31. https://doi.org/10.1186/1751-0147-50-31.

Stevenson, F. 2007. Clinical reproductive physiology of the cow. 2007. Pages 258–269 in Current Therapy in Large Animal Theriogenology. Saunders Elsevier, St. Louis, MO, USA.

Stewart A. S., S. Pratt-Phillips, and L. M. Gonzalez. 2017. Alterations in intestinal permeability: the role of the "leaky gut" in health and disease. 2017. J. Equine Vet. Sci. 52:10–22. https://doi.org/10.1016/j.jevs.2017.02.009.

Stockdale, C. R. 2001. Body condition at calving and the performance of dairy cows in early lactation under Australian conditions: A review. Aust. J. Exp. Agric. 41:823–829.

Stokes, M. R. 1983. Effect of sodium bicarbonate on rumen turnover in frequently fed sheep. Can. J. Anim. Sci. 63:721–725. https://doi.org/10.4141/cjas83-083.

Stone, W. C. 2004. Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. J. Dairy Sci. 87:E13–E26. https://doi.org/10.3168/jds.S0022-0302(04)70057-0.

Stumpff, F. 2018. A look at the smelly side of physiology: Transport of short chain fatty acids. Pflugers Arch. Eur. J. Phy. 470:571–598. https://doi.org/10.1007/s00424-017-2105-9.

Stumpff, F., M. I. Georgi, L. Mundhenk, I. Rabbani, M. Fromm, H. Martens, and D. Günzel. 2011. Sheep rumen and omasum primary cultures and source epithelia: Barrier function

aligns with expression of tight junction proteins. J. Exp. Biol. 214:2871–2882. https://doi.org/10.1242/jeb.055582.

Sueda, K. L., B. L. Hart, and K. D. Cliff. 2008. Characterisation of plant eating in dogs. Appl. Anim. Behav. Sci. 111:120–132. https://doi.org/10.1016/j.applanim.2007.05.018.

Sundrum, A. 2015. Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is over stressed. Animals 5:978–1020. https://doi.org/10.3390/ani5040395.

Sutton, J. D., M. Dhanoa, S. Morant, J. France, D. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and low-roughage diets. J. Dairy Sci. 86:3620–3633. https://doi.org/10.3168/jds.S0022-0302(03)73968-X.

Swartz, T. H., H. H. Schramm, J. M. Bewley, C. M. Wood, K. E. Leslie, and C. S. Petersson-Wolfe. 2018. Meloxicam administration either prior to or after parturition: Effects on behavior, health, and production in dairy cows. J. Dairy Sci. 101:10151–10167. https://doi.org/10.3168/jds.2018-14657.

Tang T. K., T. M. Hong, C. Y. Lin, M. L. Lai, C. H. Liu, H. J. Lo, M. E. Wang, L. B. Chen, W. Chen, W. Ip, D. C. Lin, J. J. Lin, S. Lin, T. Sun, E. Wang, J. L. Wang, R. Wu, C. Wu and S. Chien. 1993. Nuclear proteins of the bovine esophageal epithelium. I. Monoclonal antibody W2 specifically reacts with condensed nuclei of differentiated superficial cells. J. Cell Sci. 104:237–247. https//doi.org/10.1242/jcs.104.2.237.

Tang, T., J. Zhang, J. Yin, J. Staszkiewicz, B. Gawronska-Kozak, D. Y. Jung, H. J. Ko, H. Ong, J. K. Kim, R. Mynatt, R. J. Martin, M. Keenan, Z. Gao, and J. Ye. 2010. Uncoupling of inflammation and insulin resistance by NF-kB in transgenic mice through elevated

energy expenditure. J. Biol. Chem. 285:4637–4644. https://doi.org/10.1074/jbc.M109.068007.

Tao S., Y. Duanmu, H. Dong, J. Tian, Y. Ni, and R. Zhao. 2014a. A high-concentrate diet induced colonic epithelial barrier disruption is associated with the activating of cell apoptosis in lactating goats. BMC Vet. Res.10:235. https://doi.org/10.1186/s12917-014-0235-2.

Tao S., Y. Duanmu, H. Dong, Y. Ni, J. Chen, X. Shen, and R. Zhao. 2014b High concentrate diet induced mucosal injuries by enhancing epithelial apoptosis and inflammatory response in the hindgut of goats. PLoS ONE. 2014;9:e111596. doi: 10.1371/journal.pone.0111596.

Taschuk, R. and Griebel, P. J. 2012. Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract. Anim. Health Res. Rev. 13:129–141. https://doi.org/10.1017/S1466252312000096.

Tate, J., and G. Ward. 2004. Interferences in immunoassay. Clin Biochem Rev. 25:105–20.

Tatone E. H., J. L. Gordon, J. Hubbs, S. J. LeBlanc, T. J. DeVries, and T. F. Duffield. 2016. A systematic review and meta-analysis of the diagnostic accuracy of point-of-care tests for the detection of hyperketonemia in dairy cows. Prev. Vet. Med.130:18–32. https://doi.org/10.1016/j.prevetmed.2016.06.002.

Taylor-Edwards, C. C., D. G. Burrin, J. J. Holst, K. R. McLeod, and D. L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. J. Dairy Sci. 94:888–898. https://doi.org/10.3168/jds.2010-3540.

Tharangani, H., C. Lu, L. Zhao, L. Ma, X. Guo, W. P. Weiss, D. Bu. 2020. Estimation of between-cow variability in nutrient digestion of lactating dairy cows fed corn-based diets. Animals. 10:1363. https://doi.org/10.3390/ani10081363.

Thompson-Crispi, K. H. Atalla, F. Miglior, and B. A. Mallard. 2014. Bovine mastitis: frontiers in immunogenetics. Front. Immunol. 5:493. https://doi.org/10.3389/fimmu.2014.00493.

Thormar, H., H. Hilmarsson, and G. Bergsson. 2006. Stable concentrated emulsions of the 1-monoglyceride of capric acid (monocaprin) with microbicidal activities against the food-borne bacteria Campylobacter jejuni, Salmonella spp., and Escherichia coli. Appl. Environ. Microbiol. 72:522–526. https://doi.org/10.1128/AEM.72.1.522-526.2006.

Tivey, D.R., and M. W. Smith. 1989. Cytochemical analysis of single villus peptidase activities in pig intestine during neonatal development. Histochem. J21:601–608. https://doi.org/10.1007/BF01753361

Tóthová, C. S., O. Nagy, H. Seidel, J. Konvičná, Z. Farkašová, and G. Kováč. 2008. Acute phase proteins and variables of protein metabolism in dairy cows during the pre-and postpartal period. Acta Vet. Brno. 77:51–57. https://doi.org/10.2754/avb200877010051.

Tóthová, C., O. Nagy, and G. Kovác. 2014. Changes in the concentrations of selected acute phase proteins and variables of energetic profile in dairy cows after parturition. J. Appl. Anim. Res. 42:278– 283. https://doi.org/10.1080/09712119.2013.842485.

Tough D. F., S. Sun, and J. Sprent. 1997. T cell stimulation in vivo by lipopolysaccharide (LPS). J. Exp. Med. 185:2089–2094. https://doi.org/10.1084/jem.185.12.2089.

Trevisi, E., A. Zecconi, G. Bertoni, and R. Piccinini. 2010. Blood and milk immune and inflammatory responses in periparturient dairy cows showing a different liver activity index. J. Dairy Res. 77:310–317. https://doi.org/10.1017/S0022029910000178.

Trevisi, E., and A. Minuti. 2018. Assessment of the innate immune response in the periparturient cow. Res. Vet. Sci. 116:47–54. https://doi.org/10.1016/j.rvsc.2017.12.001.

Trevisi, E., and G. Bertoni. 2008. Attenuation of acetylsalicylate treatments of inflammatory conditions in periparturient dairy cows. Pages 23–37 in Aspirin and Health Research Progress. Nova Science Publishers, New York, NY, USA.

Trevisi, E., M. Amadori, S. Cogrossi, E. Razzuoli, and G. Bertoni. 2012. Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res. Vet. Sci. 93:695–704. https://doi.org/10.1016/j.rvsc.2011.11.008.

Trimboli, F., M. Ragusa, C. Piras, V. Lopreiato, and D. Britti. 2020. Outcomes from experimental testing of nonsteroidal anti-inflammatory drug (NSAID) administration during the transition period of dairy cows. Animals 10:1832. https://doi.org/10.3390/ani10101832.

Turk, R., C. Piras, M. Kovačić, M. Samardžija, H. Ahmed, M. De Canio, A. Urbani, Z. F. Meštrić, A. Soggiu, L. Bonizzi, and P. Roncada. 2012. Proteomics of inflammatory and oxidative stress response in cows with subclinical and clinical mastitis. J. Proteomics 75:4412–4428. https://doi.org/10.1016/j.jprot.2012.05.021.

Twardowska, A., A. Makaro, A. Binienda, J. Fichna, and M. Salaga. 2022. Preventing bacterial translocation in patients with leaky gut syndrome: Nutrition and pharmacological treatment options. Int. J. Mol. Sci. 23:3204. https://doi.org/10.3390/ijms23063204.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215–1223. https://doi.org/10.3168/jds.S0022-0302(65)88430-2.

Ulfina, G. G., S. P. Kimothi, P. S. Oberoi, R. K. Baithalu, A. Kumaresan, T. K. Mohanty, P. Imtiwati, and A. K. Dang. 2015. Modulation of post-partum reproductive performance in

dairy cows through supplementation of longor short-chain fatty acids during transition period. J. Anim. Physiol. Anim. Nutr. (Berl.) 99:1056–1064. https://doi.org/10.1111/jpn.12304.

Umphrey, J. E. and C. R. Staples. 1992. General anatomy of the ruminant digestive system. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Upadhyaya, I., J. Thanislass, A. Veerapandyan, S. Badami, and P. X. Antony. 2016. Characterization of haptoglobin isotype in milk of mastitis-affected cows. Vet. Sci. 3:*29. https://doi.org/*10.3390/vetsci3040029

Urrutia, N. L., and K. J. Harvatine. 2017. Acetate dose-dependently stimulates milk fat synthesis in lactating dairy cows. J. Nutr. 147:763–769. https: / / doi .org/ 10 .3945/ jn .116 .245001.

Urrutia, N., R. Bomberger, C. Matamoros, and K. J. Harvatine. 2019. Effect of dietary supplementation of sodium acetate and calcium butyrate on milk fat synthesis in lactating dairy cows. J. Dairy Sci. 102:5172–5181. https://doi.org/10.3168/jds.2018-16024.

van Gastelen, S., J. Dijkstra, K. Nichols, and A. Bannink. 2021a. Abomasal infusion of ground corn and ammonium chloride in early-lactating Holstein-Friesian dairy cows to induce hindgut and metabolic acidosis. J. Dairy Sci. 104:4174–4191. https://doi.org/10.3168/jds.2020-19300.

van Gastelen, S., J. Dijkstra, S. J. J. Alferink, G. Binnendijk, K. Nichols, T. Zandstra, and A. Bannink. 2021b. Abomasal infusion of corn starch and β-hydroxybutyrate in early-lactation Holstein-Friesian dairy cows to induce hindgut and metabolic acidosis. J. Dairy Sci. 104:12520–12539. https://doi.org/10.3168/jds.2021-20323.

van Knegsel, A.T.M., H. Van den Brand, J. Dijkstra, W. M. Van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reprod. Nutr. Dev. 45:665–688. https://doi.org/10.1051/rnd:2005059.

Van Kruiningen, H. J.1988. Morphologic considerations in the interpretation of gastrointestinal disorders. Toxicol. Pathol. 16:110–117. https://doi.org/10.1177/019262338801600202.

van Niekerk, J. K., M. Middeldorp, and M. A. Steele. 2018. Technical note: The development of a methodology for ruminal and colon tissue biopsying of young Holstein dairy calves. J. Dairy Sci. 101:7212–7218. https://doi.org/10.3168/jds.2018-14374.

Van Saun, R. J. 2016. Indicators of dairy cow transition risks: Metabolic profiling revisited. Tierarzt. Prax. Ausg. G. Grosstiere Nutztiere. 44:118–126. https://doi.org/10.15653/TPG-150947.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant, 2nd edition. Cornell University Press, Ithaca, New York, USA.

Van Soest, P. J., J. B. Robertson, and B. A. Lew. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Vane, J. R., and R. M. Botting. 1996. Mechanism of action of anti-inflammatory drugs. Scand. J. Rheumatol. Suppl. 102:9–21. https://doi.org/10.3109/03009749609097226.

Vanuytsel, T., S. van Wanrooy, H. Vanheel, C. Vanormelingen, S. Verschueren, E. Houben, S. Salim Rasoel, J. Tóth, L. Holvoet, R. Farré, L. Van Oudenhove, G. Boeckxstaens, K. Verbeke, and J. Tack. 2014. Psychological stress and corticotropin-releasing hormone

increase intestinal permeability in humans by a mast cell-dependent mechanism. Gut 63:1293-1299. https://doi.org/10.1136/gutjnl-2013-305690.

Váradyová, Z., I. Zelenak, and P. Siroka. 2000. In vitro study of the rumen and hindgut fermentation of fibrous materials (meadow hay, beech sawdust, wheat straw) in sheep. Anim. Feed Sci. Technol. 83:127–138. https://doi.org/10.1016/S0377-8401(99)00121-2.

Vazquez-Anon M., S. Bertics, M. Luck, R. R. Grummer, and J. Pinheiro. 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. J. Dairy Sci. 77:1521–1528. https://doi.org/10.3168/jds.S0022-0302(94)77092-2.

Vickers, L. A., D. M. Weary, D. M. Veira, and M. A. G. von Keyserlingk. 2013. Feeding a higher forage diet prepartum decreases incidences of subclinical ketosis in transition dairy cows. J. Animal Sci. 91:886–894. https://doi.org/10.2527/jas.2011-4349.

Vieira-Neto, A., F. S. Lima, J. E. P. Santos, R. D. Mingoti, G. S. Vasconcellos, C. A. Risco, and K. N. Galvao. 2016. Vulvovaginal laceration as a risk factor for uterine disease in postpartum dairy cows. J. Dairy Sci. 99:4629–4637. https://doi.org/10.3168/jds.2016-10872.

Villalba J. J. and F. D. Provenza. 1999. Effects of food structure and nutritional quality and animal nutritional state on intake behaviour and food preferences of sheep. Appl. Anim. Behav. Sci. 63:145–163. https://doi.org/10.1016/S0168-1591(98)00238-X

Vinolo, M. A., H. G. Rodrigues, E. Hatanaka, F. T. Sato, S. C. Sampaio, and R. Curi. 2011. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. J. Nutr. Biochem. 22:849–855. https://doi.org/10.1016/j.jnutbio.2010.07.009.

Voelker, J. A., G. M. Burato, and M. S. Allen. 2002. Effects of pretrial milk yield on responses of feed intake, digestion, and production to dietary forage concentration. J. Dairy Sci. 85:2650–2661. https://doi.org/10.3168/jds.2014-8999.

Waelchli, R. O., R. Thun, and H. Stocker. 1999. Effect of flunixin meglumine on placental expulsion in dairy cattle after a caesarean. Vet. Rec. 144:702e703. https://doi.org/10.1136/vr.144.25.702.

Walker, T. R., M. L. Land, A. Kartashov, T. M. Saslowsky, D. M. Lyerly, J. H. Boone, and P. A. Rufo. 2007. Fecal lactoferrin is a sensitive and specific marker of disease activity in children and young adults with inflammatory bowel disease. J. Pediatr. Gastroenterol. Nutr. 44:414–422. https://doi.org/10.1097/MPG.0b013e3180308d8e.

Wallon, C., P. C. Yang, A. V. Keita, A. C. Ericson, D. M. McKay, P. M. Sherman, M. H. Perdue, and J. D. Söderholm. 2008. Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. Gut 57:50-58. https://doi.org/10.1136/gut.2006.117549.

Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90:2788–2796. https://doi.org/10.3168/jds.2006-560.

Walsham, N., and R. Sherwood. 2016. Fecal calprotectin in inflammatory bowel disease. Clin. Exp. Gastroenterol. 9:21–29. https://doi.org/10.2147/CEG.S51902.

Walter, J., and R. Ley. 2011. The human gut microbiome: ecology and recent evolutionary changes. Annu. Rev. Microbiol. 65:41–129. https://doi.org/10.1146/annurev-micro-090110-102830

Wang, M. Y., Y. Li, M. Gao, L. W. Song, M. Xu, X. L. Zhao, Y. Jia, M. Zhao, Y. Y. Sun, and H. L. Hu. 2021. Effects of subacute ruminal acidosis on colon epithelial morphological structure, permeability, and expression of key tight junction proteins in dairy goats. J. Dairy Sci. 104:4260–4270. https://doi.org/10.3168/jds.2020-18738.

Wang, O., A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2018a. Interactions of the hindgut mucosa-associated microbiome with its host regulate shedding of Escherichia coli O157: H7 by cattle. Appl. Environ. Microbiol. 84:e01738–17. https://doi.org/10.1128/AEM.01738-17.

Wang, O., G. Liang, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger, and L. L. Guan. 2016. Comparative transcriptomic analysis of rectal tissue from beef steers revealed reduced host immunity in Escherichia coli O157: H7 super-shedders. PLoS One 11:e0151284. https://doi.org/10.1371/journal.pone.0151284.

Wang, S., R. Song, Z. Wang, Z. Jing, S. Wange, and J. Ma. 2018b. S100A8/A9 in Inflammation. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.01298.

Wang, Y., and T. A. McAllister. 2002. Rumen microbes, enzymes and feed digestion-A Review. Asian-Aust. J. Anim. Sci. 15:659–1676. https://doi.org/10.5713/ajas.2002.1659.

Washabau, R. J., M. J. Day, M. D. Willard, E. J. Hall, A. E. Jergens, J. Mansell, T. Minami, T. W. Bilzer. 2010. Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. J. Vet. Intern. Med. 24:10–26. https://doi.org/10.1111/j.1939-1676.2009.0443.x.

Weber, C., C. Hametner, A. Tuchscherer, B. Losand, E. Kanitz, W. Otten, S. P. Singh, R. M. Bruckmaier, F. Becker, W. Kanitz, and H. M. Hammon. 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and

lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96:165–180. https://doi.org/10.3168/jds.2012-5574.

Weiss, B. 2015. 2015. Optimizing and evaluating dry matter intake of dairy cows. Western Canadian Dairy Seminar Advances in Dairy Technology 27:189–200.

Wells, J. E., and J. B. Russell. 1996. Why do many ruminal bacteria die and lyse so quickly? J. Dairy Sci. 79:1487–1495. https://doi.org/10.3168/jds.S0022-0302(96)76508-6.

White H. M. 2015. The Role of TCA Cycle Anaplerosis in Ketosis and Fatty Liver in Periparturient Dairy Cows. Animals 18:793–802. https://doi.org/10.3390/ani5030384.

Wildman, E. E, G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501. https://doi.org/10.3168/jds.S0022-0302(82)82223-6.

Williams, C., D. David, and O. Iismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59:381–385. https://doi.org/10.1017/S002185960001546X.

Williams, E. J., D. P. Fischer, D. E. Noakes, G. C. W. England, A. Rycroft, H. Dobson, and I. M. Sheldon. 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68:549–559. https://doi.org/10.1016/j.theriogenology.2007.04.056.

Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. W. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63:102–117. https://doi.org/10.1016/j.theriogenology.2004.03.017.

Wolowczuk, I., C. Verwaerde, O. Viltart, A. Delanoye, M. Delacre, B. Pot, and C. Grangette. 2008. Feeding our immune system: impact on metabolism. Clin. Dev. Immunol. 2008:639803. https://doi.org/10.1155/2008/639803.

Wu, J. S. Zhu, F. Gu, T. G. Valencak, J. Liu, and H. Sun. 2022. Cross-tissue single-cell transcriptomic landscape reveals the key cell subtypes and their potential roles in the nutrient absorption and metabolism in dairy cattle. J. Adv. Res. 37:1–18. https://doi.org/10.1016/j.jare.2021.11.009.

Wu, Y., Y. Sun, R. Zhang, T. He, G. Huang, K. Tian, J. Liu, J. Chen, and G. Dong. 2021. Sodium butyrate more effectively mitigates the negative effects of high-concentrate diet in dairy cows than sodium B-hydroxybutyrate via reducing free bacterial cell wall components in rumen fluid and plasma. Toxins (Basel) 13:352. https://doi.org/10.3390/toxins13050352.

Xiao, T., S. Wu, C. Yan, C. Zhao, H. Jin, N. Yan, J. Xu, Y. Wu, C. Li, Q. Shao, and S. Xia. 2018. Butyrate upregulates the TLR4 expression and the phosphorylation of MAPKs and NK-κB in colon cancer cell in vitro. Oncol. Lett. 16:4439–4447. https://doi.org/10.3892/ol.2018.9201.

Yan, H., and K. M. Ajuwon. 2015. Mechanism of butyrate stimulation of triglyceride storage and adipokine expression during adipogenic differentiation of porcine stromovascular cells. PLoS One 10:e0145940. https://doi.org/10.1371/journal.pone.0145940.

Yang, W. Z., and K. A. Beauchemin. 2006. Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. J. Dairy Sci. 89:217–228. https://doi.org/10.3168/jds.S0022-0302(06)72086-0.

Yang, W. Z., and K. A. Beauchemin. 2007. Altering physically effective fiber intake through forage proportion and particle length: Chewing and ruminal pH J. Dairy Sci. 90:2826–2838. https://doi.org/10.3168/jds.2007-0032.

Yang, W. Z., and K.A. Beauchemin. 2009. Increasing physically effective fiber content of dairy cow diets through forage proportion versus forage chop length: Chewing and ruminal pH. J. Dairy Sci. 92:1603–1615. https://doi.org/10.3168/jds.2008-1379.

Yang, W. Z., K. A. Beauchemin, L. M. Rode. 2001. Barley processing, forage:concentrate, and forage length effects on chewing and digesta passage in lactating cows. J. Dairy Sci. 84:2709–2720. https://doi.org/10.3168/jds.S0022-0302(01)74725-X.

Yasuda, M., M. Fujino, T. Nasu, and T. Murakami. 2004. Histological studies on the ontogeny of bovine gut-associated lymphoid tissue: appearance of T cells and development of IgG+ and IgA+ cells in lymphoid follicles. Dev. Comp. Immunol. 28:357–369. https://doi.org/10.1016/j.dci.2003.09.013.

Yoshino, K., N. Katoh, K. Takahashi, and A. Yuasa. 1992. Purification of a protein from serum of cattle with hepatic lipidosis, and identification of the protein as haptoglobin. Am. J. Vet. Res. 53:951–956.

Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PloS One. 8:e80316. https://doi.org/10.1371/journal.pone.0080316

Yuan, M., N. Konstantopoulos, J. Lee, L. Hansen, Z.-W. Li, M. Karin, and S. E. Shoelson. 2001. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 293:1673–1677. https://doi.org/10.1126/science.1061620.

Zachut, M., H. Honig, S. Striem, Y. Zick, S. Boura-Halfon, and U. Moallem. 2013. Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. J. Dairy Sci. 96:5656–5669. https://doi.org/10.3168/jds.2012-6142.

Zarghi, A., and S. Arfaei. 2011. Selective cox-2 inhibitors: A review of their structure-activity relationships. Iran. J. Pharm. Sci. 10:655– 683.

Zarrin, M., O. Wellnitz, H. A. van Dorland, J. J. Gross, and R. M. Bruckmaier. 2014. Hyperketonemia during lipopolysaccharide-induced mastitis affects systemic and local intramammary metabolism in dairy cows J. Dairy Sci. 97:P3531-3541. https://doi.org/10.3168/jds.2013-7480.

Zebeli, Q., and B. U. Metzler-Zebeli. 2012. Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. Res. Vet. Sci. 93:1099–1108. https://doi.org/10.1016/j.rvsc.2012.02.004.

Zebeli, Q., J. R. Aschenbach, M. Tafaj, J. Boghun, B. N. Ametaj, and W. Drochner. 2012. Invited review: role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J. Dairy Sci. 95:1041–1056. https://doi.org/10.3168/jds.2011-4421.

Zebeli, Q., K. Ghareeb, E. Humer, B. U. Metzler-Zebeli, and U. Besenfelder, 2015. Nutrition, rumen health and inflammation in the transition period and their role on overall health and fertility in dairy cows. Res. Vet. Sci. 103:126–136. https://doi.org/10.1016/j.rvsc.2015.09.020

Zecconi, A., F. Albonico, M. E. Gelain, R. Piccinini, M. Cipolla, and M. Mortarino. 2018. Effects of herd and physiological status on variation of 16 immunological and

inflammatory parameters in dairy cows during drying off and the transition period. J. Dairy Res. 85:67–173. https://doi.org/10.1017/S0022029918000316.

Zhang Q., H. Su, F. Wang, Z. Cao, and S. Li. 2015. Effects of energy density in close-up diets and postpartum supplementation of extruded full-fat soybean on lactation performance and metabolic and hormonal status of dairy cows. J. Dairy Sci. 98:7115–7130. https://doi.org/10.3168/jds.2014-9112.

Zhang, K., G. Chang, T. Xu, L. Xu, J. Guo, D. Jin, and X. Shen. 2016. Lipopolysaccharide derived from the digestive tract activates inflammatory gene expression and inhibits casein synthesis in the mammary glands of lactating dairy cows. Oncotarget 7:9652–9665. https://doi.org/10.18632/oncotarget.7371.

Zhang, S., R. I. Albornoz, J. R. Aschenbach, D. R. Barreda, and G.B. Penner. 2013. Short-term feed restriction impairs the absorptive function of the reticulo-rumen and total tract barrier function in beef cattle. J. Anim. Sci. 91:1685–1695. https://doi.org/10.2527/jas2012-5669.

Zhao, C., G. Liu, X. Li, Y. Guan, Y. Wang, X. Yuan, G. Sun, Z. Wang, and X. Li. 2018. Inflammatory mechanism of Rumenitis in dairy cows with subacute ruminal acidosis. BMC Vet. Res. 14:135. https://doi.org/10.1186/s12917-018-1463-7.

Zimmer, D.B., J. O. Eubanks, D. Ramakrishnan, and M. F. Criscitiello. 2013. Evolution of the S100 family of calcium sensor proteins. Cell calcium, 53:170–179. https://doi.org/10.1016/j.ceca.2012.11.006.

Zotova, N. V., V. A. Chereshnev, and E. Yu Gusev. 2016. Systemic inflammation: methodological approaches to identification of the common pathological process. PLoS One 11:e0155138. https://doi.org/10.1371/journal.pone.0155138.