Relationship of high somatic cell counts in the milk prior to dry off with the incidence of periparturient diseases and milk yield in holstein dairy cows

by

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

Master of Science

in

Animal Science

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Abstract

Intramammary infections of dairy cows are characterized by elevation of milk somatic cell counts (SCC), which are mostly neutrophils. Infections can result in substantial production losses, increased costs for treatments, and veterinary services, besides discarding the antibiotic-treated milk and increased culling rates. The dry period for dairy cows occurs approximately 8 weeks prior to parturition and allows for maximization of milk production in the upcoming lactation. Dairy cows exhibit a state of immunosuppression between and around parturition resulting in high incidence of periparturient diseases. It has been suggested that bacteria and bacterial endotoxins originating from the infected mammary gland might play a significant role in the pathogenesis of periparturient diseases. Previous studies have shown that intramammary challenges with bacterial endotoxins trigger alterations in the blood and milk metabolites. Our lab has previously reported alterations in innate immune reactants and serum metabolite variables in dairy cows starting at -8 and -4 weeks prior to parturition and during diagnosis of several periparturient disease including subclinical mastitis, metritis, lameness, ketosis, milk fever and retained placenta suggesting chronic inflammatory state during the dry period.

The current study aimed to determine whether high SCC in dairy cows before dry off are related to the incidence of periparturient diseases as well as milk and blood alterations. Somatic cell counts were measured in 140 pregnant Holstein dairy cows one week before dry off, and were classified as either low SCC (<200,000 cells/mL) or high SCC (>200,000 cells/mL). All cows were monitored for the incidence of the major periparturient diseases including mastitis, metritis, lameness, ketosis, and retained placenta for the first 2 weeks after parturition. Results of the study showed that cows with high SCC before dry off had an increase in likeliness to be affected by ketosis. While not statistically significant, high SCC cows did have greater odds of being affected by metritis, lameness, and retained placenta compared to low SCC cows; however other factors could potentialy contribute to disease incidence. Intriguingly the odds ratio analysis indicated that the likeliness of cows to be affected by mastitis were similar for both groups of cows with low or high SCC.

Another important finding of this study was that cows with high SCC and affected by periparturient diseases produced significantly less milk during the first 60 DIM compared to cows with low SCC or the healthy ones. This was supported by the fact that cows with high SCC and affected by diseases had lower concentrations of lactose and increased concentrations of total milk

proteins prior to dry off. Lactose is consumed by bacteria during mammary infection and is the main osmotic metabolite that determines volume of milk and, therefore, milk yield; whereas milk proteins that increase during mammary infections are mostly related to mounting of an immune response to infectious pathogens.

Data also showed lower concentrations of glucose and cholesterol in the serum prior to dry off in cows with high SCC. Serum glucose is the main precursor of lactose synthesis and it has been shown that decreases during endotoxemia potentially related to mammary gland infection. In addition, cows with high SCC prior to dry off and diagnosed with ketosis exhibited higher concentrations of BHBA and NEFA after parturition where BHBA at +1 week was strongly correlated to SCC prior to dry off. Concentrations of BHBA in the serum were within normal ranges in cows affected by mastitis, metritis, retained placenta, and lameness. Concentrations of NEFA in the serum of cows with high SCC prior to dry off and diagnosed with mastitis, metritis, lameness, and retained placenta were higher in comparison to healthy cows. Concentrations of lactate in the serum of cows diagnosed with metritis were higher in both groups of cows with low and high SCC cows prior to dry off. Interestingly cows with low SCC prior to dry off and diagnosed with metritis exhibited greater concentrations of lactate after parturition.

Overall, results of this study indicate that high SCC prior to dry off can significantly influence the health status, metabolism, and productivity of dairy cows in their upcoming lactation. However, further research is warranted with regards to the reasons for high SCC prior to dry off and their effect on the overall health of dairy cows.

Preface

The idea, project proposal, and experimental design were developed by my supervisor Dr. Burim N. Ametaj. Dr. Ametaj supervised the training and conduction of the experiment, and development of the database for the experiment. He also supervised the laboratory analysis, and outline, writing, and editing of all the sections of this thesis. Dr. André Luiz Gracia Dias contributed to collection of samples and evaluation of clinical diseases for the whole experimental period. Dr. Erkan Pehlivan assisted with collection of samples and evaluation of clinical disease for part of the experimental period. Eduardo Barahona Rosales assisted with collection of samples, laboratory analyses, and evaluation of clinical disease for part of the experimental period. Suzanna M. Dunn provided assistance with laboratory analyses. I was responsible for collection of samples and data, as well as for the major part of sample analyses in the laboratory. The statistical analyses and manuscript composition were my original work.

This research project, of which this thesis is a part, received research ethics approval from the University of Alberta Animal Care and Use Committee for Livestock (Animal use protocol AUP#00001878) entitled ‘Developing a new technology to lower the incidence rate of several important periparturient diseases of transition dairy cows.

Acknowledgments

My sincerest gratitude towards all the people who have supported me and helped me throughout my Master's degree, which enabled me to complete an important step in my career.

First and foremost, I would like to extend a thank you to my supervisor Dr. Burim Ametaj for everything he has done for me to enhance my career. His guidance, support, and experience has provided me with further confidence in the career path I want to pursue. I am extremely grateful for the opportunity to work on projects including co-authorship for a chapter in Dr. Ametaj's book, which became my first publication. I am also extremely grateful to my supervisory committee member Dr. Vera Mazurak who is always willing to answers questions and provide suggestions during my studies. My thanks are extended to the final examiner Dr. Byeong Hwa Jeon name for their time and valuable advices on my Masters project.

My sincere gratitude towards Dr. Andre Luiz Gracias Dias who has helped a lot in the conduction of this project. His knowledge and veterinary experience has taught me a lot regarding the research field. My deepest thanks to my fellow Master's student and colleague Eduardo Barahona who has worked with me time and time again in order to complete our degrees. His patience and willingness to help is greatly appreciated. Many thanks Suzanna M. Dunn whose kindness and support these past two years has helped me through this degree. My thanks to Dr. Erkan Pehlivan for assistance with sample collection as well as Dr. Emily Eckel for her advice in improving my writing skills. My sincerest thanks to the Dairy Research and Technology Farm staff, and Sunalta Ltd. staff for their support throughout this animal trial. It was wonderful to work with all of you and your animals. I would also like to thank the undergraduate students who volunteered to assist collecting data.

My thanks to the Alberta Livestock and Meat Agency Ltd. for their financial support throughout this degree.

Finally, I want to extend a thank you to my friends and family in Atlantic Canada and Alberta. I want to extend a personal thank you to my parents Eileen and Dennis Egyedy. Your support in my career choices and assistance has helped me further my goals. My motivation and persistence is derived from your own, and has strengthened my own individuality.

March 21th 2018

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List of abbreviations

%	Percentage
-	Minus
x	Times
$	Dollar
>	Less than
<	More than
α	Alpha
β	Beta
γ	Gamma
µ	Micro
AIR	Adaptive immune response
Ag	Antigen
APP	Acute phase protein
APR	Acute phase response
BHBA	Beta-hydroxybutyric acid
CM	Clinical mastitis
d	Day
DAMPs	Damage-associated molecular patterns
DCT	Dry cow therapy
DIM	Days in milk
dL	deciliter
DMI	Dry matter intake
E. coli	*Escherichia coli*
H	Hour(s)
Hp	Haptoglobin
Ig	Immunoglobulin
IL-1	Interleukin-1
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8

IL-10	Interleukin-10
IIR	Innate Immune Response
IMI	Intramammary infection
IFN-γ	Interferon-gamma
kg	Kilogram
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
LIF	Leukemia inhibitory factor
M	Molarity (moles/liter)
MEC	Mammary epithelial cells
mg	milligrams
mg/dL	Milligrams/deciliter
mRNA	Messenger ribonucleic acid
MUN	Milk urea nitrogen
NEFA	Non-esterified fatty acids
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PAMPs	Pattern-associated molecular patterns
PMN	Polymorphonuclear neutrophil
PRRs	Pattern recognition receptors
Strep. agalactiae	*Streptococcus agalactiae*
Staph. aureus	*Staphylococcus aureus*
Strep. dysgalactiae	*Streptococcus dysgalactiae*
Strep. uberis	*Streptococcus uberis*
SAA	Serum amyloid A
SCC	Somatic cell counts
SCM	Subclinical mastitis
TLRs	Toll-like receptors
TNF-α	Tumour necrosis factor-alpha
TS	Total solids
TSLP	Thymic stromal lymphopoietin
µM	micromolar

WBC	White blood cell

Chapter 1 Literature Review

1.1 Mammary gland infections in dairy cows

1.1.1 Brief description of mammary gland infection

Mastitis is defined as an inflammation of the mammary gland of dairy cows caused by pathogenic bacteria. It is often associated with bacterial infection of the udder and is subdivided into subclinical mastitis (SCM; with no visual signs of infection) and clinical mastitis (CM; with visual signs of infection in the udder and milk; Shamra, 2007; Koeck et al., 2012). Subclinical and clinical mastitis negatively affect both milk quality and yield making mastitis a major economic concern for the dairy industry (Seegers et al., 2003). Indeed, a case of mastitis costs as an average US$253 (Pinzón-Sánchez et al., 2011). Production losses to dairy producers in the US alone can reach up to $2 billion dollars due to mastitis infection within a herd (Harmon, 1994a). According to the Government of Canada (2017) the number of cows culled in 2016 for mastitis reasons in Canada was 27,708. Given that the cost of a cull cow is C$880 then the economic loss to dairy industry only from mastitis is roughly CAD$24 million. This does not take into account financial losses due to other factors such as treatment, milk loss, labor for treatments, veterinary bills, medication cost, discarded milk, as well as many others.

1.1.2 Types of Mastitis

As previously described, mastitis is classified as either subclinical or clinical based on the presence of symptoms. Duration and severity of the disease has also been used to further categorize mastitis as chronic, subacute, acute, and peracute. A summary of the symptoms, somatic cell counts (SCC), and severity of each mastitis type is provided in Table 1-1.

1.1.3 Subclinical mastitis

Cows affected by subclinical mastitis do not display symptoms of infection such as alterations in milk appearance or udder swelling. However, SCM is associated with an increase in SCC, decreased milk production, presence of infectious pathogens in the milk, and alterations in

milk composition (Tylor et al., 1990). Subclinical mastitis is the most common udder infection found in Holstein dairy cows, affecting up to 36-50% of a herd (Wilson et al., 1997; Pitkala et al., 2004). Subclinical mastitis can also result in milk losses of up to 70% for dairy producers due to non-visible symptoms unless regular measurements of SCC are part of the post-partum management of the cows (Kirk and Bartlett, 1988). Cows affected by SCM do not reach their maximum milk yield potential; therefore, negatively affecting the profitability of dairy farms. Milk somatic cell counts are the main indicator in determining a subclinical mammary gland infection. A SCC in the milk of mammary glands of less than 100,000 cells/mL is considered to be a healthy mammary gland (Kromker et al., 2001; Djabri et al., 2002), while values of SCC >100,000 cells/mL are suggestive of mastitis. In Canada, a threshold concentration of 200,000 cells/mL is used for distinguishing between healthy udders from the diseased ones (Madouasse et al., 2010). There are several factors that can influence the SCC including cow's age, breed, stage of lactation, and milk yield (Nyman et al., 2014).

1.1.4 Clinical mastitis

Based on duration of disease CM is classified as: chronic, subacute, acute, and peracute (Table 1; Klocke et al., 2007; Nickerson, 2011). During CM symptoms of disease are visible on the mammary gland including swollen or hardened udder, heat, pain, and changes in the milk consistency (Roberson, 2012). Chronic cases are the least severe, compared to the other types of CM, as there is a change in milk consistency visible and infection can persist for long periods of time where culling is often recommended (Klocke et al., 2007; Nickerson, 2011; Roberson, 2012). During subacute mastitis there is a slight inflammation and swelling to the mammary gland accompanied by a change in milk consistency (Klocke et al., 2007; Nickerson, 2011). Subacute mastitis is the most common type of CM affecting around 10-50% of a herd. The onset of an acute clinical infection is relatively quick, and symptoms include the mammary gland appearing red, swollen, hard, and changes in milk consistency. Cows with acute CM also experience systemic symptoms including pain, reduced appetite, reduced rumen function, increase heart rate, fever, depression, and weakness. Peracute mastitis has an extremely severe and rapid onset often resulting in mortality of the animal (Nickerson, 2011). Both acute and peracute clinical infections are extremely severe for the cow as infection affects the animal systemically.

1.1.5 Mode of infection by bacteria that cause intramammary infections

Bacterial invasion of the mammary gland is the primary cause of an intramammary infection (IMI) in dairy cows. There are multiple microbial pathogens including Gram-negative and Gram-positive organisms that invade the mammary gland. Pathogens can invade the mammary gland directly from the environment or from animal-to-animal contact, and this is referred to as environmental mastitis and contagious mastitis (Crist et al., 1997).

Environmental mastitis is primarily caused by pathogens located within the cow's environment (Crist et al., 1997), including the soil, contaminated water, manure, and bedding. The most common environmental mastitis-causing pathogens include coliform organisms such as *Escherichia coli*, and environmental streptococci such as *Streptococcus uberis*. Approximately 70-80% of CM cases are mainly caused by coliform organisms. Coliform infections have been found to have a short duration of 10 days within the mammary gland (Harmon, 1994a). However, studies have found that 1.5% of *E. coli* infections have duration of over 100 days (Crist et al., 1997). The difference in duration could be based on the serotype of the pathogen that is causing infection. In comparison to streptococci organisms, the duration has been found to be 3 times longer within the mammary gland (Harmon, 1994a).

In contagious mastitis the mode of infection for bacteria is from udder-to-udder, typically by the milker or milk machines. This is why it has been extremely emphasized to wash, dry, and dip each teat during milking time in order to reduce spreading. The main pathogen of concern for contagious mastitis is *Staphylococcus aureus* (Crist et al., 1997), which has shown to be one of the more difficult pathogens to control and treat (Barkema et al., 2006). In a study done by McDougall et al. (2007) it was found that the majority of CM cases were caused by *S. uberis* and *S. aureus*.

1.1.6 Importance of somatic cell counts for diagnosis of mammary gland infections

There are various methods used for diagnosis of mastitis in dairy cows. The most efficient method of is by determining the SCC in the milk and presence of inflammation (Smith and Hogan, 1999). In healthy mammary glands somatic cells present consist of macrophages, neutrophils, lymphocytes, and shedded mammary epithelial cells (Dairyman's Digest, 2009; Napel et al.,

2009). Various countries worldwide have different regulations with regards to the level of somatic cells acceptable in the milk either on a bulk-tank or per cow basis. For example, the limit for raw milk SCC in Canada is 400,000 cells/mL (Shamra et al., 2011) while the International Dairy Federation (1997) states that SCC should be less than 200,000 cells/mL. The United States Department of Agriculture (2012) allows a SCC of 750,000 cells/mL, while other countries such as Brazil have a limit of 1,000,000 cells/mL. According to Kromker et al. (2001) SCC of dairy cows should be 100,000 cells/mL or less; anything exceeding would indicate presence of infection.

Determining cows with a high SCC can be done by lab analysis or using the California Mastitis Test (CMT). The CMT was developed by Schalm and Noorlander (1957) and is now used worldwide for identifying mastitis in dairy-producing animals. The benefit of using a CMT is that it's an easy method to quickly determine SCC in milk collected from individual quarters (Sanford et al., 2006). A sample of milk from each quarter is deposited into a well where detergent is then added causing lysis to the external membrane (lipoprotein membrane) of the somatic cells exposing the gel-like DNA. Indication of a mastitis infection is based on the gel consistency and color that is formed where the darker the color indicates high SCC (Pradieé et al., 2012).

Detecting a mammary gland infection through the use of CMT has shown to be correlated with the quantity of SCC present in the milk, making CMT a beneficial method for determining mastitis (Schalm and Noorlander, 1957). The procedure and the scoring system for a CMT was described in a study by Bhutto et al. (2012) who performed a 1:1000 dilution of milk samples with 3% sodium lauryl sulphate and broocresol. Samples were separated into wells where the plate was then rotated. The rotation of the plate lead to a color change or gel formation, and a score was given based of the strength of the color. The scoring system for a CMT is on a scale of 0-4 where 0 indicates no reaction, 1 for a slight reaction, 2 for a weak positive, 3 for a confirmed positive, and 4 for a high positive.

Dairy cows approaching dry off are susceptible to new IMI (intramammary infections); therefore, the CMT can be a useful tool in determining cows with high SCC prior to dry off. Poutrel and Rainard (1981) were able to accurately determined 80% of new IMI using the CMT. In the same study by Bhutto et al. (2012) it was reported that cows had significantly higher CMT scores at dry off rather than a week prior to dry off. However, the CMT has been found to produce less reliable scores in determining cows with mastitis post-partum (Poutrel and Rainard, 1981). This is a major drawback as the incidence of disease in dairy cows is higher after parturition.

1.1.7 Additional factors that cause elevation of somatic cells in mammary gland

Other factors should be taken into consideration when looking at SCC to determine mammary gland infections including stage in lactation, age, breed, stress level, and season (Shamra et al., 2011). According to Kehrli and Shuster (1994) somatic cells can increase within 6 hours following bacterial invasion. Specific mammary quarters have also been found to be highly susceptible to bacterial infections (Harmon, 1994b). For example, Harmon (1994b) indicates hind quarters have a higher SCC compared with the front ones, and Dhakal (2006) found that the right quarters had a higher SCC than the left quarters. Therefore, the position of the mammary quarters could potentially increase the cow's susceptibility to mammary gland infections.

1.2 Role of the dry period in dairy cows

1.2.1 Background and importance of the dry period on cow's performance

The dry period can be defined as the period in which the cow is no longer lactating but is undergoing nutritional and metabolic changes, and also changes to the mammary gland (Dingwell et al., 2001). The dry period is important for cows to prepare for next calving and lactation. Previous literature found that cows with a dry period have increased milk yield in the next lactation compared to cows with no dry period (Swanson and Poffenbarger, 1979; Jones 2009). During the dry period, the mammary gland regenerates the milk-secreting tissue that was damaged from previous lactation (Jones, 2009; Sjaastad et al., 2016).

Additionally, the length of the dry period can also significantly influence milk yield in the next lactation. The dry period typically begins at 60 d prior to parturition; however, DHI recommends the dry period to be a minimum of 40 d prior to parturition (Jones, 2009). Studies have found that cows with a dry period of 40 d significantly reduced milk yield in the next lactation, compared to cows dried at 60 d (Jones, 2009). Cows that are continuously milked with no dry period experience a 33% drop in production in the next lactation (Swanson and Poffenbarger, 1979). Funk et al. (1987) found that cows with a dry period of 60-69 d had an increase in milk yield by 459 kg following parturition. A study by Watters et al. (2008) showed that cows with a shorter dry period of 34 d had a significant decrease in milk yield by 2.1 kg/d

compared to cows with a dry period of 55 d following parturition. Other studies have also confirmed that cows with a 40 d dry period had a decrease in milk yield (Watters et al., 2008). We can therefore conclude that if maximum production is to be achieved cows should have approximately 60 d dry off period or greater.

All cows entering the dry period are administered an antibiotic that helps prevent infection of the mammary gland. The use and benefits of antibiotic treatments at dry off includes administering antibiotics in higher dosages without having to discard milk, reducing infectious pathogens, regeneration of damaged mammary tissue, and reduced incidence of CM (Jones, 2009). Further discussion on the use, benefits, and drawbacks of antibiotics will be addressed in Section 1.7. The mammary gland also undergoes various physiological changes when dry cow therapy is administered. Such changes will prepare the mammary gland for parturition and next lactation.

1.2.2 Physiological changes to mammary gland during the dry period

A cow entering the dry period experiences various physiological changes including nutritional, metabolic, and to the mammary gland (Oliver and Sordillo, 1988; Dingwell et al., 2001). There are 3 stages through which the mammary gland undergoes during the dry period in preparation for the next lactation (Arnold, 2012). These stages are involution, steady state involution, and colostrogenesis.

Involution occurs during the first 3 weeks of the dry period, and the mammary gland ceases milk synthesis. Regression of the alveolar epithelial cells occurs when milk builds up in the mammary gland, increasing pressure and decreasing secretion (Oliver and Sordillo, 1988; Arnold, 2012). Atrophy of epithelial cells by the increasing pressure from milk accumulation is known as pressure atrophy (Senger, 2012). Pressure atrophy is defined as a force that causes the secretory cells to cease milk secretion (Senger, 2012). The increase in pressure on the alveolar milk-secreting cells lowers blood flow to the capillaries and the mammary gland appears swollen (Sjaastad et al., 2016). In the alveolar epithelium milk secretion decreases and secretory cells becomes non-functional for the duration of the dry period (Senger, 2012). Additionally, local macrophages and neutrophils remove the apoptotic mammary epithelial cells and pathogens present in the mammary gland (Sjaastad et al., 2016).

During involution there is a build-up of a keratin plug within the teat canal that functions as a protective barrier to prevent bacteria from entering the mammary gland during the dry period (Arnold, 2012). However, the involution stage poses a high risk of bacterial infection due to accumulation of milk and bacteria not being removed, thereby increasing potential risk of infection during the dry period. It takes approximately 21-30 days for the involution process to complete. Previous studies have shown that at approximately 10 d into the dry period, 50% of the quarters have not formed the keratin plug in the teat canal making the quarter susceptible to bacterial invasion. Around 5% of quarters were found to be open at 60 d. Dry cow therapy treatments are administered at the beginning of the dry period; however, they are not effective throughout the entire dry period meaning that cows are not completely protected from new IMI occurring at parturition. Full protection of the mammary gland can be achieved by administering dry cow therapy in combination with a teat sealant. The combination of dry cow therapy with a teat sealant has shown to lower the incidence of new IMI by 7.3% (Arnold, 2012).

Senger (2012) indicates that the involution stage is crucial during the lactation cycle of dairy cows. In relation to milk yield a shorter dry period resulting in a decrease milk yield for the next lactation could be due to lack of restoration of the milk-secreting tissues. Thus, maximum production can be achieved in the subsequent lactation if the cow has a sufficient dry period, ensuring tissue regeneration.

The second stage in the dry off period is the steady state involution (also known as involuted), which is where the mammary gland is protected from entrance of bacteria from outside, and resistant to infection due to the keratin plug in the streak canal (Arnold, 2012).

Colostrogenesis is the third and final stage of the dry period, and also known as the transition period (Arnold, 2012). This stage occurs towards the end of gestation where the mammary gland undergoes physiological changes opposite to that of involution. In response to hormones prolactin, adrenal cortical hormones, and placental lactogen secreted from the pituitary gland, adrenal gland, and placenta, respectively, alveoli are stimulated to begin milk synthesis (Senger, 2012). The secretory tissue undergoes differentiation and intense growth along with secretion of proteins, lipids, and lactose as part of colostrum (Oliver and Bushe, 1986; Sordillo et al., 1987; Sordillo & Nickerson, 1988). Bacterial infection can increase during colostrogenesis as a result of breakdown of the keratin plug within the teat canal, thus increasing exposure to foreign pathogens. Leukocytes, which are white blood cells including neutrophils, have been shown to be

under a state of immunosuppression during the transition period (Kehrli et al., 1989). Approximately 95% of new IMI have been shown to occur 2-3 weeks prior to parturition (Arnold, 2012).

1.2.3 Other physiological changes during dry period and susceptibility to new infections

During the dry period the fetus will enter into its final stages of growth (Dingwell et al., 2001). Two-thirds of fetal growth is completed during the dry period, and often will cause body maintenance to prioritize the fetus over the dam. This can cause high metabolic stress towards the end of gestation results in immunosuppression and increased susceptibility to new IMI and other metabolic diseases (Goff and Horst, 1997; Mallard et al., 1998; Ametaj et al., 2005). It has also indicated that incidence of new IMI is highest during involution stage of the dry period and towards the end of gestation (Oliver and Bushe, 1986). Moreover, Natzke et al. (1975) indicates a positive correlation between cows dried off with a pre-existing mammary gland infections to the incidence of new IMI during the dry period. This could possibly be due to increased stress around parturition, and leakage of colostrum from the udder thus allowing bacteria to enter the mammary gland. Although bacterial infection increases around parturition, administration of dry cow therapy at dry off is beneficial in lowering the risk of new IMI.

1.3 Impact of mammary gland infection on a dairy herd

The incidence of a mammary gland infection in a dairy herd has tremendous impact on milk production, cow health, and economic loss. Milk production can suffer in the subsequent lactation from a mammary gland infection if cows have a short dry off period, and tissue regeneration is not fully achieved. During lactation the milk production can decrease in cows with mastitis as bacteria utilize the milk secreting cells. A decrease in milk production also results in economic loss for producers. Furthermore, mammary gland infections can impact the health of the cow including display of abnormal behavior and systemic signs, depending on severity of infection (Siivonen et al., 2011).

1.3.1 Mammary gland infections on milk production

It has been well established that an IMI results in decline of milk production. More specifically, the physical damage exerted on the mammary epithelial cells as a result of infection causes a reduction in both the synthesis and secretion of milk (Petrovski and Stefanov, 2006). The lactating bovine mammary gland is composed of a network of ducts that terminate at the alveolar clusters. The alveolar clusters are lined with mammary epithelial cells which secrete milk. Connection of the mammary epithelial cells is achieved by the apical junction complex which is composed of adherens and tight junctions (McManaman and Neville, 2003). Tight junctions link adjacent epithelial cells by forming a narrow, continuous seal surrounding each cell at the apical border. The main function of tight junctions is to coordinate the movement of materials between cells and preventing leakage of milk components into the systemic circulation (Nguyen and Neville, 1998; Petrovski and Stefanov, 2006).

During infection bacteria release endotoxins that induce an influx of leukocytes into the mammary gland, and secretion of inflammatory mediators (Petrovski and Stefanov, 2006). The influx of leukocytes into the mammary gland results in disruption of the tight junctions causing the mammary epithelial cells to lose their integrity and decrease milk synthesis (Auldist et al., 1995; Nguyen and Neville, 1998; Bruckmaier et al., 2004). Permeability of the blood-milk barrier is also increased as a result of infection leading to a decrease in volume and milk components (Shuster et al., 1991). Additionally, the role of lactose in the mammary gland is for osmotic regulation of milk volume. The reduction in lactose synthesis can therefore contribute to further decline in milk production (Auldist and Hubble, 1998).

Physical damage to the mammary epithelium is not the only cause for the decline in milk production during mammary gland infections. Affected mammary quarters can cause a decline in milk production in healthy mammary quarters as a result of systemic infection (Shuster et al., 1991). The secretion of inflammatory compounds including cytokines and arachidonic acid can alter the stimulatory or inhibiting hormone concentration causing reduced milk precursor uptake. However, the decline in milk production is evident in affected quarters as opposed to healthy ones since inflammation is localized within the sick quarters. Moreover, secretion of local inflammatory mediators, leukocytosis, and mammary edema in the affected quarters can further decline milk production (Shuster et al., 2011). Cows may also experience a decline in milk yield during other periparturient diseases such as uterine infections (LeBlanc, 2002), ruminal acidosis (Nocek, 1997),

lameness (Warnick et al., 2001), ketosis (Gordon et al., 2013), and retained placenta (Dervishi et al., 2016a).

1.3.2 Mammary gland infections on milk composition

An infection of the mammary gland not only affects the milk production in dairy cow but alters the composition of milk. Milk is composed of various proteins, fats, carbohydrates, minerals, vitamins, hormones, enzymes, ions, cells, and water. Concentrations of milk constituents tends to fluctuate during mammary gland infections depending on the pathogen present, immune response, and severity of infection.

During a mastitis infection the concentration of protein in milk will either increase or decrease. The increase in protein in the milk may be a influx of blood-borne proteins including serum albumin and immunoglobulins into the milk in response to bacterial endotoxins and tight junction disruption (Shuster et al., 1991; Auldist et al., 1995; Auldist and Hubble, 1998). This is mainly due to the increase in humoral or antibody-mediated immunity that results in an increase in immunoglobulins which are important for combating infection, and will be further discussed in Section 1.5.2 (Shuster et al., 1991; Auldist et al., 1995; Audist and Hubble, 1998).

Lactoferrin has also shown to increase during mammary gland infections (Petrovski and Stefanov, 2006). Lactoferrin is an iron-binding protein synthesized by the mammary epithelial cells and functions in competing with bacteria for free iron in order to decrease bacterial growth (Ward et al., 2002). Lactoferrin can also bind to bacterial membrane surfaces thereby altering the integrity and permeability of the cell walls resulting in cell destruction (Diarra et al., 2002). According to Smith and Oliver (1981) the concentrations of lactoferrin have a 100-fold increase during the involution stage of the dry period and less during lactation. Additionally, transferrin also an iron-binding protein taken up from the blood will increase during infection (Ollivier-Bousquet, 1998).

Caseins are phosphorylated proteins that account for 80% of the total protein found in the mammary gland (Sjaastad et al., 2016). The main function of caseins is providing amino acids to the newborn and binding of calcium and phosphorous within the Golgi apparatus of the mammary epithelial cells forming casein micelles which are important for skeletal growth in neonates (Sjaastad et al., 2016). Casein concentrations tend to decrease during infection unlike lactoferrin,

immunoglobulins, and serum albumin which increase. The decrease in casein is largely due to secreted proteinases by infectious pathogens and leukocytes, or the blood as a result of disruption of the blood-milk barrier (Auldist and Hubble, 1998).

Milk protein also consists of whey proteins including α-lactalbumin, β-lactoglobulin, serum albumin, and immunoglobulins. Both α-lactalbumin and β-lactoglobulin decrease in cows with a mammary gland infection largely due to the decline in synthesis and secretory activity (Auldist and Hubble, 1998). Studies have observed a reduction in both α-lactalbumin and β-lactoglobulin in milk of high SCC cows (Rogers et al., 1989). The reduction in these proteins could be in part due to the decrease in synthesis and secretory function as well as protein leakage from the mammary gland (McFadden et al., 1988). Auldist and Hubble (1998) suggest that the decline in α-lactalbumin and β-lactoglobulin could be due to protein leakage as a result of tight junction disruption. McFadden et al. (1988) observed elevated concentrations of α-lactalbumin in the blood of high SCC dairy cows. The role of α-lactalbumin in the mammary gland is in the synthesis of lactose whereby binding the regulatory unit of lactose synthase induces synthesis (Sjaastad et al., 2016). Therefore, decreased synthesis of α-lactalbumin can result in a decreased synthesis of lactose in high SCC cows.

Additional enzymes found in milk have also shown to be increased during a mastitis infection (Holdaway, 1990; Auldist and Hubble, 1998). The enzyme plasmin, a caseinolytic enzyme derived from plasminogen found in the blood (Petrovski and Stefanov, 2006). The main function of plasmin is for dissolving blood clots; in the milk plasmin cleaves β-casein into γ-casein (Holdaway, 1990; Auldist and Hubble, 1998; Petrovski and Stefanov, 2006). It has been suggested that elevated plasmin activity in high SCC is attributed to leakage into the mammary gland from the blood (Zachos et al., 1992). Neutrophil granules contain various bactericidal peptides including defensins, enzymes, and neutral and acidic proteases that are able to destroy various mastitis-causing pathogens (Linde et al., 2008). These proteases along with plasmin are able to permit chemotaxis of cells to the area of inflammation during immune response (Moussaoui et al., 2004). The increase in plasmin is therefore, important in the immune host response IMI.

Although much of the research has mainly focused on milk protein alterations during mammary gland infections, conflicting reports have found milk fat to decrease (Auldist and Hubble, 1998) while others have reported an increase in milk fat (Shuster et al., 1991; Bruckmaier et al., 2004). Synthesis of milk fat occurs in the rough endoplasmic reticulum of mammary

epithelial cells (Sjaastad et al., 2016). Bruckmaier et al. (2004) reported an increase in fat content, a reduction in lactose synthesis, and therefore a decline in milk production. Holdaway (1990) also indicated the decline in milk synthesis will eventually cause milk fat to decline. Moreover, unlike lactose which leaks from the milk along with water; milk fat remains contained within the alveolar lumen due to the large size of lipid droplets unable to move through the disrupted tight junctions (Holdaway, 1990). Leukocytes produce lipase enzymes during infections that can act on the fat globules causing oxidation of fatty acids and breakdown of triglycerides (Holdaway, 1990; Auldist et al., 1995; Audist and Hubble, 1998). Additionally, the phagocytotic ability of neutrophils can result in further decline of milk fat (Opdebeeck, 1982) along with the decrease in synthesis and secretory function of the mammary gland (Auldist and Hubble, 1998). Higher SCC in cows has also been associated with spontaneous lipolysis of milk fat (Holdaway, 1990).

Lactose is the main carbohydrate found in the milk, and is a disaccharide consisting of glucose and galactose molecule (Sjaastad et al., 2016). Lactose synthesis occurs within the Golgi apparatus of the mammary epithelial cells. Approximately 60-70% of plasma glucose is used for lactose synthesis (Sjaastad et al., 2016). As briefly mentioned in Section 1.3.1 on milk production, lactose is the osmotic regulator for milk volume in lactating mammary glands. During a mammary gland infection, the concentration of lactose tends to decline partly due to the damaged mammary epithelium resulting in reduce lactose synthesis (Auldist and Hubble, 1998; Bruckmaier et al., 2004). The reduction in lactose is caused by the increased gap of the tight junctions resulting in lactose being transported through the paracellular pathway out of the mammary gland and into the systemic circulation (Auldist et al., 1995). Bruckmaier et al. (2004) stated the decline in lactose concentration in the milk is dependent on the amount of damage to the tight junctions. Other sources have also observed elevated levels of lactose in both blood and urine in cows with mastitis (Auldist and Hubble, 1998; Nguyen and Neville, 1998; Bruckmaier et al., 2004). Specific bacteria are able to ferment lactose causing further decline of lactose concentration (Auldist et al., 1995).

Mammary gland infections result in alterations of mineral concentrations in the milk. Potassium is the most abundant mineral found in milk (Auldist et al., 1995) present in high concentrations compared to sodium (Holdaway, 1990). During mastitis, potassium leaks into blood from the mammary gland via the paracellular pathway causing a decrease in its concentration (Auldist et al., 1995; Auldist and Hubble, 1998). Conversely, sodium leaks from the blood into the mammary gland causing an increase in sodium concentrations in the milk (Auldist et al., 1995;

Auldist and Hubble, 1998). Additionally, chloride concentrations in the milk also increase during mastitis likely due to the influx of blood constituents into the mammary gland (Auldist and Hubble, 1998).

1.3.3 Mammary gland infections on dairy cow health

Animals will often display abnormal behavior that indicates presence of disease (Siivonen et al., 2011). Invasion by pathogenic organisms is energy-demanding for the cow, especially when the immune system must be altered to combat infection and recovery. Infection can cause a decrease in normal behavior including socialization, grooming, and feeding behaviors (Johnson, 2002). Acute mastitis causes visible symptoms of disease such as a decrease in rumination (Siivonen et al., 2011). Siivonen et al. (2011) found that cows with endotoxin-induced acute mastitis displayed poor appetite and a decrease in rumen function.

Additionally, it has been observed that sick animals are reluctant to lie down and those with a swollen udder and fever spend more time standing (Siivonen et al., 2011). Danzter (2001) also points out that less lying down time is indication of disease. Lying behavior is extremely important for dairy cows (Jensen et al., 2004) as it provides time for the cow to ruminate which can maximize milk production. If a cow is sick then there is reluctance to lie down and ruminate, negatively influencing milk production. The pain sensation in the cow's udder during infection could explain the reluctance to lie down but rather stand in order to avoid putting pressure on the swollen mammary gland (Siivonen et al., 2011). Fogsgaard et al. (2011) also found similar behavioral changes in cows with *E. coli*-induced mastitis including a decrease in feeding, rumination, grooming, and lying behavior. Changes in the normal behavior can be used as an indication for pain (Weary et al., 2006).

1.3.4 Economic implications of mammary gland infections

Infection of the mammary gland also contributes to economic loss for dairy producers due to the decrease in milk production. Subclinical mastitis which is a non-symptomatic form of mastitis infection can result in a 70% decrease in milk production (Kirk and Bartlett, 1988). Crist

et al. (1997) indicates that the economic loss due to mastitis can be up to $184 on a per cow basis, and $18,400 per 100 cow herd. The National Mastitis Council (1996) estimates 66% of production losses from a decrease in milk production due to a mastitis infection. The other 34% of losses would be for treating the sick cow including veterinary bills, cost of treatments, extra labor, animal replacement, and discarding milk (National Mastitis Council, 1996). The economic loss from cows with mastitis is tremendous. Therefore, producers should routinely check their herd for mastitis and take necessary measures to prevent further production loss.

1.4 Microbial pathogens that cause mammary gland infections

1.4.1 Gram-positive pathogens

Staphylococcus aureus is a major mastitis-causing pathogen producing both subclinical and clinical infections, and occurring more frequently in tie-stall housing systems (Barkema et al., 2006; Olde Riekerink et al., 2008). The incidence of *S. aureus* infections has been found higher during early lactation and decrease further into lactation (Persson Waller et al., 2009). Sol et al. (1997) sampled 143 *S. aureus* infected quarters 7 d prior to antibiotic treatment, day of treatment, 16 d and 30d after treatment. According to the authors it was found at 30 d after treatment 34% of infected quarters were still culture-negative (Sol et al., 1997). *Staphylococcus aureus* is able to colonize within the mammary gland and release lipoteichoic acid (LTA), a constituent of the cell wall (Ryan and Ray, 2004). Lipoteichoic acid can result in necrosis to the milk secreting tissue thereby decreasing milk production (Kerro Dego et al., 2002).

Immune response has shown to be slower during *S. aureus* infection due to limited release of pro-inflammatory cytokines (Bannerman et al., 2004). Cytokine expression in a *S. aureus* infection has shown to be 5% lower compared to *E. coli* infection (Guntler et al., 2010). Riollet et al. (2000) observed lack of pro-inflammatory cytokine expression including interleukin- 1 (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-8 (IL-8) in *S. aureus* infected milk. Rainard et al. (2008) observed an increase in the concentrations of IL-1 and chemokines but no changes in TNF-α concentration. During immune response IL-1 and TNF-α are important mediators of the inflammatory response within the host. Additionally, elevated TNF-α concentrations produce inflammatory symptoms including heat, redness, swelling, and pain (Khan, 2008). The main function of IL-8 is a potent chemoattractant in neutrophil migration and degranulation, enhancing

microbicidal activity of neutrophils, and stimulating phagocytosis (Alnakip et al., 2014). Lara-Zarate et al. (2011) suggested *S. aureus* is able to impair the nuclear factor κB (NF-κB) system and decrease cytokine expression. Nuclear factor κB is known as a transcription factor that increases pro-inflammatory cytokine production and inducible nitric oxide synthase (Eckel and Ametaj, 2016).

Another characteristic of *S. aureus* is the ability to produce a biofilm during pathogenesis which protects the pathogen from phagocytosis by neutrophils and macrophages (Kerro Dogo et al., 2002). Chronic mastitis infections have also been found to be caused by Gram-positive bacteria possibly due to the formation of the biofilm (Gilbert et al., 2013). A recent study by Giovannini et al. (2017) compared the physiological and behavioral effects of pain in cows infused with LPS and LTA. Their findings displayed a higher degree of pain and discomfort in LPS-infused cows compared to LTA (Giovannini et al., 2017). Naturally occurring *Staphylococcal* mastitis was found to induce chronic subclinical cases compared to acute clinical cases caused by *E. coli* (Wall et al., 2016). These findings further support LTA's role in chronic mastitis infections, and slower immune response. Indeed, LTA has shown to elicit a weaker effect on vascular permeability compared to LPS (Rainard et al., 2008; Giovannini et al., 2017).

Streptococcus uberis is another Gram-positive pathogen found in pasture, free-stall, and tie-stall based systems (Olde Riekerink et al., 2008; Petersson-Wolfe, 2012). Countries including Canada, United States, the Netherlands, and the United Kingdom have found *S. uberis* to produce 14 to 26% clinical symptoms, where 33% occurrence is greatest in the United Kingdom (Hogan and Smith, 1997). Interestingly 50-60% of *S. uberis* infections were found in cows housed in straw yards (NIRD, 1970; NIRD, 1974). Research of *S. uberis* is lacking but there have been various speculations on the exact mechanisms. Different strains of *S. uberis* have also been found to be resistant to phagocytosis by the neutrophils, allowing for colonization and clinical symptoms to be produced (Leigh, 1999). It is speculated that *S. uberis* strains produce a capsule for protection against neutrophils and macrophages; however, the mechanism remains unclear (Leigh and Field, 1991). Thomas et al. (1994) found *S. uberis* established within the secretory alveoli and ductular tissues suggesting colonization occurs in the milk secreting tissues. *Streptococcus uberis* also produces the LTA endotoxin similar to that of *S. aureus* (Leight et al., 2010).

1.4.2 Gram-negative pathogens

Gram-negative bacteria are major pathogens involved in environmental mastitis, where coliform pathogens are the predominate organisms including *Escherichia, Klebsiella,* and *Enterobacter* (Koneman et al., 1983). *Escherichia coli* harbors within the gastrointestinal tract of ruminants often at normal, non-pathogenic coliform forming unit (cfu) levels (Hogan and Smith, 2003). *Escherichia coli* has also been found in uterine tract of cows with uterine infections (metritis) (Sheldon et al., 2002). Interestingly, coliform pathogens are able to multiply within the mammary gland without adhering to the epithelial tissue surfaces, possibly due to the ability to utilize lactose, the main carbohydrate in milk. The mammary gland itself has low oxygen levels making this an ideal environment for coliform colonization. These factors can enable coliform bacteria numbers to increase, which are positively correlated to severity of the mammary infection. For example, coliform population can reach 10^8 cfu/mL of milk (Hogan and Smith, 2003) whereas some Gram-negative bacteria such as *Serratia* and *Pseudomonas* are not lactose fermenters and typically don't exceed 10^4 cfu/mL of milk (Hogan and Smith, 2003).

Susceptibility to new IMI increases during the first 2 weeks of the dry period and 2 weeks prior to parturition (Oliver and Bushe, 1986). Certain serotypes of bacteria also require iron, which is necessary for their growth and survival, but is bound, during dry-off, by lactoferrin (Smith and Schanbacher, 1976). As previously discussed in section 1.3.2 on protein alterations during mammary gland infections, lactoferrin increases during involution and remains constant throughout the dry period until colostrogenesis when it decreases. Bacteria such as *Klebsiella pneumoniae* and *Enterobacter* are able to overcome the iron-binding abilities by lactoferrin, and thereby cause mammary gland infection during the dry off period (Todhunter et al., 1991).

The release of lipopolysaccharide (LPS) is triggered by death of the pathogens or the toxins are released in the form of vesicles (Mashburn-Warren et al., 2008). Lipopolysaccharide can also translocate from the mammary gland into the systemic circulation, and contribute to clinical symptoms including fever, dehydration, anorexia, and diarrhea (Hogan and Smith, 2003). Hakogi et al. (1989) found an 18-fold increase in plasma LPS concentration in cows with mastitis compared with healthy cows. Dosogne et al. (2002) performed intramammary infusions of LPS, which increased plasma LPS levels in mastitis-affected cows with concentrations from 55-134 pg/mL compared to healthy cows of 10 pg/mL. Both studies confirm that infusion of LPS into the

mammary gland is able to translocate into blood circulation. Eckel and Ametaj (2016) also suggested 3 potential sources of LPS that translocate into systemic circulation including rumen, mammary gland, and reproductive tract. The mechanisms of endotoxin translocation into systemic circulation will be discussed further in section 1.6.

1.5 Immune response during mammary gland infections

During infection by bacteria the host triggers an immune response in order to combat infection. The innate immune response is the non-specific initial response by the host that recognizes the pathogens and triggers release of inflammatory mediators including cytokines. The adaptive immune response is the secondary response by the host. This response is much more prolonged as it functions in memorizing a particular bacterial strain in order to prevent further bacterial invasion. Both innate and adaptive immunity play major roles in the host defense during bacterial infection.

1.5.1 Innate immunity

The innate immune response is the first line of defense against invading bacteria. More specifically, activation of innate immunity in the mammary gland is triggered by recognition of invading organisms and the initiation of an inflammatory response (Rainard and Riollet, 2006). Innate immune response of the mammary gland includes a series of cellular (e.g., leukocytes) and humoral defenses (e.g., cytokines, complement system, lactoferrin, transferrin, lysozyme, and the lactoperoxidase/myeloperoxidase systems) as well as oligosaccharides, gangliosaccharides, reactive oxygen species, acute phase proteins (APPs), ribonucleases, and various antimicrobial proteins and peptides (Rainard and Riollet, 2006).

Macrophages are the most predominant immune cells in both healthy and unhealthy mammary glands (Lee et al., 1980; Napel et al., 2009). Proportion of macrophages varies depending on the stage of lactation. In early lactation the number of macrophages is highest at 68% of all SCC, and decreases to 21% in late lactation (Park et al., 1992). During early involution and in colostrum, the macrophages do not exceed more than 30% (Lee et al., 1980). Blood monocytes migrate to the mammary gland, and differentiate into macrophages within the mammary tissues (Alnakip et al., 2014). They migrate at a slower rate in comparison to neutrophils

due to their large nuclei that provides more of a challenge to move between the endothelial cells (Paape et al., 2000). Macrophages function in a similar manner to neutrophils where they engulf bacteria, foreign material, milk components, and cellular debris (Denis et al., 2006). These cells also play a role in the adaptive immune response by processing and presenting antigens (Ag) to lymphocytes (Fitzpatrick et al., 1992). Bovine macrophages contain receptors for immunoglobulin G1 (IgG1) and immunoglobulin G2 (IgG2) to aid in the adaptive immune response (Rossi and Kiessel, 1977). Macrophages in the mammary gland also exhibit a bactericidal effect, which is found to be more effective during the dry off period then during lactation (Denis et al., 2006).

Neutrophils are present in healthy mammary glands. They are important during early inflammatory stages and serve as the second line of defense during an infection (Alnakip et al., 2014). The migration of additional neutrophils into the mammary occurs during IMI but also during milk removal (Pappe and Guidry, 1969). Numbers of neutrophils have been found to increase during early and late lactation (Miller et al., 1993). Additionally, 40-80% of SCC were comprised of neutrophils in early involutional secretions, then, decrease on the 2^{nd} and 4^{th} week into the dry period. Once the mammary gland has become fully involuted, neutrophil numbers returns to lactational values (Lee et al., 1980). Bovine neutrophils have a multilobulated nucleus which allows for easy migration across the endothelium of the mammary gland by diapedesis without causing damage to the mammary epithelium (Paape et al., 2000). Neutrophils function in phagocytosis of foreign invaders and remove them from infected area. As previously discussed in section 1.3.2 of this review, neutrophils have shown to mistakenly engulf milk components including milk fat globules (Opdebeeck, 1982), and secrete proteinases resulting in proteolysis of milk proteins such as casein (Auldist and Hubble, 1998; Prin-Mathieu et al., 2002). Additional humoral components are secreted by neutrophils including cytokines, chemokines, and hydroxyl radicals that damage the mammary epithelium and decrease milk production (Prin-Mathieu et al., 2002). Once neutrophils have completed their task, they undergo apoptosis (programmed cell death) and are removed from the mammary gland by macrophages (Paape et al., 2002; Paape et al., 2003).

1.5.2 Adaptive immunity

Adaptive immunity is a specific response to bacterial infection mediated by lymphocytes which includes T and B lymphocytes, and Natural Killer (NK) cells. Antigens (Ag) binds to membrane-bound receptors of lymphocytes, which in turn alter the function of that particular cell. The purpose of adaptive immunity is to elicit a faster response to a previously exposed threat (Riollet et al., 2000) by memory lymphocytes (Stelwagen et al., 2009). Conditions in which adaptive immunity responds at a slower rate occurs when the host has never been previously exposed to a particular threat (Stelwagen et al., 2009). Not much is known about the role of lymphocytes within the mammary gland, and populations tend to vary during lactation (Park et al., 1992; Taylor et al., 1994; Shafer-Weaver and Sordillo, 1997).

T lymphocytes are categorized into αβ T-cells and γδ T-cells (Alnakip et al., 2014). The αβ T-cells include $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T-cells, where T-cells expressing the $CD8^+$ receptor are predominant within mammary gland (Shafer-Weaver et al., 1996). Activation of $CD8^+$ T cells is mediated by the major histocompatibility complex (MHC) class I molecules, which are present on majority of cells (Broere et al., 2011). Taylor et al. (1994) suggested that $CD8^+$ T cells may remove the damaged mammary epithelial cells, further increasing susceptibility to infection. The $CD4^+$ T cells are activated by MHC class II molecules from Ag-presenting cells, and are important for secreting various immunoregulatory compounds (Riollet et al., 2000). $CD4^+$ T cell concentrations are lower in milk compared to $CD8^+$ T cells, but higher in the blood (Shafer-Weaver et al., 1996). Cytotoxicity may also be mediated by γδ T-cells, which are found in secretions and the parenchyma of the mammary gland (Mackay and Hein, 1991). It has been suggested that γδ T-cells may also provide a barrier to the mucosal microenvironments to protect against infectious pathogens; indicating γδ T-cells potential role in antibacterial immunity (Paape et al., 2000).

B lymphocytes play a role in humoral immunity by secreting antibodies (Ab) during infection. Levels of B lymphocytes within the mammary gland remain constant during lactation and also during bacterial infection, unlike T lymphocytes which tend to fluctuate (Shafer-Weaver et al., 1996; Paape et al., 2000). Recognition of Ag by B lymphocytes is done via MHC class II molecules which bind the Ag, internalize, and process it whereby specific Ab are produced. Antibodies produced are termed immunoglobulins including IgG1, IgG2, IgM, IgA, IgD, and IgE where IgG is the predominant antibody in the milk of dairy cows (Sjaastad et al., 2016).

Natural killer cells reside in the bone marrow, spleen, lymph nodes, and tonsils (Roitt et al., 2001). They are involved in the non-specific response that recognize and cause lysis of foreign

cells by various mechanisms including Ab-dependent cell-mediated cytotoxicity, release of cytolytic factors, receptor mediated Ag-recognition, granule exocytosis, and secretion of toxic molecules that induce apoptosis of altered cells (Paape et al., 2000). Natural killer cells ability to destroy both Gram-negative and Gram-positive bacteria has been demonstrated in various studies; therefore, NK cells could be crucial in preventing IMIs (Shafer-Weaver and Sordillo, 1996).

1.5.3 Recognition of infectious bacteria within the mammary gland

Microbial surfaces contain various molecules that alert the immune system for presence of an infectious organism. Such molecules are termed pathogen-associated molecular patterns (PAMPs), and are recognized by pattern recognition receptors (PRRs; Medzhitov et al., 1997). There are 3 known categories of PRRs which include secreted PRRs, membrane-bound PRRs, and phagocytic PRRs (Gao et al., 2008). Secreted PRRs are proteins including complements, pentraxins, peptidoglycan-recognition proteins, and lipid transferases, which are produced by hepatocytes that destroy bacteria via phagocytosis. Pattern recognition receptors are found on the surfaces of macrophages, neutrophils, and dendritic cells that bind bacteria and remove from the host. Common PRR's would include macrophage mannose receptors, β-glucan, and scavenger receptors (Gao et al., 2008).

The most common type of PRR that recognizes PAMPs and damage-associated molecular patterns (DAMPs) are toll-like receptors (TLRs), which are the most heavily involved in the innate immune response. The central role of TLRs is binding of bacteria and secreting pro-inflammatory compounds. Macrophages, dendritic cells, and neutrophils have majority of TLRs present on the cell surfaces further indicating the role of these phagocytes during innate immunity (Cruvinel et al., 2010). Binding of bacteria to the TLRs triggers the secretion of pro-inflammatory cytokines initiating an inflammatory response. Macrophages are known to have the most abundant production of cytokines during infection and inflammation. Macrophages and other leukocytes release cytokines such as TNF-α and IL-1; both of which are important during inflammatory response (Cruvinel et al., 2010).

1.5.4 Alterations in serum components during mastitis infections

Apart from alterations in milk composition during mammary gland infections, there are various serum metabolites, carbohydrates, and fatty acids involved in the immune responses that undergo marked changes during infection. Additionally, serum concentrations have also shown to be altered during other periparturient diseases (Dervishi et al., 2015; Zhang et al., 2015; Dervishi et al., 2016a; Dervishi et al., 2016b; Zhang et al., 2016).

Tumor necrosis factor-α and IL-1 have shown to be major indicators of diseases in dairy cows. The main functions of IL-1 are enhancing recruitment of neutrophils and their phagocytic and bactericidal abilities, stimulate secretion of additional cytokines and chemokines (e.g., IL-1, IL-6, IL-8, IL-12, TNF-α), and mediate the acute phase response (APR) (Alnakip et al., 2014). Tumor necrosis factor-α possesses similar pro-inflammatory properties to IL-1 including neutrophil recruitment and enhancing neutrophil activity, and mediating the APR (Alnakip et al., 2014). Additionally, TNF-α will stimulate the endothelial cells to express adhesion molecules (Alnakip et al., 2014). Both TNF-α and IL-1 can cause systemic effects to the host including fever, increased heart rate, and loss of appetite (Cruvinel et al., 2010). Dervishi et al. (2015) observed significant increases in TNF-α in serum of cows with SCM at 4 weeks prior to calving and also during disease diagnosis. In both naturally occurring and experimentally-induced *E. coli* mastitis, there is a significant increase in TNF-α in both milk and serum of cows (Hoeben et al., 2000; Hisaeda et al., 2001). The increased concentrations of both TNF-α and IL-1 in the milk implies a relation to neutrophil recruitment in the mammary gland and development of SCM (Blum et al., 2000). Other studies on innate immunity and disease also found increased levels of pro-inflammatory cytokines in cows with subclinical mastitis, retained placenta, metritis, ketosis, and lameness (Dervishi et al., 2015; Zhang et al., 2015; Zhang et al., 2016; Dervishi et al., 2016a; Dervishi et al., 2016b).

Tumor necrosis factor-α and IL-1 are important pro-inflammatory cytokines in activating the APR which involves the secretion of APPs including haptoglobin (Hp) and serum amyloid A (SAA) from liver hepatocytes (Blum et al., 2000; Riollet et al., 2000). The study by Dervishi et al. (2015) additionally found serum SAA concentrations to be greater in cows with SCM throughout the entire experimental period. The authors speculated that elevated TNF-α and SAA throughout the study means that IMI had begun at dry off and that cows were in a state of endotoxemia throughout the entire dry period (Dervishi et al., 2015). The function of SAA is to bind high-density lipoproteins and remove endotoxin from the host through the liver (Ametaj et al., 2005).

Serum amyloid A is also present in the mammary epithelial cells of infected mammary glands and may be important in protecting the tissues against pathogenic bacteria in the early stages of infection (Ametaj et al., 2011).

Haptoglobin also plays a major role in innate immunity as it binds free hemoglobin to decrease the availability of iron, which bacteria require for growth (Wassell, 2000). Haptoglobin was observed to be lower at -8 and -4 weeks prior to parturition possibly making cows more susceptible to disease during the dry period (Dervishi et al., 2015). Previous studies on lameness, metritis, and ketosis witnessed significant increases in Hp during the week of diagnosis (Dervishi et al., 2015; Zhang et al., 2015; Zhang et al., 2016). Dervishi et al. (2015) suggests that lower concentrations of Hp in the blood of SCM cows prior to calving is a result of Hp moving into the mammary gland to assist in immune response. Aside from binding of free hemoglobin, Hp can also facilitate neutrophil recruitment, free radical quenching, help tissue repair and regeneration during inflammation (Quaye, 2008).

Apart from innate immunity alterations during disease, there have also been alterations in the carbohydrate and lipid metabolism in dairy cows with SCM. Cows with SCM post-partum were shown to have significantly higher levels of serum lactate at -8 weeks relative to calving (Dervishi et al., 2015). Research has found that concentrations of lactate in the blood can serve as a useful indicator on the severity of illness (Allen and Holm, 2008). Moreover, assessment of lactate concentrations within the milk can also be a useful indicator for udder health (Davis et al., 2004). Davis et al. (2004) previously reported that there is a close relationship between SCC and high lactate in the milk, further supporting lactate as an indicator for udder health. Levels of non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) also have shown to be elevated at -4 weeks in dairy cows diagnosed with SCM (Dervish et al., 2015). Elevation in NEFA and BHBA is a result of negative energy balance and reduced in feed intake which causes mobilization of fatty acids in the adipose tissue and conversion of NEFA into ketone bodies in the liver (Gordon, 2013). Elevation in BHBA and NEFA concentrations have been associated with incidence of periparturient disease including ketosis, displaced abomasum, milk fever, mastitis, and retained placenta (Schröder and Staufenbial, 2006; Duffield and LeBlanc, 2009, LeBlanc, 2010).

1.6 Relation of mastitis to other periparturient diseases

It is being proposed that mastitis possibly contributes to the development of other periparturient diseases in dairy cows. These diseases include retained placenta, metritis/endometritis, lameness, and ketosis; however, there has been no research conducted on whether mastitis does in fact contribute to other diseases. Previous studies have found an elevation in SCC of dairy cows diagnosed with periparturient disease (Zhang et al., 2015; Zhang et al., 2016; Dervishi et al., 2016a; Dervishi et al., 2016b). Eckel and Ametaj (2016) have suggested that endotoxins, such as LPS and LTA, can translocate from 3 different regions of cattle including the rumen, reproductive tract, and mammary gland. Reports from Hakogi et al. (1989) and Dosogne et al. (2002) both found plasma LPS increased during experimentally-induced or naturally-occurring mastitis. Translocation of LPS is suggested to occur by two possible mechanisms (Mani et al., 2012). The first mechanism is paracellular transport where endotoxin disrupts the epithelium tight junctions, increase permeability, and allow for passage of endotoxin across the epithelium into the bloodstream (Rainard and Riollet, 2006; Mani et al., 2012). The second possible mechanism of endotoxin transport is transcellular transport where endotoxin binds to TLR's located on the epithelial surfaces where it is internalized, transported to the Golgi apparatus, and chylomicrons are produced which are transported into systemic circulation (Ghoshal et al., 2009). Passage of endotoxin via paracellular and/or transcellular transport could provide some evidence on how bacteria causing an IMI could enter into systemic circulation and cause or enhance severity of other diseases.

1.6.1. Retained Placenta

The incidence of retained placenta has increased substantially over the years, occurring in 2-5% of dairy cows post-partum (Opsomer, 2015). Normal expulsion of the fetal membranes should be within 6 hours following parturition. The placenta is considered retained if cow fails to expel the fetal membranes after 24 hours. Multiple factors increase the likeliness of a cow developing retained placenta including abortion, short gestation, twins and dystocia. Retained placenta additionally contributes to increased development of metritis and/or endometritis post-partum, increased interval of time to first heat, increased number of days open, decreased fertility, delayed uterine involution, and increased number of times bred. Incidence of retained placenta

also has been linked to dairy cows developing other periparturient diseases including ketosis, metritis, endometritis, and mastitis (Opsomer, 2015). Retained placenta is associated with a decrease in milk production (Lucey et al., 1986). Dervishi et al. (2016a) found elevations of blood metabolites including TNF-α, IL-1, IL-6, SAA, and lactate beginning at -8 and -4 weeks prior to parturition, indicating inflammatory state and activation of innate immunity. Concentrations of Hp also increased 10-fold in retained placenta cows during diagnosis week (Dervishi et al., 2016a). Ametaj et al. (2010) suggests that *E. coli* LPS has some involvement in low milk yield of dairy cows with retained placenta. Increased concentrations of TNF-α during gram-negative bacterial infections inhibit prolactin production in the pituitary gland thereby decreasing production (Theas et al., 1998). Lactate levels have also been noted to increase in cows with retained placenta (Dervishi et al., 2016a). Lactate has previously been elevated during diseases like mastitis, and origins for this metabolite may come from the mammary gland or muscle tissue (Davis et al., 2004).

1.6.2 Metritis and Endometritis

Metritis and endometritis refer to inflammation of the uterine tract where metritis is the inflammation of the uterus, and endometritis is inflammation of the endometrium (Opsomer, 2015). Metritis and endometritis are mainly caused by bacterial infection, however inflammation of the uterine tract post-partum is considered normal. Visual indication of metritis is a purulent or reddish-brown discharge, often with a foul odor, that occurs within 21 d post-partum (Sheldon et al., 2006). Metritis incidence in dairy cows has been reported to be around 18.5% (Drillich et al., 2001), but in some farms can reach up to 40% of the cows (Markusfeld, 1987). Studies performed using American and European dairy herds have also found that cows with retained placenta were at 6 times more likely to develop metritis, and those with metritis had a 2-fold higher risk for developing ketosis (Opsomer, 2015).

Metritic cows also exhibited activation of innate immunity and subsequent inflammation prior to calving (Dervishi et al., 2016b). Multiple studies have found pro-inflammatory cytokines IL-6 and TNF-α were upregulated in cows with metritis (Kasimanickam et al., 2014; Dervishi et al., 2016b), while Dervishi et al. (2016b) observed lower levels of IL-1. The lowered levels IL-1 could be caused by negative feedback exerted by IL-6 type cytokines (Jordan et al., 1995). Pro-

inflammatory cytokines have also shown to activate APP and SAA as early as -8 weeks prior to parturition (Dervishi et al., 2016b). The involvement of SAA in uterine tissue damage has been observed after the fetus and placenta are expelled from the uterus (Chan et al., 2010). There is also evidence that Hp is involved in pathogenesis of multiple reproductive diseases (Dubuc et al., 2010). It has additionally been observed that metritis can decrease milk production and increase SCC within the mammary gland (Dervishi et al., 2016b).

To our best knowledge, there have been no studies conducted on the association of SCC and metritis; however, some studies have observed a negative correlation of mastitis on pregnancy success (Moore et al., 1991; Hansen et al., 2004). Moore et al. (1991) reported that cows with CM were more likely to develop irregular estrous cycles. Fetal abortion was also 3 times higher in cows that developed CM within the first 45 DIM (Risco et al., 1999). Endotoxins have also been found to be present in tissues of the ovary (Herath et al., 2007), endometrium (Herath et al., 2009), and hypothalamus (Nugent et al., 2002). Herath et al. (2007) found that follicular growth was disrupted with bacterial infection while Hearth et al. (2009) found LPS prolonged the luteal phase with an increase in estrogen production. Intramammary infections have also been linked to hypothermia (fever) induction and embryo loss as a result pro-inflammatory cytokines released from the mammary gland (Hansen et al., 2004).

1.6.3 Ketosis

Ketosis is a metabolic disorder commonly occurring during the early and peak lactation period (3-6 week's post-partum) (Duffield, 2000; Çağdaş, 2013). Ketosis first appears in subclinical form, characterized by an elevation of ketone bodies (β-hydroxybutyric acid (BHBA), acetoacetate, and acetone) in the urine, milk, and blood. Subclinical ketosis (SCK) affects approximately 40% of dairy cows in North America, often reaching 80% in some dairy herds (Duffield, 2000). Around 2-15% of SCK cases can progress to clinical ketosis (CK) characterized by excess ketone bodies in the blood, urine, and milk, decreased appetite, decreased milk production, significant decrease in body condition, and dry manure (Gordon et al., 2013). Cows tend to experience a NEB during early lactation, where a decrease in feed intake cannot compensate the high demand for milk production, resulting in ketosis (Çağdaş, 2013).

Additionally, cows at peak lactation require large amounts of energy for milk production resulting in NEB and reduced feed intake (Çağdaş, 2013).

Previous research has shown an association between ketosis and incidence of other periparturient diseases including mastitis and metritis (Erb and GroÈhn, 1988). Moreover, alterations in blood metabolites suggest that innate immunity as well as carbohydrate and lipid metabolism may have a role in the development of ketosis (Zhang et al., 2016). Zhang et al. (2016) observed a significant increase in BHBA, lactate, IL-6, TNF-α, Hp, and SAA in dairy cows beginning at -8 and -4 weeks prior to calving, indicating activation of innate immunity. Elevation in IL-6 was observed in human subjects during hyperketonemia, possibly due to IL-6 effects on multiple metabolic pathways including oxidative stress, oxidation of fatty acids, lipoprotein metabolism, and protein degradation (Loor et al., 2007). In addition, Kushibiki et al. (2003) suggested that elevated concentrations of IL-6 and TNF-α and depressed appetite could stimulate breakdown of adipose tissue leading to insulin resistance and lipolysis.

In vitro studies have also determined a correlation between elevated plasma BHBA and *E.coli* mastitis (Kremar et al., 1993). Intramammary infusion of LPS has been previously demonstrated to affect metabolism, immune response, and overall performance of dairy cows (Bruckmaier et al., 1993). Several studies have provided evidence that LPS challenge induces both a metabolic response and mRNA abundance of inflammatory mediators (Bruckmaier et al., 1993; Vernay et al., 2012). Moreover, infusion of BHBA has resulted in an increase in APP mRNA abundance within the mammary gland (Zarrin et al., 2014a). Zarrin et al. (2014b) induced hyperketonemia by BHBA infusion for 56 h, and then infused LPS within the mammary gland in mid-lactating dairy cows. The authors observed a decline in plasma glucose concentrations, and confirmed that the mammary tissues used BHBA as an alternative energy source. Reduction in glucose concentrations could negatively impact the immune system and reduce cow performance if glucose concentrations are depleted for long periods of time (Zarrin et al., 2014b). While this study established metabolic effects of induced hyperketonemia and LPS-infusion in the mammary gland, it still remains unclear whether mastitis is associated with ketosis.

1.6.4. Laminitis

Laminitis is defined as the aseptic inflammation of the corium layer commonly occurring in both horses and bovines. Laminitis is one of the top 3 majoring occurring diseases of dairy cows following infertility and mastitis (Weaver and Jean, 2005). Physical characteristics of cows affected by laminitis include abnormal gait, arched back, abnormal claw morphology, favoritism on one leg, decreased body condition, decreased feed intake, and decreased milk production (Boosman et al., 1991). Inflammation in the hoof region is also a major indicator of laminitis. The relationship between grain-overload and development of subclinical ruminal acidosis to the incidence of laminitis has been well established; however, the pathogenesis of laminitis still remains poorly understood (Ametaj et al., 2010). Furthermore, research has primarily focused on lameness contributing to a mastitis infection, but not mastitis contributing to lameness.

Cook et al. (2004) conducted research on the lying and standing behaviors of dairy cows. It was found that cows with lameness spent 4.31 h more per day standing instead of lying (Cook et al., 2004). Lying time for cows on mattress surfaces was previously reported to be 12-14 h per day (Chaplin et al., 2000; Tucker et al., 2003). Additionally, lameness in the hoof area of cattle also causes extreme pain (Whay et al., 1998). Indeed, the standing and lying process could potentially be more challenging for a lame cow as a result of pain in the hoof (Nordlund and Cook, 2003). It has also been observed that lame cows are at higher risk of slipping (Cook et al., 2004).

A study by Archer et al. (2011) investigated the relationship between locomotion scores and SCC in 7 UK dairy herds. Their results indicated a negative association between SCC and locomotion score, where lame cows had lower SCC compared to non-lame cows (Archer et al., 2011). Archer et al. (2011), then, concluded that lame cows that spent more time standing decreased exposure of the mammary gland to infectious pathogens, thus maintaining a low SCC. To our best knowledge, this is the only study that aimed to establish a relationship between SCC and lameness. While these findings observed the overall behavior and locomotion scoring of lame cows, they did not however look at the changes in metabolites that are associated with lameness and mastitis.

It was previously demonstrated the upregulation of blood metabolites in dairy cows with lameness to be used as potential biomarkers for predicting lameness incidence (Zhang et al., 2015). Zhang et al. (2015) reported increased serum concentrations of TNF-α, IL-1, and IL-6 in dairy cows at -8 and -4 weeks relative to calving. Previous reports in horses suggested that development of clinical lameness would result in an increase of IL-6 in blood (Boontham et al., 2003). In

previous reports, it was shown that IL-6 was a potent biomarker for cows with mastitis, retained placenta, and metritis (Hagiwara et al., 2001; Ishikawa et al., 2004). This indicates that IL-6 may play an important role in clinical stages of lameness and other metabolic diseases. It was also suggested that endotoxins might play a role in stimulating cytokine production and increased Hp and SAA levels (Emmanuel et al., 2008). Zhang et al. (2015) also reported increased SCC levels in lame cows, and a positive correlation between lactate, IL-6, TNF-α, and SAA, further suggesting that mammary gland infection prior to dry off could contribute to development of diseases post-partum.

1.7 Current approaches to treatment of mammary gland infections

1.7.1 Application of antibiotics and bacterial resistance

Treatment and prevention of disease in food-producing animals has been maintained through the use of antibiotics; which is one of the most effective ways in reducing IMI. The highest risk for a cow to develop a new IMI has been shown to be at the beginning of dry off and around parturition (Oliver and Bushe, 1986). Dry cow therapy (DCT) is an antibiotic treatment administered to cows at dry off. The benefits of DCT are related to decreasing the number of infectious organisms within the mammary gland, thus reducing the incidence of new IMI post-partum (Jones, 2009). According to Jones (2009), farmers who do not treat their cows with DCT increase the incidence rate of new IMI by 10-15%. Dry cow therapy has shown to be 90-93% effective against *S. agalactiae* infections, 70-80% on *S. aureus* infections, and 70-90% against environmental streptococci bacteria. In comparison to treating mastitis infections during lactation, the dosage of antibiotic is less compared to DCT, due to the risk of antibiotic residues within the milk. Therefore, cows receiving DCT at dry off is more beneficial since higher dosages can be administered, and incidence of new IMI is decreased.

Recent suggestions have been put forward that antibiotic use for treatment of disease within the agriculture industry is resulting in increased antibiotic resistance by infectious organisms (Oliver and Murinda, 2012). Indeed, the mechanisms in which bacteria become resistant to antibiotics includes preventing entry or exporting of the drug, secretion of enzymes that alter or destroy the antibiotic, or make changes to the antimicrobial target (Holmes et al., 2016). Countries such as the United Kingdom, Denmark, and Norway have implemented a ban on certain

antimicrobials in swine and poultry systems with the goal of decreasing populations of resistant organisms. Conflicting reports showed that some populations of resistant organisms had decreased after the ban, while other populations had remained unchanged (Smith, 1975; Aarestrup et al., 2001). An example is the resistance of *S. aureus* against penicillin (Nickerson, 2011), which has made it increasingly difficult to control and treat the sick animals. Cows treated for a staphylococcal infection during lactation have shown to be <50% effective at reducing infectious pathogens (Jones, 2009). Oliver and Sordillo (1988) concluded that DCT is not entirely effective at decreasing the incidence of new IMI. There is now an increasing interest for new alternative methods in reducing IMI, and also reducing other periparturient diseases of dairy cows.

1.7.2 Probiotics, the new alternative for treatment of mastitis

The use of probiotics has become increasingly popular for treatment and prevention of disease. The definition of probiotics according to the FAO-WHO are "*live microorganisms which when administered in adequate amounts confer a health physiological benefit on the host"*. The interest in probiotic use for treatment of disease is a result of bacterial resistance to antibiotics, and also the consumer's desire for antibiotic-free food.

Microorganisms naturally thrive within the bodies of mammals, and are often beneficial to the host. In humans, there is a tremendous quantity of beneficial bacteria that are important in maintaining human health (Blaser, 2014). Isolation of bacterial strains is through the use of molecular techniques on a strain-specific level (van Loveren et al., 2012). This is important to determine whether a particular bacterial strain is safe for probiotic use (Amir et al., 2016). For example, isolated *Lactobacillus* has been able to reduce eczema in early childhood (Zuccotti et al., 2015), and serious infection in premature newborns (AlFaleh and Anabrees, 2014). It is also possible that the "beneficial bacteria" in human milk may possibly lower mastitis incidence in humans (Amir et al., 2016).

Conflicting results have been reported on whether probiotic use lowers the incidence of IMI in dairy cows (Klostermann et al., 2008). *Lactobacilli* have been identified within the bovine teat canal, and have become one of the most popular groups of bacteria used in probiotic treatments for mastitis (Giannino et al., 2009; Espeche et al., 2012). Infusion of *Lactobacillus lactis* into the mammary was found to significantly increase cytokine and chemokine levels in the mammary

gland (Klostermann et al., 2008). Additionally, neutrophil concentrations also increased significantly following *Lactobacillus* infusion (Klostermann et al., 2008). Interestingly, an in vitro study found lacticin 3147, produced by *Lactococcus lactis* DPC3147, could inhibit mastitis-causing pathogens (Ryan et al., 1998). A combination of lacticin 3147 with a bismuth-based teat sealant was found to lower *S. dysgalactiae* infections in dry cows (Ryan et al., 1999), and additionally lower *S. aureus* infections in lactating cows (Twomey et al., 2000; Crispie et al., 2005). Klostermann et al. (2008) found that resuspended freeze-dried *Lactococcus lactis* DPC3147 was able to treat clinically mastitic cows. While research on effectiveness of probiotics is still new, probiotic use has become increasingly popular approach in treating clinical disease.

1.8 Summary

The incidence of a mammary gland infection is highly problematic within the dairy industry as it results in production loses for producers and poor cow performance. Both Gram-negative and Gram-positive organisms are able to produce endotoxins that contribute to mammary gland infections and activation of the immune system. There have been multiple studies on immunosuppression during the transition period, and disease incidence post-partum. To our best knowledge, there is no research yet on the association of mammary gland infections at the time of dry off being related to other periparturient diseases in dairy cows. The incidence of mammary gland infection at dry off and its possible association with periparturient diseases including metritis, laminitis, ketosis, retained placenta, and milk fever will be the subject of interest.

1.9 Hypotheses

1. Cows with high SCC prior dry off is associated with periparturient disease (metritis, retained placenta, ketosis, mastitis, and lameness).

2. Cows with high SCC at dry off exhibit alterations in milk composition and have lower milk production post-partum.

3. Cows with high SCC at dry off exhibit alterations in serum metabolites that are related to incidence of periparturient disease.

1.10 Objectives

1. To determine whether high SCC prior to dry off is associated with post-partum diseases (metritis, retained placenta, ketosis, mastitis, and lameness).

2. To determine whether high SCC is associated with changes in the milk composition and lower milk production.

3. To determine whether high SCC in the mammary gland at dry off are associated with alteration in serum metabolites.

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Table 1-1. Subclinical vs clinical mastitis including the four categories of clinical mastitis (chronic, subacute, acute, and peracute).

Subclinical Mastitis			Clinical Mastitis			
Symptoms	**SCC Level[1]**	**Severity**	**Category**	**Symptoms**	**SCC Level[1]**	**Severity**
Decreased production, increased SCC, changes in milk components, presence of pathogens.	>200	Can only be determined by laboratory tests. Non-visible symptoms causing potential risk of infecting entire herd.	**Chronic**	Visible alterations in the milk (clots, flakes, water), no inflammation or other visible sings.	>200	Long-term infection in which culling is recommended.
			Subacute	Minor udder inflammation, heat, changes in milk (clots, flakes, water-like appearance), some sensitivity to udder.	>200	Typically non-life threatening.
			Acute	Sudden onset, redness/ swelling, hardness, pain, abnormal milk, decreased production, fever, decrease appetite, decrease rumen function, rapid pulse, dehydration, weakness, depression.	>200	Life-threatening and causes extreme discomfort.
			Peracute	Very rapid onset with similar symptoms to acute but more severe.	>200	Decrease in cow's overall condition, life threatening.

[1]Somatic cell counts in 10^3 cells/ml

(References: Kirk and Bartlett, 1988; Tylor et al., 1990; Klocke et al., 2007; Nickerson, 2011; Roberson, 2012).

Chapter 2 Association of high somatic cell counts prior to dry off to the incidence of periparturient diseases in Holstein dairy cows

ABSTRACT

Intramammary infections (mastitis) of dairy cows along with other periparturient diseases have become problematic within the dairy industry as they result in production loss. The main objective of this study was to determine whether high somatic cell counts (SCC) in cows before dry off are related to the incidence of other periparturient diseases. Additionally, we determined whether milk composition and milk production are affected by high SCC prior to dry off. Somatic cell counts from milk samples were determined prior to dry off from cows (n = 140) and were used to classify cows in the study as high (>200,000 cells/ml) or low (<200,000 cells/ml) SCC. Milk composition was analyzed prior to dry off and at +1 and +2 weeks postpartum. Results showed that high SCC before dry off were significantly related to incidence of ketosis. High SCC cows also showed increased likelihood of retained placenta, metritis, and lameness post-partum; however, it was not statistically significant. Lactose in milk was lower for high SCC cows while protein was lower after parturition. Milk production was lower for high SCC cows after parturition especially for retained placenta, ketosis, and mastitis. In conclusion, cows with high SCC before dry off were more likely to have incidence of periparturient diseases after parturition.

2.1 Introduction

Intramammary infection (IMI) of dairy cows, known are mastitis, is defined as inflammation of the mammary gland as a result of bacterial infection. Infection of the udder can produce either subclinical mastitis (no visible symptoms) or clinical mastitis (visible symptoms). Clinical symptoms of an IMI include increased SCC, decrease milk yield, altered milk appearance and composition as well as swelling, heat, and pain of the udder (Roberson, 2012). Approximately 50% of cows in a dairy herd can be affected by subclinical IMI (Pitkala et al., 2004). A mastitis infection can result in production loss of $184 per case, and up to $18,400 per 100 cows (Crist et al., 1997).

Elevation in milk SCC has been the most common and effective method in determining IMI in dairy cows. Somatic cells in milk consist of leukocytes, primarily macrophages and neutrophils (Napel et al., 2009). It has been suggested that SCC in healthy mammary glands are

less than 100,000 cells/mL, and counts greater than that indicate presence of infection (Djabri et al., 2002). In Canada, presence of an IMI is indicated when SCC exceed 200,000 cells/mL (Madouasse et al., 2010).

Milk yield losses can be significantly impacted by IMI within a dairy herd. The decrease in milk production during infection is a result of increase permeability of the blood-milk barrier, damage to the mammary tissue (Shuster et al., 1991), and decreased lactose synthesis (Auldist and Hubble, 1998). Additionally, the decline in milk production occurs during other diseases including uterine infections (LeBlanc, 2002), retained placenta (Dervishi et al., 2016b), ruminal acidosis (Nocek, 1997), lameness (Warnick et al., 2001), and ketosis (Gordon et al., 2013).

Milk composition also becomes altered during an IMI. The decline in milk fat can be attributed to neutrophils mistakenly engulfing milk fat globules during phagocytosis (Opdebeeck, 1982), and oxidation by lipase enzymes from leukocytes (Holdaway, 1990; Auldist et al., 1995; Audist and Hubble, 1998). Lactose is the main carbohydrate of bovine milk and functions as an osmotic regulator for milk volume. Lactose is synthesized from one galatoce molecule, which is derived from glucose taken up by the blood, and one glucose molecule also taken up by the blood (Sjaastad et al., 2016). Potentially the decline in lactose in the milk could be related to a decline in glucose in the blood. Lactose concentrations decrease during IMI as a result of the increased gap between the tight junctions causing lactose leakage into the blood circulation, which has been confirmed by several authors (Shuster et al., 1991; Auldist et al., 1995). Mastitis has been associated with a decrease in the synthesis of milk protein including casein and whey proteins including α-lactalbumin (Auldist and Hubble, 1998). In contrast, concentration of serum albumin, immunoglobulins, and lactoferrin increase during IMI as a part of the immune response (Shuster et al., 1991; Auldist et al., 1995; Auldist and Hubble, 1998).

Pregnant lactating dairy cows enter into a dry period occurring roughly 8 weeks prior to the next parturition, where the mammary gland is no longer secreting milk. The dry period is beneficial for dairy cows to maximize milk production in the next lactation cycle (Dingwell et al., 2001). However, the incidence of new IMI for dairy cows has shown to be highest at the beginning of the dry period and prior to parturition (Smith et al., 1985). Current treatment protocols at dry off including use of antibiotics and a teat sealant, have been shown to lower the incidence rate of IMI by 7.3%, during the dry period (Arnold, 2012). However, dairy cows undergo various metabolic changes as parturition approaches associated with immunosuppression and increasing

susceptibility to periparturient diseases (Goff & Horst, 1997; Mallard et al., 1998; Ametaj et al., 2005).

Common metabolic diseases occurring in post-partum dairy cows include ketosis, retained placenta, metritis, mastitis, and lameness. Ketosis is characterized by elevation of ketone bodies (e.g., β-hydroxybutyric acid) in the urine, blood, and milk (Gordon et al., 2013). Retained placenta is characterized by the inability to expel fetal membranes within 24 h post-partum. Metritis is a bacterial infection of the uterus resulting in inflammation of the uterine tract (Opsomer, 2015). Lameness is characterized by inflammation of the corium and germinal layer accompanied by visible symptoms including abnormal gait and claw issues (Boosman et al., 1991). Archer et al. (2011) reported a negative relationship between SCC and locomotion scoring in cattle.

Research conducted by our lab found SCC to be high during diagnosis week of several periparturient diseases. Their results found a significant increase in SCC during diagnosis of disease, but the SCC were within the normal ranges except for subclinical mastitis where SCC were >200,000 cells/mL (Dervishi et al., 2015). It has been suggested that bacterial endotoxins might play a role in the etiopathology of multiple periparturient diseases (Ametaj et al., 2010; Eckel and Ametaj, 2016) and there has been reports that endotoxins can translocate from mammary gland into the systemic circulation (Hakogi et al., 1989; Dosogne et al., 2002).

We hypothesized that cows with high SCC prior to dry off could be more susceptible to the incidence of various periparturient diseases; have alterations in milk composition and produce less milk than the healthy counterparts. Therefore, the main objectives of this study were to determine whether cows with SCC higher than 200,000 cells/mL (indication of mammary subclinical infection) prior to dry off are associated with higher incidence of periparturient diseases during the first 2 weeks after parturition. Additionally, relationships between SCC and alterations in milk composition prior to dry off and during the first 2 weeks after parturition will be evaluated; and whether high SCC will be related to milk yield.

2.2 Materials and Methods

2.2.1. Animals and experimental design

The study was conducted at 2 farms located in Edmonton and Ponoka, Alberta, Canada. For this study a total of 140 pregnant Holstein dairy cows consisting of 82 multiparous and 58

primiparous were used. The number of cows used per each dairy farm were 104 and 36 cows in Edmonton and Ponoka, respectively. The university dairy farm in Edmonton uses a tie-stall system and the conventional farm in Ponoka uses a free-stall parlour system. All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock. Proper care of each animal was followed in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Cows used in the study were randomly selected (heifers were excluded) and had to meet the following criteria: 1) pregnant, and 2) entering the dry period. Approximately one week prior to dry off each cow was sampled, and then sampled again at +1 and +2 weeks post-partum. Milk samples were obtained at each time point, and approximately 20-mL was transferred into a DHI vial for laboratory analysis. The rest of the milk sample was kept for other analysis and stored at -20 °C. The 30-mL DHI vial of milk was transported to the Central Milk Testing Lab located in Edmonton, AB, Canada (CanWest DHI, Canada) and analyzed by mid-infrared spectroscopy (MilkoScan 605; A/S Foss Electric, Hillerød, Denmark). Milk samples were analyzed for concentrations of fat, protein, lactose, milk urea nitrogen (MUN), total solids (TS), and somatic cells counts (SCC). The fat:protein ratios (FPR) were calculated by dividing total fat% by the total protein% per cow.

Fecal scores were determined per fecal sample on a scale of 1-5 where a score of 1 = diarrhea; 2 = appears runny and does not form a distinct pile 3 = optimal score, porridge-like appearance, will stack up at 4-5 cm; 4 = manure is thicker and will stick to shoe, stacks up to more than 5 cm; 5 = firm fecal balls (Hutjens, 1999). Body condition scoring was done for all animals at each sampling time point in accordance to Elanco's Body Condition Scoring in Dairy Cattle on a scale of 1-5 with intervals of 0.25 points.

Additional information including lactation, age, previous 305 days in milk yield, and date of the drying off were recorded. DairyComp Software was used to determine the length of the dry period (days) per animal. Expected calving and actual calving dates were recorded and whether calving was early or late relative to expected calving date. Daily milk weights (kg) were recorded up to the first 60 days in milk (DIM).

2.2.2 Clinical observations for periparturient disease and SCC groupings with disease diagnosis

Health status of each cow was monitored for clinical signs of disease on a daily basis by trained staff members. All periparturient diseases and treatments were recorded throughout the experimental period. Breeding and culling records were recorded for the first 6 weeks after parturition. Animals were removed from the study if mortality/culling occurred during the dry period or post-partum where samples could not be obtained. External symptoms were observed including alertness, appetite, fecal consistency, and body condition score (BCS).

Post-partum mastitis was determined based on whether SCC was >200,000 cells/mL. Retained placenta was diagnosed based on whether cows failed to expel fetal membranes within 24 h post-partum. Metritis was evaluated using vaginal mucus score by Metricheck device (Simcro, New Zealand). The devise was disinfected with Nolvasan (Zoetis, Kalamazoo, Michigan, United States) containing 2% chlorhexidine diacetate and ethanol. Cows were disinfected with 16% iodine solution (Vetoquinol N.-A, Inc., Lavaltrie, Quebec, Canada), and then metricheck was inserted into reproductive tract to obtain vaginal mucus. Evaluation of mucus was done according to Sheldon et al. (2006) where 0 = clear or translucent mucus; 1 = mucus containing flecks of white or off-white puss; 2 = discharge containing ≤ 50% puss or off-white mucopurulent material; 3 = discharge containing ≥ 50% purulent material typically white or yellow or sanguineous. Metritis was diagnosed if mucus score was 3. Lameness was diagnosed on a locomotion scoring system following farm standard operating procedures (Sprecher et al., 1997). Locomotion scoring was done following Zinpro Locomotion Scoring of Dairy Cattle guidelines on a scale of 1-5 where 1 = normal; 2 = mildly lame; 3 = moderately lame; 4 = lame; and 5 = severely lame. Diagnosis of ketosis was accomplished by KetoStix strip (Bayer Corp. Diagnostics Division, Tarrytown, NY, US) detecting urinary acetoacetate (AcAc) as well as clinical signs including reduce appetite, poor body condition, and treatment according to farm standard operating procedures. Measuring of urinary AcAc was based on color intensity found on KetoStix package, and scored from 5 categories KetoStix strips where Negative = 0 mmol/L; Trace = 0.5 mmol/L; Small = 1.5 mmol/L; Moderate = 4 mmol/L; Large = 8 mmol/L; Large = 16 mmol/L.

The experimental design for this study was a nested case-control design (Figure 2-1) where SCC in the milk prior to dry off was used to classify cows into two groups: 1) Low SCC (< 200,000 cells/mL) and 2) High SCC (> 200,000 cells/mL), and evaluated for incidence of the 5 periparturient diseases including mastitis, metritis, lameness, retained placenta, and ketosis. Cows were diagnosed for mastitis based on farm standard operating procedures. For comparisons

between groups for analysis, cows diagnosed with post-partum disease were labeled as low-disease (LD) and high-disease (HD) based on the SCC prior to dry off and whether they were diagnosed with one of the 5 diseases being studied (e.g. Cow with >200,000 cells/ml prior to dry off diagnosed with metritis after parturition was considered a high-disease animal). Healthy cows were identified if their SCC were <200,000 cells/mL with no incidence of disease throughout the experimental period. Healthy cows were used as a comparison to the sick groups and were classified as the Healthy group (HG).

2.2.3 Statistical analysis

In this study, cows were blocked by the SCC in the milk that was determined prior to dry off, and were assigned a group of low SCC and high SCC. Cows with SCC of < 200,000 cells/mL were classified as low, and cows with a SCC of > 200,000 cells/mL were classified as high. Further blocking was done based on whether cows were diagnosed with post-partum disease, and were classified as LD and HD (see Section 2.2.2 for explanation). The study is a nested case-control design where cows were blocked into two groups based on the SCC determined at prior to dry off, and then further blocked based on diagnosis of periparturient disease. We ran separate analysis for each disease.

Binary data for incidence of disease were analyzed using the crosstabs function in MedCalc 18.2.1 software (Acacialaan, Ostend, Begium). The odds ratio was calculated for entire population using the crosstabs function in order to determine the likelihood of high SCC cow's incidence of post-partum disease compared to low SCC.

Frequency for cows diagnosed with disease was calculated using the FREQ procedure in SAS 9.2 software (SAS Institute Inc., Cary, NC). The frequency was calculated for the number of cows diagnosed with disease that were low SCC, high SCC, and healthy. Additionally, the frequency of cows with either single or multiple diseases after parturition was calculated. The percentages were calculated out of the total population (n=140).

For analysis of milk composition, we selected 15 healthy low-SCC cows throughout duration of the study as the control to compare to each disease of interest, and were classified as the healthy group (HG). Selection of the control group had to have similar BCS, fecal score, milk composition, dry period length, and was completely healthy throughout study period. From each

SCC group that were diagnosed with post-partum disease we randomly selected 7-8 cows from the low SCC group and classified as Low-Disease (LD), and 7-8 from the high SCC group classified as High-Disease (HD). Animals selected from diseased groups had to only been diagnosed with 1 disease throughout study period (e.g., a cow was diagnosed for metritis, but only had metritis and no other disease). However, in order to have a sufficient number of sick animals for comparison purposes, we had to select cows with less than 3 diseases. For the metritis group, all cows were only diagnosed with metritis during the experimental period. In the mastitis HD group there were a total of 5 cows diagnosed with 2 diseases, and all other cows diagnosed with mastitis were only diagnosed with 1 disease. For lameness, the LD group had 5 of 7 cows diagnosed with 2 diseases, and the HD group had 2 of 7 diagnosed with 2 diseases and 2 cows were diagnosed with 3 diseases. For ketosis, all cows in the LD group were diagnosed with only ketosis while the HD had 4 out of 7 cows diagnosed with 2 diseases. For retained placenta, the LD had 4 out of 7 diagnosed with 2 diseases, and the HD group had 3 out of 7 cows diagnosed with 2 diseases, and 4 out of 6 cows diagnosed with 3 diseases but none with only retained placenta. Therefore, number of diseases per cow was taken into consideration when fitting the statistical model. The exception was the metritis group where all cows were diagnosed for just metritis during the study; therefore, the number of diseases was excluded from the statistical model for metritis.

Milk composition and milk production data were analyzed using SAS 9.2. Normality of data was firstly tested using the UNIVARIATE procedure for each group and each variable, however, the data did not follow a normal distribution. We therefore used the GLIMMIX procedure with repeated measures for non-normal distribution data. Effect of farm and cow was considered a random effect in the model statement. The covariance structure was modeled according to the smallest Akaike information criterion (AIC) and the Bayesian information criterion (BIC) values generated. The effect of health status was forced into the model statement since we were interested in determining whether alterations in milk composition and production were different between groups. Therefore, our main model was as follows:

$$Y_{ijkl} = \mu + H_i + e_{ijkl}$$

where μ = the overall population mean; H_i = the fixed effect of health status i (i = 1-3, healthy cows compared to LD and HD groups separately), and e_{ijkl} = the residual error. Additional model fixed effects for week, parity, and number of diseases were investigated along with their corresponding interactions for each milk component per disease group. A backwards elimination

from a saturated model was performed if effect was not significant on the response variable. There was no significance in the 3-way and 4-way interactions for all milk components and FPR per disease, and they were removed from the statistical model.

For analysis of milk production data, total milk production was calculated and compared to previous 305 DIM productions between the groups. Data was firstly tested for normality using the UNIVARIATE procedure and was found to not follow a normal distribution. All milk production data, then, were analyzed using the GLIMMIX procedure. The model for total milk yields for the first 60 DIM included the effect of health status, previous 305 DIM production, and the interaction between health status and previous production. The interaction effect was significant only for mastitis and was removed from the statistical model for metritis, retained placenta, ketosis, and lameness. The effect of previous production was significant for metritis, mastitis, and ketosis and therefore it was kept in the statistical model. Previous yield was not significant for retained placenta and lameness, and therefore was not included in the model. The mean and SEM for milk production over the 60 DIM was calculated for each group for each disease. For all data, significance was declared at $P < 0.05$ and tendency at $0.05 \leq P \leq 0.10$.

2.3 Results

2.3.1 Frequency of cows diagnosed with post-partum disease

The frequency for incidence of periparturient disease for SCC groups, parity, and number of diseases is displayed in Table 2-1 and Figure 2-1. Cows diagnosed with single or multiple diseases incidence rate is displayed in Figure 2-2. Approximately 22.14% (n=31) of the total population had no incidence of disease after parturition. Of the 22.14% healthy, roughly 17.14% (n=24) were low SCC and were identified as healthy cows, and 5.00% (n=7) were high SCC prior to dry off.

For mastitis, 29.29% (n=41) of the total population was diagnosed with disease where 20.00% (n=28) were low SCC and 9.29% (n=13) were high SCC prior to dry off; 7.86% (n=11) were diagnosed with only mastitis, and 21.43% (n=30) had multiple diseases, besides mastitis.

The total population that was diagnosed with metritis was 42.14% (n=59), where 27.14% (n=38) had low SCC and 15.00% (n=21) had high SCC prior to dry off; 12.14% (n=17) were

diagnosed with metritis only, and 30.00% (n=42) were diagnosed with multiple diseases including metritis.

For lameness, 15.71% (n=22) of the total population were diagnosed with the disease where 10.71% (n=15) were low SCC and 5.00% (n=7) were high SCC prior to dry off; 2.14% (n=3) were diagnosed with lameness only, and 13.57% (n=19) were diagnosed with multiple diseases, besides lameness.

For retained placenta, 14.29% (n=20) of the total population was diagnosed with the disease where 8.57% (n=12) were low SCC and 5.71% (n=8) were high SCC prior to dry off; 2.14% (n=3) were diagnosed with retained placenta only, and 12.14% (n=17) had multiple disease, besides retained placentas.

For ketosis, 32.14% (n=45) of the total population were diagnosed with the disease where 17.14% (n=24) were low SCC 15.00% (n=21) were high SCC prior to dry off; 12.86% (n=18) were diagnosed with only ketosis, and 19.29% (n=27) were diagnosed with multiple diseases, besides ketosis.

2.3.2 Odds ratio for the incidence of disease for dairy cows

In order to determine whether cows with high SCC prior to being dried off is related to incidence of disease, we calculated the odds ratios for the likelihood of cows with high SCC compared to low SCC prior to dry off to be diagnosed with post-partum disease of the total population (n=140) (Table 2-2).

Overall data showed cows with high SCC prior to dry off had increased odds in the incidence of ketosis after calving (Table 2-2). Indeed, the odds of cows with high SCC developing ketosis was 166% (or 2.66 odds ratio) more compared to low SCC cows ($P = 0.01$). Although the odds ratio for cows with high SCC prior to dry off and incidence of metritis, retained placenta, and lameness were not statistically significant, cows with high SCC showed to have increased odds in the incidence of post-partum disease. Cows with high SCC before dry off had a 43.0% (1.43), 56.0% (1.56), and 31.0% (1.31) increased odds to be affected by metritis, retained placenta, and lameness, respectively. In comparison to cows with low SCC prior to dry off, the odds ratio is less than 1 for all 5 diseases indicating incidence of an event to occur is less likely due to SCC and other factors may be invovled.

The odds of cows being diagnosed with post-partum mastitis was not statistically significant among groups, where both low and high SCC cows prior to dry off showed to have increased odds of 2.0%.

2.3.3 Somatic cell counts

Data related to milk composition between the healthy group (HG), LD, and HD cows among diseases prior to dry off as well as at +1 weeks and +2 weeks can be found in Tables 2-3, 2-4, and 2-5, respectively (See graphs in Appendix B). The average SCC (multiplied by 10^3 cells/mL) prior to dry off among all diseases showed the HD group to be significantly greater than the LD and HG ($P < 0.01$) (Table 2-3). This is to be expected as we grouped cows prior to dry off based on concentration of SCC in the milk. Comparison of the means at +1 week for retained placenta showed a tendency with the HD group having a mean of 1,359.86 ± 578.00, compared to 54.07 ± 78.73 and 41.57 ± 101.06 ($P = 0.06$) in the HG and LD group, respectively (Table 2-5). Similarly, at +2 weeks, cows in the HD group had significantly higher SCC of 223.86 ± 73.47 compared to the LD and HG of 43.57 ± 32.41 and 37.58 ± 22.99, respectively ($P = 0.02$) (Table 2-5).

Interestingly, cows diagnosed with mastitis showed a greater mean of 939.37 ± 411.81 in the LD group compared to the HD group of 666.50 ± 346.88 at +2 weeks, although not statistically significant ($P = 0.23$) (Table 2-4). At +1 week cows demonstrated elevated SCC in the LD group (1,993.63 ± 798.24) and the HD group (1,396.00 ± 667.96), compared to healthy cows (54.07 ± 96.00) ($P = 0.15$) (Table 2-4). The number of SCC in cows diagnosed with metritis, ketosis, and lameness were not significant at + 1 week ($P = 0.99$; $P = 0.40$; $P = 0.47$, respectively).

2.3.4 Lactose

Similar to the number of SCC prior to dry off, discussed in the previous section, concentrations of lactose in the milk prior to dry off were found to be significantly lower in all disease groups ($P < 0.05$) with the HD group demonstrating the lowest lactose concentration (Table 2-3; (See graphs in Appendix B). Cows diagnosed with metritis demonstrated decreased lactose concentrations in the HD group at all three time points ($P = 0.01$). Comparisons of the means prior

to dry off showed that the HD in the ketosis group had the lowest lactose concentration of 3.73 ± 0.15 compared to the other HD groups of metritis, retained placenta, lameness, and mastitis groups which displayed similar means (Table 2-3). At +1 week concentration of lactose for ketosis group of cows showed that the HD group was still numerically lower than concentrations for the healthy and LD groups of cows ($P = 0.25$), and had a tendency at +2 weeks ($P = 0.09$) (Table 2-4 and 2-5, Appendix B). There was also a tendency demonstrated in the HD retained placenta group where cows with high SCC prior to dry off had a mean lactose concentration of 3.96 ± 0.17 at +1 week compared to 4.31 ± 0.18 and 4.44 ± 0.12 in the LD and healthy group ($P = 0.10$) (Table 2-3). At +2 week there were no significant differences in the concentration of lactose for retained placenta cows ($P = 0.42$) (Table 2-5). Cows in the mastitis group with high SCC before dry off had the lowest concentration of lactose at all 3 time points where time period prior to dry off and +1 week were significantly lower ($P < 0.01$) (Table 2-3). At +2 week, the HD group of mastitic cows demonstrated lower lactose concentration of 4.32 ± 0.12 compared to 4.60 ± 0.13 in the low group and 4.55 ± 0.10 in the HG but the difference did not reach significance ($P = 0.25$) (Table 2-5). There were no significant differences in the lactose concentration at +1 and +2 weeks in the lameness group among the HG, LD, and HD cows, although the HD group consistently had the lowest mean.

2.3.4 Protein

Comparisons of the means for protein concentration in the milk were significant at all 3 time points for lameness ($P < 0.01$). Protein concentrations for lameness were found to be significant at all 3 time points ($P < 0.01$) (Table 2-3, 2-4, 2-5; See graphs in Appendix B). Interestingly, the HD group had the highest mean protein concentration prior to dry off (4.10 ± 0.15) while the HG and LD groups were lower (3.41 ± 0.10 and 3.79 ± 0.14) (Table 2-3). After parturition, protein concentration decreased for the HD group compared to HG and LD (3.29 ± 0.12 vs. 3.70 ± 0.10 vs. 3.87 ± 0.13) (Table 2-4), and further decreased at +2 weeks for the HD group (2.93 ± 0.10 vs. 3.38 ± 0.09 vs. 3.39 ± 0.11) (Table 2-5). Similar to lameness, cows diagnosed with retained placenta in the HD group showed a tendency at prior to dry off and had greater mean protein concentration compared to the LD and HG (3.91 ± 0.18 vs. 3.73 ± 0.14 vs. 3.40 ± 0.11) (Table 2-3). At +1 week the mean concentration of protein was similar among all 3

groups ($P = 0.97$) (Table 2-4), while at +2 weeks the HD group had the lowest mean concentration for protein (3.09 ± 0.15) but was not significant ($P = 0.27$) (Table 2-5). Ketosis showed no significant differences in the mean protein concentration among the groups prior to dry off ($P = 0.55$) (Table 2-3). However, the HD group did exhibit higher mean protein concentrations prior to dry off of 3.64 ± 0.12 compared to healthy of 3.49 ± 0.08 and the LD group of 3.55 ± 0.11 (Table 2-3). At +1 week the HD group showed significantly lower mean protein concentration (3.26 ± 0.11) (Table 2-4), and decreased further at +2 weeks (2.80 ± 0.10) ($P < 0.01$) (Table 2-5). There was no statistical significance for protein concentration in the milk for metritis and mastitis for all 3 time points.

2.3.5 Fat, fat:protein ratio, milk urea nitrogen, and total solids

The mean concentrations of fat, FPR, MUN, and TS are shown in Table 2-3, 2-4, and 2-5. Overall fat concentration in the metritis group prior to dry off showed a tendency where the HD group had the greatest mean fat concentration of 6.52 ± 0.96 compared to the HG and LD groups of 4.13 ± 0.56 and 4.79 ± 0.82, respectively ($P = 0.09$) (Table 2-3). Mean fat concentrations showed no statistical significance at +1 ($P = 0.60$) and +2 weeks ($P = 0.26$) for the metritis group (Table 2-4 and 2-5 respectively). Fat content was not different between the three groups among retained placenta, ketosis, lameness, and mastitis for all 3 time points. The FPR showed no statistical significance except for the ketosis group at +2 weeks ($P < 0.01$) where the HD group exhibited the highest FPR of 1.71 ± 0.19 compared to the HG and LD groups of 1.02 ± 0.10 and 1.21 ± 0.15, respectively (Table 2-5). Moreover, concentrations of MUN and TS were not significant between the groups for all diseases analyzed for each of the sampling time points.

2.3.6 Milk production

Data for milk production (kg) are presented as total yields for 60 DIM in Table 2-6. Overall comparisons of the mean total milk production showed that cows in the HD group had a decrease in milk production compared to the LD and HG. Cows with high SCC prior to dry off that were diagnosed with retained placenta had a mean total milk production of 2,042.99 ± 216.57 compared to the low SCC cows of 2,285.54 ± 216.57 and the HG of 2,716.81 ± 153.14 ($P = 0.04$). Cows in

the HD group diagnosed with mastitis had a mean total yield of 1,970.88 ± 177.47 compared to the LD group of 2,652.61 ± 158.63 and the HG of 2,742.51 ± 122.88 ($P < 0.01$). The effect of previous 305 DIM yields as well as the interaction between health status and previous yield showed significance for mastitis ($P < 0.01$). The high SCC cows diagnosed with ketosis had a mean yield of 2,301.81 ± 152.20 compared to the LD group of 2,672.33 ± 139.34 and the HG of 2,789.97 ± 107.43 ($P = 0.05$). The effect of previous 305 DIM yields was also significant for ketosis ($P < 0.01$). Lameness HD group had a mean yield of 2,528.36 ± 122.83 compared to the LD group of 2,633.04 ± 122.83 and HG of 2,716.81 ± 86.85 but was not statistically significant ($P = 0.46$). Comparisons of means among the total milk yields for metritis was not statistically significant ($P = 0.84$), however the HD group did have a lower mean yield (2,683.27 ± 93.00) compared to the LD group (2,748.07 ± 89.65) and healthy cows (2,748.21 ± 68.90). The effect of previous 305 DIM yield was significant on the total yield after calving ($P = 0.02$).

Differences in milk yields between HG, LD, and HD groups among each disease can be found in Table 2-7. The differences in daily milk yield showed that HD cows would produce less milk compared to the LD cows and HG. Daily milk losses per cow with high SCC compared to healthy can be 1.07, 12.86, 11.23, 8.14, and 3.14 when diagnosed with metritis, mastitis, retained placenta, ketosis, and lameness respectively. Likewise, HD compared to LD showed that daily milk decrease of high SCC cows before dry off diagnosed with disease to be 1.08, 11.36, 4.04, 6.18, and 1.74 for metritis, mastitis, retained placenta, ketosis and lameness respectively.

2.4 Discussion

We hypothesized that high SCC in the milk before dry off is related to the incidence of post-partum disease, as well as alterations in milk composition and milk yield. Indeed, results revealed that cows with high SCC had a higher incidence of periparturient disease, most notably ketosis. Analysis of milk composition revealed that cows with high SCC had lower lactose concentrations prior to dry off for all diseases analyzed. Moreover, protein concentrations were greater in the HD group prior to dry off, decreasing on the first week after parturition, further decreasing on the second week. Milk SCC after parturition were higher in the HD group for retained placenta, while the LD group for mastitis had high SCC after parturition compared to the HD group. Milk production was lower for the HD group for all diseases where mastitis, retained placenta, and ketosis were found to be significant.

2.4.1 Relation of SCC to incidence of periparturient disease

To our best knowledge, this is the first study to establish a relationship of high SCC prior to dry off to the incidence of periparturient disease in dairy cows. The most interesting finding of this study was that the incidence of ketosis was significantly greater for high SCC cows before dry off. Indeed, a cow with high SCC prior to dry off was 166% more likely to develop ketosis during the first 2 weeks after parturition compared to low SCC cows. A possible explanation for high SCC cows prior to dry off being more susceptible to the incidence of ketosis or other periparturient disease could be due to circulating endotoxins (ET). The reason for this assumption is based on Eckel and Ametaj (2016) suggestion of 3 sources of bacterial endotoxins within dairy cows including the mammary gland, reproductive tract, and the rumen. We speculate that before administration of antibiotic treatment at dry off ET could potentially be translocating out of the mammary gland into the systemic circulation of the high SCC cows. This means that while the antibiotic is treating the mammary gland at dry off, systemic circulation may potentially be contaminated with bacterial ET before antibiotic treatment.

The potential of ET to translocate from mammary gland into the systemic circulation is supported by previous studies of both naturally-occurring and experimentally-induced mastitis where lipopolysaccharide (LPS) concentrations were found to increase within the plasma of mastitic dairy cows (Hakogi et al., 1989; Dosogne et al., 2002). A study by Zebeli et al. (2011) reported that parenteral administration of increasing doses of LPS around calving increased blood β-hydroxybutyric acid (BHBA) at -10 d prior to parturition. It is well known that increasing levels of BHBA is associated with ketosis in dairy cows (Työppönen and Kauppinen, 1980). Additionally, Zhang et al. (2016) found elevations of serum BHBA at -4 weeks prior to calving in pre-ketotic cows suggesting that ET insults could be a potential contributor to the elevation of BHBA. Therefore, the incidence of ketosis in high SCC cows could be attributed, among others, to translocation of ET into the systemic circulation prior to dry off and during dry period contributing to elevation in ketone bodies throughout the dry period and increasing susceptibility to ketosis before parturition.

The odds ratio demonstrated that cows with high SCC prior to dry off have an increased likeliness in the incidence of metritis, retained placenta, and lameness by 43.0%, 56.0%, and 31.0%

respectively (Table 2-2). However, the p-values were not statistically significant meaning other factors could potentially be involved in the incidence of disease after parturition. Retained placenta has been known to occur with abortion, twins, dystocia, and short gestation. In addition, cows with retained placenta are also likely to have incidence of metritis, which is plausible since some cows in this study were affected by multiple diseases. Bacterial infection of the uterus following parturition may also be a contributing factor to metritis incidence (Opsomer, 2015). Feeding of large quanities of grain has been strongly associated with incidence of lameness (Bergsten, 2003). Elevated SCC before dry off may have a role in incidence of metritis, retained placenta, and lameness including bacterial endotoxins from high SCC mammary glands; however further investigation is required on this matter.

Inflammatory insult and bacterial endotoxins, arising from high SCC cows, could be contributing or increasing the likeliness in the incidence of these 3 diseases. For lameness, both systemic and local administrations of LPS have resulted in lesions within the corium and epidermis of the hoof region, suggesting a role for ET in the pathogenesis of disease (Boosman et al., 1991). Intermitent and increasing doses of LPS over 3 consecutive weeks relative to parturition were associated with higher incidence of retained placenta (Zebeli et al., 2011). Zebeli et al. (2011) suggested neutrophil exposure to LPS could potentially result in LPS tolerance causing reduced TLR4 expression by cells and weakening of the host immune response. Development of LPS tolerance could explain why high SCC cows were 56% likely to develop retained placenta.

For metritis, the reproductive tract has been proposed as a source of ET along with the mammary gland and rumen of dairy cows (Eckel and Ametaj, 2016). Mammary gland infections may however, influence the reproductive tract in dairy cows. For example, induction of mastitis by infusing *Escherichia coli* (*E. coli*) LPS in cows has been reported to lower follicular estrogen, androstenedione, and progesterone by 40, 13, and 35%, respectively (Lavon et al., 2011). In *Staphylococcus aureus* (*S. aureus*) induced mastitis Lavon et al. (2011) demonstrated a reduction by 56% in concentrations of circulating estrogen. Additionally, hormone release from the hypothalamus and pituitary gland can become hampered by systemic ET. This was demonstrated in ewes by Battagila et al. (2000) where LPS delayed or blocked the luteinizing hormone and follicular-stimulating hormone surges, interrupting the pre-ovulatory increase in estrogen. Likewise, this type of delayed response in dairy cows can result in poor reproductive performance.

Therefore, high SCC cows that have an increasing likelihood in the incidence of metritis may also have poor reproductive performance.

Moreover, previous research conducted by our team found alterations in innate immune reactants beginning at -8 weeks and -4 weeks prior to parturition in cows affected by retained placenta, metritis, lameness, ketosis, and mastitis (Zhang et al., 2015; Dervishi et al., 2015; Dervishi et al., 2016a; Dervishi et al., 2016b; Zhang et al., 2016). Results from previous studies indicate that dairy cows prior to calving are under a chronic inflammatory state starting as early as 2 months prior to parturition. While we did not analyze alterations in innate immune reactants before cows were dried off, it is speculated that high SCC cows are experiencing an inflammatory state at the end of their lactation cycle and throughout the dry period.

The odds ratios for mastitis incidence for LD and HD groups of cows was 0.98 and 1.02, respectively, which suggests other factors could potentially make cows susceptible to post-partum mastitis. This could include the dry cow therapy treatment not being effective at dry off, insufficient development of the sealant within the teat canal, or breakdown of the sealant before calving (Arnold, 2012).

2.4.2 Alterations in milk composition

Cows with high SCC prior to dry off diagnosed with post-partum disease had significantly higher SCC in the milk. The significant differences of SCC among the groups and between diseases were expected. The elevated SCC was continuous for the HD group that were diagnosed with retained placenta after parturition. In comparison to the LD group where the SCC after parturition remained <200,000 cells/mL. It was previously observed in cows diagnosed with retained placenta to have elevated SCC during diagnosis week, but SCC were within normal range (Dervishi et al., 2016a). In the current study cows at +1 week, which was the week of diagnosis for retained placenta, had significantly higher SCC for the HD group. At +2 weeks the SCC decreased to slightly above subclinical levels. The potential reason for the continued high SCC during the three time periods in the retained placenta group could be attributed to the number of diseases the cows were experiencing during retained placenta diagnosis. The effect of number of diseases (multiple diseases) was accounted for when modeling SCC for retained placenta; however, this effect was

not significant, suggesting other factors may be contributing to the elevated SCC after parturition in cows with retained placenta.

Somatic cell counts in the mastitis group increased for both the LD and HD groups after parturition. It is possible that the LD group could have incidence of new IMI during the dry off period, consequently having elevated SCC in the milk after parturition. Arnold (2012) has stated that 95% of all new IMI occur around 2-3 weeks prior to parturition. It has been indicated that the incidence of IMI is highest at the beginning of the dry off period and towards the end of gestation (Smith et al., 1985). In addition, the type of bacterial strain could be a factor in low SCC cows becoming sick, or the high SCC cows before dry off still having high incidence of mastitis. For example, bacterial infection by *S. aureus* were found to be higher in early lactation (Persson Waller et al., 2009). Additionally, host immune response to *S. aureus* infections was found to be weaker (Bannerman et al., 2004). The slow response could be due to the biofilm formation by *S. aureus*, which protects the pathogen from phagocytosis by neutrophils (Cucarella et al., 2004). It would be intriguing to conduct bacterial strain analysis of milk microbiota in low and high SCC cows to determine the type of pathogens that could potentially be causing mastitis.

Cows in the HD group prepartum that were diagnosed with lameness, ketosis, or metritis after parturition showed to have normal SCC in the milk after parturition. It was previously demonstrated in cows diagnosed with metritis, ketosis, and lameness to have higher SCC during diagnosis of disease compared with healthy cows; however, the number of SCC were within the normal ranges, below 200,000 cells/mL, which is in agreement with the current study (Dervishi et al., 2016b, Zhang et al., 2015; Zhang et al., 2016). For lameness, authors have suggested decrease in milk SCC for the HD group could be attributed to the pain sensation associated with inflammation of the hoofs, and cows may stand more instead of lying because the latter is extremely painful to lame cows (Cook et al., 2003; Nordlund and Cook, 2003); however this remains controversial. Archer et al. (2011) aimed to establish a relationship between milk SCC and lameness. The authors of the study found lame cows had lower SCC compared to non-lame cows, and concluded that lame cows spend more time standing than lying thereby decreasing exposure of the mammary gland to the bedding bacteria (Archer et al., 2011).

Alterations in lactose concentrations were observed in the HD group for all diseases. The mammary gland is composed of a network of alveoli that are lined with mammary epithelial cells (MEC), which are the cells that secrete milk; and are connected by tight junctions to prevent milk

leakage (McManaman and Neville, 2003). During infections there is an influx of leukocytes into the mammary gland in order to remove the pathogens, which results in increased gap between the epithelial cells (Auldist et al., 1995; Bruckmaier et al., 2004). The increase gap facilitates leakage of lactose into the systemic circulation, which is supported by several authors who observed elevations in lactose concentration within the blood and urine of mastitic cows (Auldist et al., 1995; Bruckmaier et al., 2004). Other factors that could cause lactose decline include the ability of some specific bacterial serotypes to use lactose for their needs (Auldist et al., 1995) and the physical damage to the MEC causing reduced lactose synthesis. In addition, lactose functions as a main osmotic regulator for milk synthesis (Auldist and Hubble, 1998); therefore, if lactose were to decline then milk production would decline in high SCC cows.

Furthermore, it is suggested that pro-inflammatory cytokines along with pathogens and their associated ET play a role in the synthesis of lactose. For example, tumor necrosis factor-α (TNF-α), a potent pro-inflammatory cytokine, has been shown to influence lactose secretion pathway by causing a downregulation of lactose synthesis-related genes such as α-lactalbumin gene, and degradation of glucose transporter-1 (GLUT1) from the basolateral membrane (Kobayashi et al., 2016). Therefore, the decrease in lactose of high SCC cows could be attributed to elevation of inflammatory mediators during infection, and the suppression of genes responsible for lactose synthesis.

We also observed alterations in milk protein concentrations for lameness at all time points, ketosis at +1 and +2 weeks, and retained placenta showing a tendency prior to dry off. Interestingly, protein concentration within the HD group was greater prior to dry off then decreased after parturition for all diseases. Protein concentration during a mammary gland infection tends to increase as a result of the influx of blood-borne proteins including serum albumin and immunoglobulins as part of the immune response (Shuster et al., 1991; Auldist et al., 1995; Auldist and Hubble, 1998). A study using proteomics method in identifying proteins in bovine milk of mastitic cows identified proteins involved in the immune response including lactoferrin, transferrin, fibrinogen, apolipoprotein AI, glycosylation-dependent cell adhesion molecule-1, peptidoglycan recognition proteins, and cathelicidin-1 (Boehmer et al., 2010). Additionally, lactoferrin (iron-binding protein) has been found to increase 100-fold during the involution stage of the dry period (Smith and Oliver, 1981) in order to prevent usage of iron by iron-utilizing

bacteria (Ward et al., 2002). It was previously demonstrated that lameness was associated with a decline in milk protein as well as a significant decline in milk fat (Penev and Stankov, 2015).

Moreover, the main proteins of milk have shown to decrease during infection which includes casein and whey proteins such as α-lactalbumin and β-lactoglobulin. The decrease in milk proteins can be caused by secreted proteinases from bacteria, leakage of proteins from the mammary gland, and decrease in synthesis as a result of damage of the MEC (Auldist and Hubble, 1998). Additionally, secretion of pro-inflammatory cytokine TNF-α in rats was reported to suppress both transcriptional and posttranscriptional gene expression for β-casein (Shea-eaton et al., 2001). Under normal conditions TNF-α is important for proliferation and differentiation of mammary epithelial cells in the mammary glands of rats (Ip et al., 1992). In dairy cows, elevation of TNF-α along with other pro-inflammatory cytokines is important for the host immune response against infection; and therefore could inhibit milk protein expression.

Strong associations have been shown in milk protein concentrations and energy balance where low milk protein reflects negative energy balance (NEB) and poor reproductive performance (Fahey, 2008). Negative energy balance is strongly associated with ketosis, especially during early lactation where energy demand for milk production is high (Çağdaş, 2013). We observed decreased milk protein after parturition in cows diagnosed with ketosis where the HD group exhibited the lowest concentrations. This decrease in milk protein could indicate that cows are undergoing NEB and have insufficient feed intake. Moreover, neutrophil granules contain both enzymes and antibacterial peptides that are important for removing bacteria during infection, but also can modify milk protein synthesis during infection (Sordillo et al., 1997).

Additionally, ET or pro-inflammatory cytokines translocated from mammary gland of high SCC cows at dry off may have a role in NEB, and indirectly contribute to low milk protein resulting in the incidence of ketosis. Systemic circulation of pro-inflammatory cytokines trigger expression of acute phase proteins from the liver (Ametaj et al., 2011). Reports in LPS-induced mastitis models showed an induction of the transcriptome response by the liver, and increase in acute phase protein genes (Minuti et al., 2015). Zhang et al. (2016) observed an upregulation of TNF-α and serum amyloid A in ketosis cows during the week of diagnosis of disease as well as elevations occurring at -8 and -4 weeks before calving. This study has confirmed that the incidence of ketosis is significantly related to high SCC prior to dry off, and the decrease in milk protein concentration in the HD group, which further supports this speculation.

In the current study, there was a significant difference for the FPR in the HD group diagnosed with ketosis at +2 weeks. The FPR has been suggested as an indicator for diagnosing cows with ketosis and other diseases (Heuer et al., 1999). Several authors had proposed different cut-off values for diagnosis of ketosis using the FPR values. For example, Heuer et al. (1999) found that cows had an increased risk for clinical ketosis with an FPR of > 1.5. The authors also found there was a greater incidence of other post-partum diseases including displaced abomasum, ovarian cysts, lameness, and mastitis at that cut off value (Heuer et al., 1999). Other authors reported increased incidence of retained placenta, displaced abomasum, metritis, endometritis, and increased culling risk with an FPR > 2.0 at 7 DIM (Toni et al., 2001). The HD group that were diagnosed with ketosis had greater FPR prior to dry off (1.33 ± 0.19), +1 (1.57 ± 0.20), and at +2 weeks (1.71 ± 0.19) compared to the HG and LD groups, which further supports that high SCC cows before dry off are susceptible to incidence of ketosis.

Alterations in milk fat composition showed no differences between groups for 4 diseases, while metritis showed a tendency prior to dry off. Milk fat concentrations where found to be greater in the HD group of pre-metritic cows. Previously milk fat concentrations were shown to be lower in cows during the week of diagnosis of metritis (Dervishi et al., 2016b). It was found that milk fat during mammary gland infections is increased while lactose is reduced (Bruckmaier et al., 2004). The increased gaps between the tight junctions results in leakage of milk components from the mammary gland; however, milk fat globules are too large to move through the tight junctions and remain within the mammary gland (Holdaway, 1990). The increase in milk fat concentrations in the HD group prior to dry off may be attributed to the decrease in other milk components during infections, and less likely related to incidence of metritis.

In this study, there were no differences between the three groups for concentrations of MUN and TS in the milk among all diseases analyzed.

2.4.3 Alterations in milk production

Milk production was demonstrated to be lower for the first 60 DIM in all diseases analyzed with retained placenta, mastitis, and ketosis being the most significant in production loss in cows with high SCC prior to dry off (Table 2-4). Previously, we showed a decline in daily milk production in cows diagnosed with metritis, mastitis, retained placenta, lameness, and ketosis

(Zhang et al., 2015; Dervishi et al., 2015; Dervishi et al., 2016a; Dervishi et al., 2016b; Zhang et al., 2016). Additionally, the incidence of new IMI can significantly impact both the synthesis and secretion of milk, causing milk decline (Petrovski and Stefanov, 2006). The decrease in milk production with high SCC can be attributed to the bacterial infection resulting in an influx of leukocytes in the mammary gland and secretion of inflammatory mediators causing disruption of the tight junctions (Auldist et al., 1995; Bruckmaier et al., 2004). Furthermore, the reduction in lactose synthesis can contribute to milk production loss (Auldist and Hubble, 1998), which was observed in the HD group of this study.

In addition, prolactin from the anterior pituitary gland is important in regulation of both the cellular and humoral immune responses. Towards the end of gestation there is an increase in prolactin secretion, which stimulates proliferation of the alveoli within the mammary gland, which is reflected by an increase in milk production after parturition (Sjaastad et al., 2016). It's been reported that external LPS causes activation of the hypothalamic-pituitary-adrenocortical axis and secretion of pro-inflammatory cytokines (Turnbull and River, 1999). Additionally, pro-inflammatory cytokines have been shown to inhibit prolactin secretion in rodents (Mandrup-Poulsen et al., 1995). Therefore, it is speculated that high SCC cows that have lower milk production after parturition may be related to suppression of prolactin secretion in pituitary gland.

The dry period is important for regeneration of milk-secreting tissues within the mammary gland (Sjaastad et al., 2016). Our finding with regards to lower milk yield in cows with high SCC suggest that inflammation in the mammary gland prior to dry off affects milk yield and composition of the next lactation. In addition, high SCC cows also showed to have a decrease in daily milk yield when compared to low SCC and healthy cows. This is especially important for producers as cows with high SCC before dry off may cause tremendous economic losses in the following lactation. Additional negative impacts of high SCC on the farm profitability include the costs of treatments and extra labour, increased culling rate, and discarding of milk (National Mastitis Council, 1996). Therefore, better management of late lactating dairy cows before the dry off period may be necessary in order to increase future production and lower disease incidence. This would include testing cows for SCC before cows are dried off. More research is required on the etiology of high SCC cows in relation to incidence of periparturient disease in order to develop a better understanding of the pathomechanisms involved in the disease process and thereby improve dairy cow health during the periparturient period and decrease production loss.

2.5 Conclusions

Results from this study indicate that dairy cows with high SCC prior to dry off were highly susceptible to incidence of ketosis after parturition. Low protein concentrations after parturition were strongly significant in high SCC cows diagnosed with ketosis possibly related to a NEB status. Additionally, significant differences were found in the FPR for the HD group at +2 weeks in cows that were diagnosed with ketosis. Albeit not significant, the incidence of metritis, retained placenta, and lameness were more likely to occur in cows with high SCC before the dry off; however other factors may also be contributing to the incidence of disease. Milk composition was shown to be altered in high SCC cows where lactose was lower at all sampling time points, and protein concentrations where higher prior to dry of and lower after parturition. Somatic cell counts were significantly greater for high SCC cows prior to dry off for all diseases. After parturition, SCC were greater for the HD group with retained placenta, which could be related to other factors. Somatic cell counts after parturition for ketosis, metritis, and lameness groups were within normal ranges (<200,000 cells/mL), while SCC were not significant between LD and HD groups diagnosed with mastitis. Milk production after parturition was also found to be significantly lower for cows with high SCC prior to dry off that were diagnosed with mastitis, ketosis, and retained placenta. Although not statiscally significant, milk production for high SCC cows was numerically lower for metritis and lameness, indicating milk yield potentially could be affected after parturition if cows are dried off with high SCC.

2.6. Acknowledgements

We acknowledge the financial support of the funding agency Alberta Livestock and Meat Agency Ltd. (ALMA) for this project. Dr. Burim N. Ametaj was the principal investigator of this study who contributed to conceptualization and designing of this research work, as well as organizing and managing the entire study. Dr. Ametaj supervised all lab analyses, and contributed in writing, editing as well as in improving the quality of this manuscript. We also acknowledge Dr. Vera Mazurak for contributing for the editing and improving the quality of this manuscript. We acknowledge the assistance of Dr. Andre Luiz Gracias Dias, Dr. Erkan Pehlivan, and Eduardo Barahona for collection of samples. We are also grateful to the technical staff of the Dairy Research

and Technology Centre, University of Alberta, and the staff of Sunalta Ltd., Ponoka, Alberta for their help and care to the cows.

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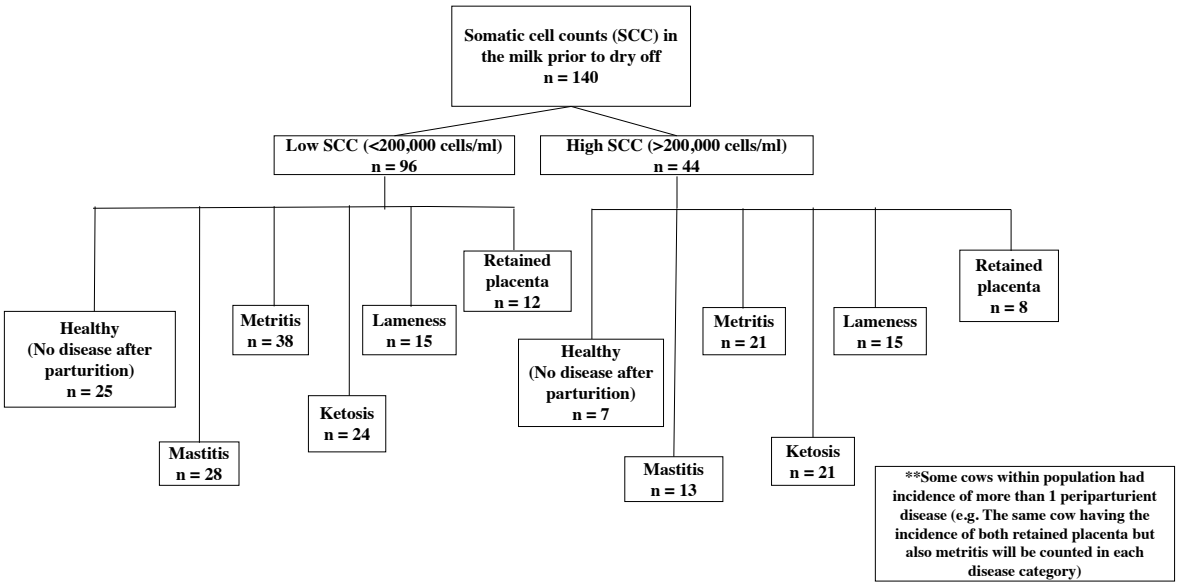

Figure 2-1. Flow diagram for the incidence rate of diseases for cows grouped based on somatic cell counts (SCC) in the milk obtain at prior to dry off (approximately 1 week before the expected date of dry off). Cows with SCC <200,000 cells/mL were sorted into the Low SCC group, and cows with SCC >200,000 cells/mL were sorted into the High SCC group. After parturition, cows from each group were evaluated for each of the 5 diseases of interest (i.e. mastitis, metritis, ketosis, lameness, and retained placenta) with the total number of cows per each disease indicated in each box in the diagram. Healthy cows were identified if no incidence of disease occured. *Note: Some cows diagnosed with more than one disease after parturition may have been counted more than once (i.e., the same cow could have been classified as retained placenta and metritis).

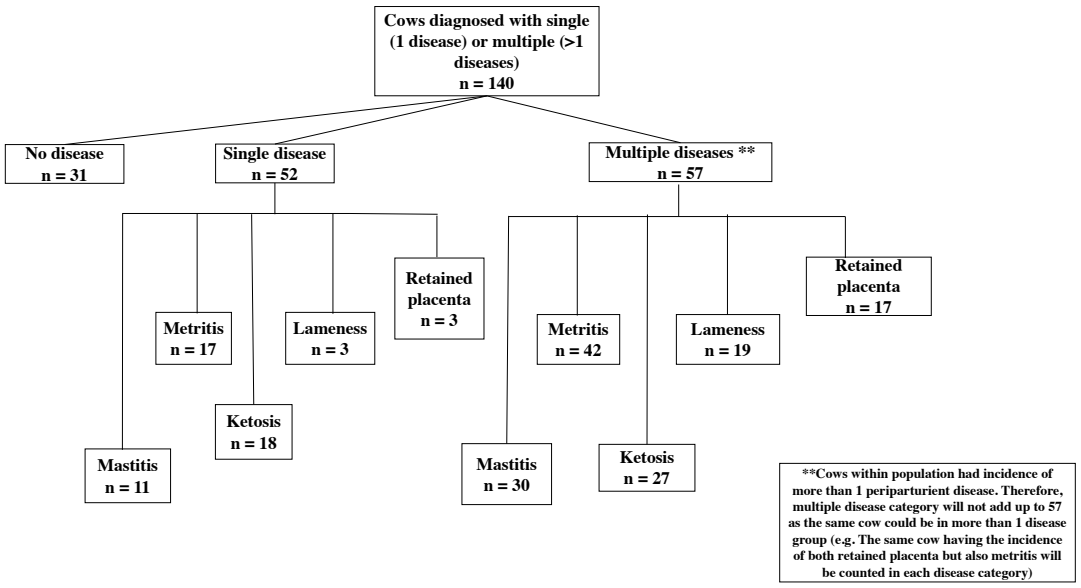

Figure 2-2. Flow diagram for the incidence rate of each of the 5 periparturient diseases (i.e. mastitis, metritis, ketosis, lameness, retained placenta) for cows diagnosed with Single disease (1 disease only), Multiple diseases (2 or more diseases), and No diseases. Note: Cows may have been diagnosed with 1 or more diseases after parturition (i.e., the same cow could have been classified as retained placenta and metritis).

Table 2-1. Frequencies of the incidence rate for cows diagnosed with periparturient disease between SCC groups and number of diseases.

Disease	SCC Group*			Number of Diseases*[4]	
	Low[1]	High[2]	Total[3]	Single	Multiple
Metritis	38 (27.14)	21 (15.00)	59 (42.14)	17 (12.14)	42 (30.00)
Retained Placenta	12 (8.57)	8 (5.71)	20 (14.29)	3 (2.14)	17 (12.14)
Mastitis	28 (20.00)	13 (9.29)	41(29.29)	11 (7.86)	30 (21.43)
Ketosis	24 (17.14)	21 (15.00)	45 (32.14)	18 (12.86)	27 (19.29)
Lameness	15 (10.71)	7 (5.00)	22 (15.71)	3 (2.14)	19 (13.57)
Healthy	24 (17.14)	7 (5.00)	31 (22.14)	-	-

[1]Low: SCC prior to dry off <200,000 cells/ml, percentage of total cows diagnosed with disease
[2]High: SCC prior to dry off >200,000 cells/ml, percentage of total cows diagnosed with disease
[3]Total cows diagnosed with disease and percentage of total population
[4]Number of diseases: cows diagnosed with 1 disease (single) and > 2 diseases (multiple), exception are healthy cows which had no incidence of disease.
*Percentages (brackets) are calculated out of the total population (n=140). Note that cows were diagnosed with multiple diseases, and there is overlap within other disease groups, except for SCC groupings and single disease (i.e. The same cow diagnosed with retained placenta and metritis is considered multiple). Therefore, sum of the percentages will not equal 100.

Table 2-2. Comparisons of odd ratios between low SCC and high SCC cows that were diagnosed with post-partum disease.

	Odds Ratio				
Disease	Low[1]	95% CI[2]	High[3]	95% CI	P-value
Metritis	0.70	0.34 - 1.44	1.43	0.69 - 2.96	0.33
Retained Placenta	0.64	0.24 - 1.71	1.56	0.59 - 4.13	0.38
Mastitis	0.98	0.45 - 2.15	1.02	0.47 - 2.23	0.96
Ketosis	0.38	0.18 - 0.80	2.66	1.26 - 5.65	0.01
Lameness	0.96	0.36 - 2.57	1.31	0.39 - 2.76	0.94

[1]Low: SCC prior to dry off < 200,000 cells/ml.
[2]95% CI: 95% Wald confidence limits.
[3]High: SCC prior to dry off > 200,000 cells/ml.

Table 2-3. Alterations in milk composition (fat, protein, fat:protein ratio, milk urea nitrogen (MUN), total solids (TS), and somatic cell counts (SCC)) prior to dry off in dairy cows diagnosed with post-partum disease.

Disease	Health	Milk Composition prior to dry off						
	Status	SCC 10^3 cells/ml	Fat%	Protein%	FPR	Lactose%	MUN mg/dL	TS%
Metritis[4]	HG[1]	66.40 ± 17.07[a]	4.13 ± 0.56[a]	3.49 ± 0.09[b]	1.19 ± 0.1	4.46 ± 0.09[a]	12.71 ± 1.06	13.08 ± 0.58
	LD[2]	15.10 ± 0.85[a]	4.79 ± 0.82[ab]	3.75 ± 0.12[a]	1.26 ± 0.22	4.47 ± 0.13[a]	12.23 ± 1.43	14.06 ± 0.82
	HD[3]	509.00 ± 64.70[b]	6.52 ± 0.96[b]	3.75 ± 0.12[a]	1.73 ± 0.26	4.01 ± 0.12[b]	14.39 ± 1.55	15.10 ± 0.85
	P-value	<0.01	0.09	0.11	0.17	0.01	0.55	0.15
Retained Placenta[5]	HG[1]	66.40 ± 28.99[a]	4.13 ± 0.36	3.40 ± 0.11[a]	1.19 ± 0.11	4.46 ± 0.09[a]	13.06 ± 0.99	13.08 ± 0.41
	LD[2]	47.29 ± 35.82[a]	4.59 ± 0.55	3.73 ± 0.14[a]	1.23 ± 0.16	4.47 ± 0.13[a]	14.49 ± 1.34	13.86 ± 0.63
	HD[3]	851.86 ± 152.03[b]	4.77 ± 0.56	3.91 ± 0.18[b]	1.26 ± 0.16	4.06 ± 0.13[b]	13.24 ± 1.49	13.67 ± 0.62
	P-value	<0.01	0.57	0.06	0.93	0.05	0.65	0.52
Mastitis[6]	HG[1]	66.40 ± 50.03[a]	4.13 ± 0.37	3.49 ± 0.07[a]	1.19 ± 0.11	4.46 ± 0.09[a]	12.71 ± 1.14	13.08 ± 0.41
	LD[2]	74.63 ± 72.62[a]	3.89 ± 0.50	3.61 ± 0.10[a]	1.07 ± 0.15	4.61 ± 0.13[a]	13.10 ± 1.59	13.09 ± 0.56
	HD[3]	1554.50 ± 331.44	4.28 ± 0.52	3.69 ± 0.10[b]	1.16 ± 0.15	4.07 ± 0.12[b]	12.46 ± 1.55	13.09 ± 0.56
	P-value	<0.01	0.86	0.24	0.82	0.01	0.96	1.0
Lameness[7]	HG[1]	67.79 ± 10.51[a]	4.25 ± 0.42	3.41 ± 0.10[a]	1.19 ± 0.12	4.37 ± 0.10[a]	13.03 ± 1.05	13.21 ± 0.51
	LD[2]	65.00 ± 14.56[a]	3.78 ± 0.56	3.79 ± 0.14[b]	1.03 ± 0.16	4.54 ± 0.14[a]	14.05 ± 1.54	12.90 ± 0.71
	HD[3]	351.29 ± 33.86	4.62 ± 0.62	4.10 ± 0.15[c]	1.15 ± 0.17	3.98 ± 0.13[b]	13.23 ± 1.50	13.55 ± 0.73
	P-value	<0.01	0.61	<0.01	0.73	0.01	0.85	0.82
Ketosis[8]	HG[1]	66.40 ± 52.32[a]	4.13 ± 0.39	3.49 ± 0.08	1.19 ± 0.12	4.46 ± 0.11[a]	12.71 ± 1.03	13.08 ± 0.46
	LD[2]	98.13 ± 87.09[a]	3.98 ± 0.53	3.55 ± 0.11	1.12 ± 0.16	4.43 ± 0.16[a]	11.07 ± 1.32	12.98 ± 0.62
	HD[3]	1292.71 ± 337.92	4.87 ± 0.63	3.64 ± 0.12	1.33 ± 0.19	3.73 ± 0.1[b]	10.82 ± 1.39	13.2 ± 0.67
	P-value	<0.01	0.49	0.55	0.68	<0.01	0.47	0.96

[1]Health group (n=15): cows that were low SCC and no incidence of disease throughout study period
[2]Low-disease: SCC < 200,000 cells/mL prior to dry off
[3]High-disease: SCC > 200,000 cells/mL prior to dry off
[4]Metritis: LD (n=8), HD (n=8)
[5]Retained placenta: LD (n=7), HD (n=7)
[6]Ketosis: LD (n=8), HD (n=7)
[7]Lameness: LD (n=7), HD (n=7)
[8]Mastitis: LD (n=8), HD (n=8)
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 2-4. Alterations in milk composition (fat, protein, fat:protein ratio, milk urea nitrogen (MUN), total solids (TS), and somatic cell counts (SCC)) at +1 weeks after parturition in dairy cows diagnosed with post-partum disease.

Disease	Health	Milk Composition at +1 weeks						
	Status	SCC 10^3 cells/ml	Fat%	Protein%	FPR	Lactose%	MUN mg/dL	TS%
Metritis[4]	HG[1]	54.07 ±10.80	4.56 ± 0.57	3.67 ± 0.07	1.25 ± 0.16	4.43 ± 0.05[a]	13.11 ± 0.61	13.86 ± 0.58
	LD[2]	51.38 ± 14.42	4.50 ± 0.77	3.84 ± 0.10	1.19 ± 0.21	4.42 ± 0.06[a]	11.26 ± 0.77	13.96 ± 0.80
	HD[3]	53.00 ± 14.65	5.49 ± 0.86	3.55 ± 0.10	1.57 ± 0.25	4.19 ± 0.06[b]	12.66 ± 0.82	14.74 ± 0.80
	P-value	0.99	0.60	0.12	0.41	0.01	0.20	0.66
Retained Placenta[5]	HG[1]	54.07 ± 78.73[a]	4.56 ± 0.49	3.81 ± 0.24	1.25 ± 0.15	4.44 ± 0.12[a]	14.09 ± 1.27	13.86 ± 0.50
	LD[2]	41.57 ± 101.06[ab]	4.80 ± 0.73	3.73 ± 0.28	1.29 ± 0.22	4.31 ± 0.18[ab]	14.72 ± 1.53	13.96 ± 0.74
	HD[3]	1359.86 ± 578.00[b]	5.68 ± 0.79	3.78 ± 0.34	1.60 ± 0.24	3.96 ± 0.17[a]	12.28 ± 1.59	14.91 ± 0.76
	P-value	0.06	0.46	0.97	0.42	0.10	0.57	0.49
Mastitis[6]	HG[1]	54.07 ± 96.00	4.56 ± 0.49	3.67 ± 0.17	1.25 ± 0.13	4.44 ± 0.05[a]	13.11 ± 0.75	13.86 ± 0.48
	LD[2]	1993.63 ± 798.24	4.54 ± 0.66	3.68 ± 0.23	1.24 ± 0.18	4.40 ± 0.07[a]	12.02 ± 1.00	13.87 ± 0.67
	HD[3]	1396.00 ± 667.96	4.94 ± 0.69	4.04 ± 0.24	1.32 ± 0.19	4.15 ± 0.06[b]	12.15 ± 1.00	14.37 ± 0.67
	P-value	0.15	0.89	0.42	0.94	<0.01	0.61	0.80
Lameness[7]	HG[1]	47.86 ± 9.63	4.50 ± 0.42	3.70 ± 0.10[a]	1.25 ± 0.12	4.37 ± 0.05	13.46 ± 0.79	13.91 ± 0.48
	LD[2]	62.57 ± 15.58	4.89 ± 0.72	3.87 ± 0.13[a]	1.04 ± 0.17	4.45 ± 0.06	12.03 ± 1.06	13.55 ± 0.67
	HD[3]	69.29 ± 16.39	4.89 ± 0.67	3.29 ± 0.12[b]	1.28 ± 0.96	4.38 ± 0.06	13.19 ± 1.10	13.12 ± 0.66
	P-value	0.47	0.85	<0.01	0.57	0.63	0.57	0.63
Ketosis[8]	HG[1]	54.07 ± 12.39	4.65 ± 0.48	3.67 ± 0.08[a]	1.25 ± 0.12	4.44 ± 0.04	13.11 ± 0.79	13.86 ± 0.50
	LD[2]	61.38 ± 18.08	4.03 ± 0.63	3.48 ± 0.10[a]	1.11 ± 0.16	4.43 ± 0.05	12.53 ± 1.06	13.03 ± 0.66
	HD[3]	87.00 ± 23.01	4.28 ± 0.65	3.26 ± 0.11[b]	1.57 ± 0.20	4.33 ± 0.06	13.47 ± 1.17	14.99 ± 0.73
	P-value	0.40	0.73	0.01	0.20	0.25	0.83	0.55

[1]Health group (n=15): cows that were low SCC and no incidence of disease throughout study period
[2]Low-disease: SCC < 200,000 cells/mL prior to dry off
[3]High-disease: SCC > 200,000 cells/mL prior to dry off
[4]Metritis: LD (n=8), HD (n=8)
[5]Retained placenta: LD (n=7), HD (n=7)
[6]Ketosis: LD (n=8), HD (n=7)
[7]Lameness: LD (n=7), HD (n=7)
[8]Mastitis: LD (n=8), HD (n=8)
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 2-5. Alterations in milk composition (fat, protein, fat:protein ratio, milk urea nitrogen (MUN), total solids (TS), and somatic cell counts (SCC)) at +2 weeks after parturition in dairy cows diagnosed with post-partum disease.

Disease	Health	Milk Composition at +2 weeks						
	Status	SCC 10^3 cells/ml	Fat%	Protein%	FPR	Lactose%	MUN mg/dL	TS%
Metritis[4]	HG[1]	35.53 ± 8.49	3.38 ± 0.22	3.32 ± 0.06	1.02 ± 0.07[a]	4.58 ± 0.03[a]	12.67 ± 0.78	12.34 ± 0.23
	LD[2]	22.63 ± 9.27	3.31 ± 0.30	3.36 ± 0.08	0.99 ± 0.09[a]	4.66 ± 0.05[a]	12.06 ± 1.04	12.37 ± 0.31
	HD[3]	30.13 ± 10.70	3.94 ± 0.32	3.16 ± 0.08	1.25 ± 0.10[b]	4.45 ± 0.04[b]	13.12 ± 1.09	12.64 ± 0.32
	P-value	0.64	0.26	0.17	0.10	0.01	0.78	0.73
Retained Placenta[5]	HG[1]	37.58 ± 22.99[a]	3.44 ± 0.41	3.46 ± 0.12	1.02 ± 0.08	4.55 ± 0.09	12.77 ± 1.10	12.38 ± 0.49
	LD[2]	43.57 ± 32.41[a]	3.57 ± 0.55	3.37 ± 0.14	1.07 ± 0.13	4.50 ± 0.11	11.06 ± 1.12	12.45 ± 0.64
	HD[3]	223.86 ± 73.47[b]	4.56 ± 0.62	3.09 ± 0.15	1.34 ± 0.14	4.36 ± 0.11	11.89 ± 1.44	13.34 ± 0.66
	P-value	0.02	0.28	0.27	0.15	0.42	0.53	0.48
Mastitis[6]	HG[1]	37.58 ± 67.26[b]	3.44 ± 0.35	3.33 ± 0.09	1.02 ± 0.10	4.55 ± 0.10	13.21 ± 1.00	12.38 ± 0.36
	LD[2]	939.37 ± 411.81[a]	3.50 ± 0.43	3.31 ± 0.10	1.07 ± 0.14	4.60 ± 0.13	12.03 ± 1.17	12.55 ± 0.45
	HD[3]	666.50 ± 346.88[a]	4.04 ± 0.46	3.30 ± 0.10	1.23 ± 0.15	4.32 ± 0.12	10.23 ± 1.08	12.77 ± 0.45
	P-value	0.23	0.54	0.96	0.47	0.25	0.16	0.80
Lameness[7]	HG[1]	34.29 ± 15.58	3.35 ± 0.26	3.38 ± 0.09[a]	1.02 ± 0.08	4.57 ± 0.03	12.66 ± 0.88	12.28 ± 0.28
	LD[2]	91.29 ± 35.95	3.68 ± 0.45	3.39 ± 0.11[a]	0.98 ± 0.12	4.52 ± 0.05	11.80 ± 1.20	12.37 ± 0.40
	HD[3]	58.71 ± 28.83	4.18 ± 0.42	2.93 ± 0.10[b]	1.31 ± 0.14	4.45 ± 0.05	12.96 ± 1.26	12.45 ± 0.40
	P-value	0.28	0.27	<0.01	0.13	0.16	0.78	0.94
Ketosis[8]	HG[1]	35.53 ± 9.04	3.33 ± 0.28	3.32 ± 0.07[a]	1.02 ± 0.10[a]	4.58 ± 0.04[a]	12.67 ± 0.79	12.34 ± 0.28
	LD[2]	41.50 ± 13.38	3.31 ± 0.39	3.12 ± 0.10[b]	1.21 ± 0.15[a]	4.55 ± 0.05[a]	11.16 ± 1.01	12.49 ± 0.39
	HD[3]	44.43 ± 14.80	3.91 ± 0.42	2.80 ± 0.10[c]	1.71 ± 0.19[b]	4.42 ± 0.05[b]	11.96 ± 1.12	13.11 ± 0.43
	P-value	0.85	0.46	<0.01	<0.01	0.09	0.52	0.32

[1]Health group (n=15): cows that were low SCC and no incidence of disease throughout study period
[2]Low-disease: SCC < 200,000 cells/mL prior to dry off
[3]High-disease: SCC > 200,000 cells/mL prior to dry off
[4]Metritis: LD (n=8), HD (n=8)
[5]Retained placenta: LD (n=7), HD (n=7)
[6]Ketosis: LD (n=8), HD (n=7)
[7]Lameness: LD (n=7), HD (n=7)
[8]Mastitis: LD (n=8), HD (n=8)
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 2-6. Total milk yields comparison for 60 days in milk (DIM) among diseases and between healthy, low-disease, and high-disease groups.

	Mean total yield for 60 DIM (kg)			*P*-value		
Disease	HG[1]	LD[2]	HD[3]	HS[4]	PY[5]	HS x PY[6]
Metritis	2748.21± 68.90	2748.07 ± 89.65	2683.27 ± 93.00	0.84	0.02	NS[7]
Retained placenta	2716.81 ± 153.14	2285.54 ± 216.57	2042.99 ± 216.57	0.04	NS	NS
Mastitis	2742.51 ± 122.88	2652.61 ± 158.63	1970.88 ± 177.47	<0.01	<0.01	0.01
Lameness	2716.81± 86.85	2633.04 ± 122.83	2528.36 ± 122.83	0.46	NS	NS
Ketosis	2789.97 ± 107.43	2672.33 ± 139.34	2301.81 ± 152.20	0.05	<0.01	NS

[1]Healthy group (n=15): cows that were low SCC and no incidence of disease throughout study period.
[2]Low-disease: SCC < 200,000 cells/mL prior to dry off.
[3]High-disease: SCC > 200,000 cells/mL prior to dry off.
[4]HS = effect of health status.
[5]PY = effect of previous yield for 305 DIM.
[6]HS x PY = effect of health status and previous yield.
[7]NS = no significance; variable showed no significance in the statistical model and was removed.

Table 2-7. Milk yield differences between groups for each disease for daily yield and total yield for 60 DIM per cow and for 100 cows.

		Disease				
	Group	Metritis	Mastitis	Retained placenta	Ketosis	Lameness
Difference in total milk	LD[1] vs. HG[2]	-0.14	-89.90	-431.27	-117.64	-83.77
yield for 60 DIM per	HD[3] vs. HG	-64.49	-771.63	-673.82	-488.16	-188.45
cow (kg/60d)[4]	HD vs. LD	-64.80	-681.73	-242.55	-370.52	-104.68
Difference in daily	LD vs. HG	-0.0023	-1.50	-7.19	-1.96	-1.40
milk yield per cow	HD vs. HG	-1.07	-12.86	-11.23	-8.14	-3.14
(kg/d)[5]	HD vs. LD	-1.08	-11.36	-4.04	-6.18	-1.74
Difference in daily	LD vs. HG	-0.23	-150	-719.00	-196	-140
milk yield per 100	HD vs. HG	-107	-1,286	-1,123	-814	-314
cows (kg/d)[6]	HD vs. LD	-108	-1,136	-404	-618	-174
Difference in total milk	LD vs. HG	-13.80	-9,000	-43,140	-11,760	-8,400
yield for 60 DIM	HD vs. HG	-6,420	-77,160	-67,380	-48,840	-18,840
per 100 cows (kg/60d) [7]	HD vs. LD	-6,480	-68,160	-24,240	-37,080	-10,440

[1]Low-disease: SCC < 200,000 cells/mL prior to dry off.
[2]High-disease: SCC > 200,000 cells/mL prior to dry off.
[3]Healthy group (n=15): cows that were low SCC and no incidence of disease throughout study period.
[4]Subtraction of total yield from Table 2-6 for LD and HD, respectively from HG group to get difference in total milk yield for 60 DIM per cow; Negative values indicate how much less LD and HD are producing compared to healthy, and how much less HD are producing compared to LD.
[5]Values calculated by dividing difference in total yield by 60 days in milk (DIM).
[6]Values calculated by multiplying daily yield by 100 to determine loss per day per 100 cows.
[7]Values calculated by multiplying daily yield per 100 cows by 60 to give total milk losses for 60 DIM per 100 cows.

Chapter 3 High somatic cell counts prior to dry off is associated with serum metabolite alterations in Holstein dairy cows diagnosed with periparturient disease.

ABSTRACT

It has been well established that dairy cows exhibit alterations in carbohydrate and lipid metabolism prior to parturition, which are strongly linked to the incidence of periparturient diseases of dairy cows. Intramammary infections of dairy cows are also problematic within the dairy industry. In addition, bacterial endotoxins have been known to alter blood metabolites related to incidence of disease; and the mammary gland has been proposed as a source of such toxins, which could potentially contribute to incidence of other periparturient diseases. The main objective of this study was to investigate whether high SCC prior to dry off is related to alterations in blood variables in cows diagnosed with periparturient disease. We analyzed serum variables including glucose, cholesterol, lactate, NEFA, and BHBA of healthy, as well as low SCC and high SCC cows diagnosed with disease. Results showed high SCC cows exhibiting lower glucose, cholesterol, and lactate concentrations, and strong correlations observed among high SCC cows prior to dry off to serum metabolites for BHBA at +1 week (metritis and ketosis) and glucose at +2 (mastitis). Concentrations of NEFA in the serum showed no significant alterations except for high SCC cows with mastitis at +1 week that had increased serum NEFA. Concentrations of BHBA in the serum showed overall significance but values were within normal range except for ketosis.

3.1 Introduction

Intramammary infections or mastitis in dairy cattle is the second most frequent disease in dairy cows, resulting in roughly 27,708 animals culled in Canada for 2016 (Government of Canada, 2017). Intramammary infection is the result of bacterial invasion of the mammary gland causing an increase in SCC in the milk (Tylor et al., 1990). Elevation of milk SCC has become a useful indicator in diagnosis of IMI in dairy cows. The threshold of SCC used for diagnosis of an IMI is when SCC exceed >200,000 cells/mL (Madouasse et al., 2010), and often accompanied by other physical symptoms including inflammation of the udder, altered milk composition and consistency, and decreased milk yield (Roberson, 2012). Infections that become systemic result in fever, increased heart rate, weight loss, depression, and weakness (Nickerson, 2011).

The dry off period in dairy cows occurs approximately 60 days prior to the anticipated parturition where the mammary gland is no longer secreting milk. The dry period is beneficial in dairy production to optimizing animal welfare as well as regeneration of milk secreting tissues, thus, maximizing milk production in the next lactation (Dingwell et al., 2001). However, the incidence of new IMI is highest at the beginning of the dry period and towards parturition (Oliver and Bushe, 1986). This can be a result of failure of the sealant within the teat canal or breakdown of the sealant around parturition (Arnold, 2012). Furthermore, as parturition approaches, dairy cows undergo a state of immunosuppression associated with high incidence of other periparturient diseases after calving (Goff & Horst, 1997; Mallard et al., 1998; Ametaj et al., 2005).

Several authors have reported alterations in serum metabolites preceding clinical diagnosis of clinical disease in dairy cows. For example, elevations of blood lactate have been observed starting at -8 and -4 weeks prior to the expected day of calving, in cows diagnosed with subclinical mastitis (Dervishi et al., 2015), retained placenta (Dervishi et al., 2016a), metritis (Dervishi et al., 2016b), ketosis (Zhang et al., 2016), and lameness (Zhang et al., 2015). Lactate measurements in both blood and milk have been found to be a useful indicator in assessing overall health status and udder health (Davis et al., 2004; Allen and Holm, 2008). Furthermore, increased concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) have been linked to the incidence of displaced abomasum, milk fever, mastitis, retained placenta, and ketosis (Duffield and LeBlanc, 2009, LeBlanc, 2010). Previous research conducted by our lab has also observed increased SCC in dairy cows diagnosed with retained placenta, metritis, ketosis, and lameness; although SCC were lower than 200,000 cells/mL (Dervishi et al., 2016a; Dervishi et al., 2016b; Zhang et al., 2015; Zhang et al., 2016).

To our best knowledge, no research has aimed to establish a relationship between high SCC in dairy cows before dry off to alterations in serum metabolites in relation to incidence of periparturient diseases. Therefore, we hypothesized that cows with high SCC before dry off are associated with multiple alterations in serum metabolites, and are related to the incidence of periparturient disease. The main objectives of the present study were to determine whether cows with high SCC at dry off and that were diagnosed with periparturient disease after parturition do exhibit alterations in serum metabolites preceding clinical signs of disease.

3.2 Materials and Methods

3.2.1 Animals and experimental design

The study was conducted at 2 farms located in Edmonton, Alberta, Canada, and Ponoka, Alberta, Canada. For this study we used a total of 140 pregnant Holstein dairy cows consisting of 82 multiparous and 58 primiparous. The first farm is a tie-stall system and we used a total of 104 cows. The second farm is a free-stall parlour system and we used a total of 36 animals. All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock. Proper care of each animal was followed in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Animals used in the study were randomly selected and had to meet the following criteria: 1) pregnant, and 2) entering the dry period. Approximately one week prior to being dried off each animal was sampled, and then sampled at +1 and +2 weeks post-partum. Samples collected at each time point included serum for a total of 3 samples per animal. Blood samples were obtained via tail venipuncture into one 10-mL vacutainer blood collection tube for serum (Becton, Dickinson & Company, USA). Blood samples for serum were allowed to clot and were then centrifuged at 4000 rpm for 20 minutes. The separated serum was then transferred using a disposable transfer pipette into a sterile polypropylene culture test tube (Fisher Scientific, Toronto, ON, Canada) and stored at -20°C. Approximately 1.5-mL of the separated serum was also transferred into a microcentrifuge tube and stored at -80 °C (Fisher Scientific, Toronto, ON, Canada).

Milk samples were collected at each time point and were analyzed for SCC similarly to the previous chapter. Somatic cell counts from prior to dry off were used to group cows as low SCC (<200,000 cells/mL) and high SCC (>200,000 cells/mL).

Fecal consistency was evaluated at each time point on a scale of 1-5 where a score of 1 = diarrhea, 2 = appears runny and does not form a distinct pile 3 = optimal score, porridge-like appearance; will stack up at 4-5 cm; 4 = manure is thicker and will stick to shoe, stacks up to more than 5 cm; 5 = firm fecal balls (Hutjens, 1999). Body condition scoring (BCS) was done for all animals at each sampling time point in accordance to Elanco's Body Condition Scoring in Dairy Cattle on a scale of 1-5.

Additional information including lactation, age, previous 305 days in milk yield, and date of the dried off were recorded. DairyComp Software was used to determine the length of the dry

period (days) per animal. Expected calving and actual calving dates were recorded and whether calving was early or late relative to expected calving. Calf information was recorded for gender, single or twins, mortality, and weight (kg). Daily milk weights (kg) were recorded up to the first 60 days in milk (DIM).

3.2.2 Clinical observations for peripartuient disease

In this study, we evaluated cows for 5 periparturient diseases including mastitis, metritis, retained placenta, ketosis, and lameness. Diagnosis of disease was done in a similar procedure from the previous chapter found in Section 2.2.2. All periparturient diseases and treatments were recorded throughout the experimental period. Breeding and culling records were recorded for the first 6 weeks after parturition. Animals were removed from the study if mortality/culling occurred during the dry period or post-partum where samples could not be obtained. External symptoms were observed including appetite, fecal consistency, and body condition score (BCS).

For analysis of serum, 6 healthy low-SCC cows throughout the duration of the study were selected as the controls to compare to each of the 5 diseases. Criteria for selection of the healthy group included similar BCS, fecal score, milk composition, dry period length, and no incidence of disease throughout the study period. For each SCC group we selected 6 low-SCC cows and 6 high-SCC cows before dry off that were diagnosed with periparturient disease. Each group was classified as healthy group (HG), low-disease (LD), and high-disease (HD), as previously described in Section 2.2.2. The experimental design for this study was a nested case-control design as described in Section 2.2.2 and Figure 2-1.

3.2.4 Serum Analyses

Concentration of serum glucose was measure by an enzymatic colorimetric method using commercially available kits provided by Sekisui Diagnostics Inc. (Charlottetown, Prince Edward Island, Canada). The reaction involves phosphorylation of glucose by hexokinase in the presence of adenosine-triphosphate (ATP) yielding glucose-6-phosphate (G-6-P). Oxidation of G-6-P that occurs by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD) yielding 6-phosphogluconate and NADH. Standards for glucose were

provided in the kit, and diluted to a set detection range of 19 to 152 mg/dL. All samples were analyzed in duplicate on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corp, California, USA) at 340 nm. The intra-assay CV were maintained at ≤10%, for all assays.

Serum concentration of cholesterol was measured by an enzymatic colorimetric method using commercially available kits provided by Sekisui Diagnostics Inc. (Charlottetown, Prince Edward Island, Canada). The procedure for determining cholesterol concentration involves hydrolysis of cholesterol esters into free cholesterol by cholesterol esterase. Oxidation of free cholesterol by cholesterol oxidase yielding cholest-4-ene-3-one and hydrogen peroxide. Hydrogen peroxide couples with 4-aminoantitipyrine and p-hydroxbenzoate in the presence of peroxidase to yield a chromogen. Standards for cholesterol were provided in the kit, and diluted to a set detection range of 20.5 to 164 mg/dL. All samples were analyzed in duplicate on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corp, California, USA) at 505 nm. The intra-assay CV were maintained at ≤10%, for all assays.

Quantitative determination of serum lactate was done by an enzymatic colorimetric method using commercially available kits provided by Biomedical Research Center (Buffalo, New York, USA). The test is based on the reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to formazan. Measurement by the assay is the concentration of intracellular and extracellular L-lactate. Standards for lactate were provided in the kit, and diluted to set a detection range of 125 to 1000 μM. All samples were tested in duplicate and diluted 10-fold with distilled ice H_2O. Lactate concentrations were determined by reading the optical density values on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corp, California, USA) at 429 nm. All results for lactate were multiplied by dilution factor of 10. The intra-assay CV were maintained at ≤10%, for all assays.

Serum NEFA concentration was determined by an enzymatic colorimetric method using commercially available kits provided by Randox Laboratories Ltd. (Crumlin, County Antrim, United Kingdom). The procedure involves acylation of coenzyme A by fatty acids by acyl-CoA sythetase and production of hydrogen peroxide in the presence of Acyl-CoA oxidase. Hydrogen peroxide, in the presence of peroxidase, permits the oxidative condensation of N-Ethyl-N-(2hydroxy-3-sulphopropyl)-m-toluidine with 4-aminoantipyrine to form a purple adduct, which is proportional to NEFA concentration within the sample. Standards for NEFA were provided in the kit and diluted to set a detection range of 0.25 to 2 mM/L. All samples were analyzed in duplicate

and concentrations were determined by reading the optical density values on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corp, California, United States) at 550 nm. The intra-assay CV were maintained at ≤10%, for all assays.

Enzymatic quantification of BHBA was done by an enzymatic colorimetric method using commercially available kits provided by Cayman Chemical (Ann Arbor, Michigan, USA). The procedure involves the oxidation of D-3-hydroxybutyrate to acetoacetate under the influence of 3-hydroxybutyrate dehydrogenase. The resulting reaction also produces cofactor NAD^+ which is reduced to NADH. NADH then reacts with the colorimetric detector WST-1 in the presence of diaphorase yielding a formazan dye. Standards for BHBA were provided in the kit, and diluted to set a detection range of 0.0625 to 0.5 mM. All samples were analyzed in duplicate and concentrations were determined by reading the optical density values on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corp, California, United States) at 450 nm. The intra-assay CV were maintained at ≤10%, for all assays.

3.2.3 Statistical analysis

The experimental design for this study was a nested case-control design where cows were blocked into two groups based on the SCC determined at prior to dry off, and then further blocked based on diagnosis of each of the 5 periparturient diseases (See section 2.2.2. and Figure 2-1 for detail on experimental design). We ran separate analysis for each disease and serum parameter.

For analysis of serum parameters, 6 healthy low-SCC cows throughout duration of the study were selected as the control to compare to each disease of interest, and were classified as the healthy group (HG). Control cows had similar BCS, fecal score, milk composition, dry period length, and were completely healthy throughout the entire study period. Six cows from each of the SCC group that were diagnosed with post-partum disease were randomly selected and classified as Low-Disease (LD) and High-Disease (HD). Cows selected for the LD group had been diagnosed with 1 disease throughout the study period (e.g., a cow was diagnosed for lameness only if she was suffering from lameness and no other diseases). However, in order to have a sufficient number of sick animals for comparison purposes, cows that were diagnosed with less than 3 diseases were used for analysis. For the metritis LD group, all cows were diagnosed with metritis only during the experimental period. In the mastitis group, all cows selected for the LD group were diagnosed

with mastitis only. On the other hand, the HD group had 3 of the 6 cows diagnosed with 2 diseases. For lameness, the LD group had 4 of 6 cows diagnosed with 2 diseases, and the HD group had 2 of 6 diagnosed with 2 diseases, and 1 with 3 diseases. For ketosis, all cows in the LD group were diagnosed with ketosis only whereas the HD had 3 out of 6 diagnosed with 2 diseases. For retained placenta, the LD had 3 out of 6 diagnosed with 2 diseases, and the HD group had 3 diagnosed with 2 diseases, and 3 diagnosed with 3 diseases. The effect of multiple diseases was taken into consideration when fitting the statistical model. The exception was the metritis group where all cows were diagnosed for just metritis during the study; therefore, the number of diseases was excluded from the statistical model for metritis.

Analysis of serum parameters were analyzed using SAS 9.4 software (SAS Institute Inc., Cary, NC). Normality of data was firstly tested using the UNIVARIATE procedure for each group and each parameter. Data that followed a normal distribution were analyzed using the MIXED procedure of SAS, and non-normal data were analyzed using the GLIMMIX procedure to generate the LSMEANS, standard error of the mean (SEM), and P-values. The effect of farm and cow were considered as random effect in the model statement. The effect of week was considered a repeated measure. The covariance structure was modeled according to the smallest Akaike information criterion (AIC) and the Bayesian information criterion (BIC) values generated. The effect of health status was forced into the model statement since we were interested in determining whether serum parameters were different between healthy, low-SCC, and high-SCC cows diagnosed with post-partum disease. Therefore, our main model was as follows:

$$Y_{ijkl} = \mu + H_i + e_{ijkl}$$

where μ = the overall population mean; H_i = the fixed effect of health status i (i = 1-3, healthy cows compared to LD and HD groups separately), and e_{ijkl} = the residual error. Additional model fixed effects for week, parity, and number of diseases were investigated along with their corresponding interactions for each serum parameter per disease group. A backwards elimination from a saturated model was performed if effect was not significant on the response variable.

There was no significance in the 3-way and 4-way interactions for all serum parameters, and were removed from the statistical model. The exception would be for retained placenta, where there was a significant effect of the 3-way interaction between week, number of diseases, and parity for cholesterol and lactate concentrations, and was therefore kept in the statistical model. The 3-way interaction between week, health status, and parity was also significant for serum

cholesterol for metritis, and was kept in the statistical model. Correlation analysis was conducted using the CORR procedure in SAS between milk SCC obtained from prior to dry off to each of the 5 parameters analyzed at all 3 time points. Analysis of SCC prior to dry off was done using the GLIMMIX procedure to determine the LSMEANS and SEM between groups. For all data, significance was declared at $P < 0.05$ and tendency at $0.05 \leq P \leq 0.10$.

3.3. Results

3.3.1 Somatic cell counts

Data relating to SCC prior to dry off can be found in Table 3-1. Somatic cell counts obtain prior to dry off were used to group cows into two SCC groups, and used for correlation of serum parameters. Results found SCC to be significantly greater in the HD group diagnosed with metritis where the HD mean SCC was 559.83 ± 85.12, LD group was 65.17 ± 85.12, and HG group was 73.33 ± 85.12 ($P < 0.01$). For retained placenta, the HD group SCC prior to dry off was 782.33 ± 202.62, LD was 52.17 ± 202.62, and HG was 73.33 ± 202.62 ($P = 0.04$). For ketosis, the HD group SCC was 1,423 ± 473.96, LD was 110.33 ± 473.96, and HG was 73.33 ± 473.96 ($P < 0.01$). The HD group was lameness had a SCC of 375.00 ± 25.91 while LD was 67.00 ± 25.91 and HG was 73.33 ± 25.91 ($P < 0.01$). There was a tendency found for mastitis where the HD group had greater SCC of 1,525 ± 497.21, and the LD and HG groups were 89.83 ± 497.21 and 73.33 ± 497.21, respectively ($P = 0.09$).

3.3.2 Glucose

Data for the concentrations of glucose prior to dry off as well as at +1 week and +2 week between the HG, LD, and HD groups for all diseases analyzed are presented in Table 3-2. Results showed that concentrations of glucose in the serum of cows diagnosed with mastitis prior to dry off to be significantly different, where the HG group had the highest overall mean concentration of glucose (74.98 ± 3.25 mg/dL) versus LD and HD groups (37.59 ± 3.25 and 37.24 ± 3.25 mg/dL, respectively; $P < 0.01$). Mean concentrations of glucose prior to dry off for cows diagnosed postpartum with metritis, retained placenta, ketosis, and lameness were not significant among the groups ($P = 0.17$; $P = 0.26$; $P = 0.22$; $P = 0.38$, respectively).

At +1 week, concentration of glucose was significantly different for mastitis, metritis, ketosis, and lameness in comparison to HG. The HD group for mastitis had the lowest glucose concentration at +1 week of 32.31 ± 2.51 mg/dL compared to the LD group of 34.23 ± 2.51 mg/dL and the HG group of 64.61 ± 2.51 mg/dL ($P < 0.01$). Similarly, cows in the HD group diagnosed with metritis had the lowest concentration of glucose (45.21 ± 4.07 mg/dL), whereas the LD group and HG group had higher concentrations (69.15 ± 4.07 and 64.61 ± 4.07 mg/dL, respectively) ($P < 0.01$). For ketosis, the LD group exhibited the lowest mean glucose concentration of 49.58 ± 3.62 mg/dL compared to the HD group of 54.51 ± 3.62 mg/dL and the HG group of 64.61 ± 3.62 mg/dL ($P = 0.03$). For lameness, the HD group had the lowest mean glucose concentration of 41.43 ± 5.05 mg/dL compared to the LD group and HG group with 52.99 ± 5.05 and 64.61 ± 5.05 mg/dL, respectively ($P = 0.02$).

At +2 week, cows diagnosed with mastitis demonstrated significance in the mean glucose concentration where the LD group had a mean of 32.38 ± 5.55 and the HD group had a mean glucose of 42.71 ± 5.55, which were both lower compared to the HG group was 59.02 ± 5.55 mg/dL ($P = 0.01$). Additionally, cows from the HD group, diagnosed with metritis, were found to have the lowest mean of glucose at 47.10 ± 4.35 compared to the LD and HG group of 63.33 ± 4.35 and 59.02 ± 4.35 mg/dL ($P = 0.05$). Cows diagnosed with retained placenta, ketosis, and lameness exhibited no significant alterations in their mean glucose concentrations compared to HG cows, at +2 weeks after calving ($P = 0.87$; $P = 0.22$; $P = 0.28$).

3.3.2 Cholesterol

Data related to cholesterol concentrations between groups at all 3 sampling time points can be found in Table 3-3. Concentrations of cholesterol at prior to dry off were found to be significant between groups for cows diagnosed with mastitis, retained placenta, and lameness, while ketosis demonstrated tendency ($P < 0.01$; $P = 0.04$; $P < 0.03$; $P = 0.06$, respectively). Cows diagnosed with metritis showed no significant alterations in the concentration of cholesterol in the serum prior to dry off ($P = 0.51$). With regards to cows affected by mastitis, the HD group exhibited the lowest mean concentration of cholesterol prior to dry off (93.20 ± 7.20 mg/dL) compared to the LD group (131.80 ± 7.20 mg/dL) and the HG group (160.67 ± 7.20 mg/dL). Interestingly, the HD group for cows diagnosed with retained placenta had the highest concentration of cholesterol prior

to dry off at 179.36 ± 29.67 compared to the LD group of 162.67 ± 29.67 and the HG group of 160.67 ± 29.67 mg/dL. For lameness, the HD group demonstrated the lowest cholesterol concentrations prior to dry off of 86.20 ± 8.31 compared to the LD group and HG group of 126.93 ± 8.31 and 160.67 ± 29.67 mg/dL, respectively. Ketosis demonstrated a tendency prior to dry off where the mean concentration of cholesterol for the HD group was the lowest at 113.62 ± 12.99 and the LD and HG groups were found to be 145.09 ± 12.99 and 160.67 ± 12.99 mg/dL, respectively.

At +1 week the HD group cows that were diagnosed with mastitis were found to have the lowest mean cholesterol concentration of 60.29 ± 5.83 while the LD group and HG group had a mean concentration of 78.66 ± 5.83 and 103.05 ± 5.83 mg/dL ($P < 0.01$). Additionally, concentrations of cholesterol in cows diagnosed with lameness were found to have significantly lower cholesterol with the HD group exhibiting the lowest mean concentration of 43.79 ± 5.87 compared to the LD group of 73.34 ± 5.87 and the HG group of 103.05 ± 5.87 mg/dL ($P < 0.01$). Concentrations of cholesterol for ketotic cows were found to be significantly altered where the HD group had a mean concentration of 67.71 ± 8.03 compared to the LD group and HG group of 73.50 ± 8.03 and 103.05 ± 8.03 mg/dL, respectively ($P = 0.02$). There were no significant alterations in the cholesterol concentration at +1 week for metritis and retained placenta ($P = 0.48$; $P = 0.20$).

Differences in cholesterol concentrations at +2 weeks were found to be significant for mastitis, ketosis and lameness ($P < 0.01$; $P = 0.05$; $P < 0.01$). The mean concentration of cholesterol for the mastitis group was lowest for the HD group of 74.38 ± 7.25 compared to the LD group of 103.05 ± 7.25 and the HG group of 124.26 ± 7.25 mg/dL. For ketosis, the mean concentrations for the HD group were found to be 76.01 ± 12.50 while the LD and HG groups had mean concentrations of 100.71 ± 12.50 and 124.26 ± 12.50 mg/dL, respectively. For lameness, the HD group cholesterol concentration was 54.23 ± 7.24 while the LD and HG group had mean concentrations of 82.55 ± 7.24 and 124.26 ± 7.24 mg/dL, respectively. There were no significant alterations in the mean cholesterol concentrations for metritis and retained placenta at +2 weeks post-partum ($P = 0.22$; $P = 0.84$).

3.3.3 Non-esterified fatty acids

Comparisons of mean NEFA concentrations between the groups at all sampling points can be found in Table 3-4. Overall data for NEFA showed no significant differences between the groups prior to dry off for mastitis, metritis, retained placenta, ketosis, and lameness ($P = 0.60$; $P = 0.72$; $P = 0.36$; $P = 0.46$; $P = 0.17$). At +1 week the mean concentration of NEFA in the serum for cows diagnosed with mastitis were significantly lower in the HD group with mean concentration at 2,010 ± 213.73 and the LD and HG groups of 1,294 ± 213.73 and 1,205 ± 213.73 μM, respectively ($P = 0.03$). The mean NEFA concentration at +1 week showed no significant differences for metritis, retained placenta, ketosis, and lameness between groups ($P = 0.88$; $P = 0.40$; $P = 0.23$; $P = 0.29$). Also, there were no significant differences between groups found for mean NEFA concentrations at +2 weeks for mastitis, metritis, retained placenta, ketosis, and lameness ($P = 0.69$; $P = 0.77$; $P = 0.23$; $P = 0.46$; $P = 0.79$).

3.3.4 Lactate

Data related to concentration of lactate in the serum for all disease groups can be found in Table 3-5. At prior to dry off there was an overall significant difference in the concentrations of lactate for metritis, and a tendency for lameness. For metritis, the LD group demonstrated a higher concentration of lactate (12,528 ± 1,320 μM) compared to the HD group (10,375 ± 1,320 μM) and HG group (4,846 ± 1,320 μM) ($P < 0.01$). For lameness, the HD group had the greatest mean lactate concentration of 8,474 ± 1,033 compared to the LD and HG group of 6,864 ± 1,033 and 4,846 ± 1,033 μM, respectively ($P = 0.07$). There were numerical differences between disease groups with regards to serum lactate albeit the differences did not reach significance for mastitis ($P = 0.28$), retained placenta ($P = 0.28$), and ketosis ($P = 0.16$). However, for mastitis, the HD group had a greater mean concentration of lactate of 8,026 ± 1,483 compared to the LD group of 7,658 ± 1483 and the HG group of 4,846 ± 1,483 μM. For retained placenta, the HD group had a concentration of 8,231 ± 2,095, the LD group was 7,530 ± 2,095, and the HG group was 4,846 ± 2,095 μM. Interestingly, the LD group for ketosis demonstrated a numerically greater lactate concentration of 7,295 ± 1,103 compared to the HD group of 4,327 ± 1103 and the HG group of 4,846 ± 1,103 μM.

Lactate concentrations at +1 week demonstrated overall significance for retained placenta, while metritis had an overall tendency ($P = 0.01$; $P = 0.07$, respectively). For cows diagnosed with

retained placenta, both the HD and LD group showed no differences in their concentration of lactate at 4,594 ± 1,284 vs 4,593 ± 1,284 μM, respectively, while the HG group had a mean concentration of 5,278 ± 1,284 μM. For metritis, the LD group had the greatest mean concentration of 8,688 ± 978 compared to the HD and HG group of 7,662 ± 978 and 5,278 ± 978 μM, respectively. There was no statistical significance between groups for mastitis ($P = 0.33$), ketosis ($P = 0.42$), and lameness ($P = 0.91$) for lactate concentrations at +1 week.

An overall statistical significance for lactate at +2 weeks was demonstrated for mastitis ($P = 0.05$) and metritis ($P = 0.02$), but not for cows diagnosed with retained placenta ($P = 0.23$), ketosis ($P = 0.49$), and lameness ($P = 0.50$). For mastitis, the LD group demonstrated a higher concentration of lactate in the serum (6,683 ± 733 μM) compared to the HD group (3,868 ± 733 μM) and the HG group (5,029 ± 733 μM). For metritis, the LD group also had the highest mean lactate concentration (10,027 ± 1,089) whereas the HD and HG groups had lower mean concentrations of 6,727 ± 1,089 and 5,029 ± 1,089 μM, respectively.

3.3.5 β-hydroxybutyric acid

Concentrations of BHBA in the serum among SCC groups and disease incidence are reported in Table 3-6. Analysis of the BHBA concentrations for the HG cows found outliers at each of the time points and were removed from the statistical output. Therefore, we compared the BHBA concentrations of 5 healthy cows to the two SCC groups. At prior to dry off there was an overall statistical significance for retained placenta ($P = 0.04$) and ketosis ($P < 0.01$). For cows with retained placenta, the HD group had a mean concentration of BHBA at 808.33 ± 142.02, the LD group was 750.00 ± 142.02, and the HG group was 1,242 ± 135.86 μM. For ketosis, concentration of BHBA for the HD group was 601.67 ± 117.26, the LD group 746.67 ± 117.26, and the HG group 1,242 ± 128.45 μM. There were no statistical differences between groups for mastitis ($P = 0.14$), metritis ($P = 0.12$), and lameness ($P = 0.21$) for BHBA concentrations prior to dry off.

There were significant differences overall in the concentration of BHBA at +1 week for cows diagnosed with mastitis, retained placenta, and lameness between groups ($P = 0.03$; $P < 0.01$; $P < 0.01$, respectively). For cows diagnosed with mastitis, the LD group had a mean BHBA concentration of 785.00 ± 74.66 while the HD group had a mean of 793.33 ± 74.66 and the HG

group was 1,084. ± 81.78 μM. For retained placenta, the HD group had BHBA at 801.67 ± 85.49, the LD group at 596.67 ± 85.49, and the HG group at 1,084 ± 93.65 μM. For lameness cows, the mean BHBA for the HD was 686.67 ± 89.96 whereas the LD and HG groups were 511.67 ± 89.96 and 1,084 ± 98.54 μM, respectively. There were no significant differences observed between groups for serum BHBA for metritis ($P = 0.39$) and ketosis cows ($P = 0.51$). Although, cows in the HD group that were ketotic demonstrated a numerical difference with regards to mean BHBA of 1,823 ± 438.96 compared to the LD group of 1,282 ± 438.96 and the HG group of 1,084 ± 480.86 μM; however, the differences did not reach significance.

At +2 weeks, there was a significant difference between groups for mastitis, retained placenta, and lameness with regards to the mean BHBA concentration ($P = 0.05$; $P < 0.01$; $P = 0.01$, respectively). For metritis, there was a tendency observed between groups for serum BHBA concentrations at +2 weeks ($P = 0.09$). For mastitis, the HD group had a mean BHBA concentration of 630.00 ± 144.54, the LD group 868.33 ± 144.54, and the HG group 1,216 ± 158.33 μM. Cows diagnosed with retained placenta in the HD group had a mean BHBA concentration of 750.00 ± 105.71 while the LD group was at 515.00 ± 105.71 and the HG group at 1,216 ± 115.80 μM. For lameness, the HD group's BHBA was 725.00 ± 128.21, the LD group's was 593.33 ± 128.21 and the HG group's was 1,216 ± 140.45 μM. For metritis, the HD group had a mean of 750.00 ± 145.02 whereas the LD and HG group had means of 1,142 ± 145.02 and 1,216 ± 158.86 μM, respectively. On the other hand, cows diagnosed with ketosis showed no statistical difference between groups in the mean BHBA at +2 weeks ($P = 0.80$), albeit there was a numerical difference among the means. Cows in the HD group diagnosed with ketosis had the highest mean of BHBA at 1,683 ± 350.65 compared to the LD group of 1,457 ± 350.65 and the HG group of 1,360 ± 384.12 μM.

3.3.6 Correlations between milk SCC prior to dry off to serum variables

Results for correlation analyses between milk SCC from prior to dry off and serum variables prior to dry off as well as at +1 and +2 weeks are presented in Table 3-7, 3-8, and 3-9, respectively. Milk SCC prior to dry off showed strong positive correlations to concentrations of BHBA in the serum at +1 week for cows in the HD group diagnosed with metritis ($P < 0.01$) and ketosis ($P = 0.01$) (Table 3-6; r = 0.95; r = 0.91, respectively). There was a tendency for the HG prior to dry off showing a positive correlation between SCC and NEFA (Table 3-7; r = 0.77; $P =$

0.07), whereas cholesterol was negatively correlated to SCC (Table 3-7; r = -0.78; P = 0.07). At +1 weeks, serum cholesterol and SCC prior to dry off demonstrated a tendency for the HG group (Table 3-8; P = 0.07; r = 0.77). The LD group, diagnosed with retained placenta, showed a positive correlation between their milk SCC prior to dry off with concentration of glucose at +1 week (Table 3-8; r = 0.84; P = 0.04). Concentration of NEFA in the serum at +1 week, for cows in the HD group diagnosed with ketosis, showed a positive correlation to their SCC prior to dry off (Table 3-8; r = 0.73; P = 0.10). Lactate concentrations for lameness-diagnosed cows in the HD group at +1 week postpartum were positively correlated to SCC prior to dry off (Table 3-8; r = 0.73; P = 0.10). At +2 weeks there was a positive correlation for serum glucose and milk SCC in the HD group for mastitis (r = 0.85; P = 0.03), serum cholesterol and milk SCC in the LD group for mastitis (r = 0.74; P = 0.09), and lactate and milk SCC in the LD group for metritis (r = 0.74; P = 0.09) (Table 3-9).

A negative correlation was observed for cholesterol concentrations prior to dry off in the LD group that were diagnosed with metritis (Table 3-7; r = -0.97; $P < 0.01$). The HD group demonstrated a negative correlation between milk SCC and serum NEFA prior to dry off for metritis (Table 3-7; r = -0.76; P = 0.08). For mastitis, BHBA prior to dry off showed a negative correlation to SCC in the LD group (Table 3-7; r = -0.79; P = 0.06). The HD group of cows prior to dry off, diagnosed with ketosis, had a negative correlation between milk SCC and serum NEFA (Table 3-7; r = -0.78; P = 0.07) as well as between SCC and serum cholesterol (Table 3-7; r = -0.79; P = 0.08). At +1 week there was a negative correlation between milk SCC prior to dry off to serum glucose concentrations for the LD group cows diagnosed with mastitis (Table 3-8; r = -0.76; P = 0.08). Cholesterol concentrations at +1 week, for the HD grouped diagnosed with metritis, were found to be negatively correlated to SCC prior to dry off (Table 3-8; r = -0.75; P = 0.08). A strong negative correlation was found for BHBA at +1 week in the HD group diagnosed with retained placenta (Table 3-8; r = -0.82; P = 0.04). Finally, cholesterol concentrations at +1 week for the LD group that was diagnosed with lameness had a negative correlation between cholesterol and SCC prior to dry off (Table 3-8; r = -0.77; P = 0.07).

3.4 Discussion

We hypothesized that dairy cows with high milk SCC prior to dry off might exhibit alterations in the serum variables before being dried off and during the first two weeks postpartum

when diagnosed with periparturient disease. Indeed, results showed alterations in the serum metabolites for high SCC cows before the dry off period but also between the low SCC at dry off and affected by periparturient diseases. To our best knowledge this is the first study to establish an association between high SCC before dry off to alterations in the serum variables in cows diagnosed with periparturient disease.

Glucose is one of the most important molecules involved in various metabolic processes. For the mammary gland, glucose is important in the synthesis of lactose in the Golgi apparatus that requires glucose from the bloodstream to be converted into UDP-galactose, which then combines with another glucose molecule to form lactose (Sjaastad et al., 2016). The increased demand for glucose around parturition has been estimated to be between 1,000-1,100 g/d at 21 d prior to parturition and rapidly increases to 2,500 g/d at 21 d post-partum (Overton, 1998). This high demand for glucose is regulated by gluconeogenesis in the liver. According to Reynolds et al. (2000a) the amount of glucose released from the liver 11 d after parturition is 2,760 g/d.

In the current study, serum glucose concentrations prior to dry off for both the LD and HD groups diagnosed with mastitis were lower compared to healthy cows. Glucose concentrations for mastitis continued to decrease after parturition for both diseased groups. In the previous chapter, we showed that concentrations of lactose were lower in the HD group for all diseases analyzed when compared to the LD and HG. Based on the findings reported in the current chapter, lower lactose concentrations in the milk of cows affected by disease could be attributed to lowered glucose concentrations in the serum of cows with periparturient disease. In the previous chapter, it was observed the HD group had lower lactose in the milk which may be related to lower serum glucose.

What is the reason for lower glucose in cows affected by periparturient disease? One reason might be bacterial endotoxins. Eckel and Ametaj (2016) in their review article indicated that bacterial endotoxins can translocate into systemic circulation from three main sources including the mammary gland, rumen, and reproductive tract. Bacterial endotoxins are known to stimulate the release of pro-inflammatory cytokines including TNF-α and IL-1 (Baumann and Gauldie, 1994). Previous research reported that a challenge with intramammary endotoxin resulted in reduced expression of genes related to metabolic processes as well as reduction of liver's ability to metabolize fatty acids (Jiang et al., 2008; Jorgensen et al., 2012). Tumor necrosis factor-α also has been shown to down-regulate acetyl-CoA synthase, an enzyme that catalyzes the first reaction

in fatty acid oxidation (Weiner et al., 1991), which can result in a decrease in glucose concentration and increased systemic NEFA. We observed greater serum NEFA concentration and lower glucose in the HD group at +1 week. Cows diagnosed with ketosis postpartum and high SCC before dry off had greater concentrations of NEFA and BHBA at both +1 and +2 weeks compared to the low SCC cows. Concentration of glucose in the serum decreased by 22.39 mg/dL at +1 week as compared to prior to dry off for the HD group compared to the LD group which decreased only by 17.49 mg/dL. Alterations in free glucose in mastitic milk has been related to fluctuation in the circulating metabolites (Annison et al., 1968). Interestingly, Moyes et al. (2014) reported increases in plasma NEFA and glucose, and a decrease in BHBA following *Escherichia coli* challenge in the mammary gland.

We also observed lower glucose concentrations in the serum of cows affected by lameness in high SCC cows at +1 week after parturition. There is minimal research regarding lameness and glucose in dairy cows, and studies that were conducted have found glucose elevations in lame cows. For example, Lischer et al. (2001) found that in one third of the cows with sole ulcers blood glucose was elevated. O'Driscoll et al. (2015) had similar results in which lame cows had elevated glucose along with elevated cortisol. The onset of a stressor, such as lameness, can result in hyperglycemia with the rise in serum glucose from tissue injury (Blebuyck, 1990). In addition, stressors are associated with increased catecholamines and glucocorticoids, which can cause further elevation in glucose concentration (McDowell, 1983). Additionally, lesions within the corium and epidermis of the hoof region have been found to occur with local and systemic administrations of LPS, which suggests the role of ET in the incidence of lameness (Boosman et al., 1991). Therefore, the cause of lower glucose levels during disease of high SCC lame cows could potentially be a result of ET insult from the mammary gland, which could be leading to lameness.

Prepartum and postpartum alterations in the lipid metabolism have been the subject of interest in dairy cows. The liver is an important organ that is central in supporting metabolic processes during pregnancy and lactation (Drackley et al., 2001). Alterations in lipid metabolism have been noted to occur prior to incidence of disease. Elevated concentrations of NEFA and cholesterol before parturition were associated with greater risk of incidence of retained placenta (LeBlanc et al., 2004; Quiroz-Rocha et al., 2010). The incidence of uterine infections was also increased with higher concentrations of NEFA in dairy cows prepartum (Hammon et al., 2006). It

has been well established that concentrations of NEFA in the blood increase prepartum and remain high for approximately 2 weeks postpartum (Underwood, 1998; Drackley, 1999). Drackley (2000) indicates that cows are in a state of positive energy balance if NEFA concentrations are <0.2 mM (<200 μM). Approximately 2-3 d prior to calving NEFA concentrations increase dramatically ranging from 0.8-1.2 mM (800-1,200 μM) (Drackley, 2000). After parturition, NEFA concentrations decrease; however, if values exceed 0.7 mM following parturition is an indication of NEB (Drackley, 2000).

In the current study, cows in the HD group diagnosed with mastitis had significant alterations in serum NEFA concentrations at +1 week after parturition. Dervishi et al. (2015) also measured NEFA concentrations in cows with subclinical mastitis during diagnosis week and found no significant differences in the NEFA concentrations between groups. Moreover, Melendez et al. (2009) found that concentrations of NEFA exceeding >1.2 mEq/L (>1200 μM) at calving had a greater incidence of mastitis and milk fever. The authors also reported a higher tendency for retained placenta in cows with higher serum NEFA around calving. In the current study, the mean serum NEFA prior to dry off, for all diseases studied, were <1,000 μM. After parturition, mean NEFA concentrations in the serum exceeded >1,000 μM, where the HD group for cows with mastitis had the highest concentration at +1 week (2,010 ± 213.73 μM), suggesting that cows were experiencing a NEB. Previously, Moyes et al. (2014) observed an elevation of plasma NEFA at 18 h after an intramammary challenge with LPS. Interestingly, there was a correlation between ketosis at +1 week postpartum and SCC prior to dry off, suggesting there is an association between SCC before the dry off to serum NEFA after calving.

Besides serving as precursors of milk fatty acids NEFA are also oxidized by the liver. The bovine liver oxidizes NEFA by either: 1) complete oxidation through the tricarboxylic acid (TCA) cycle yielding acetyl CoA, 2) synthesis and storage of triacylglycerols (TAG), 3) oxidation of TAG into very low density lipoproteins (VLDL), 4) production of ketone bodies when maximum TAG accumulation is reached (Spain and Scheer, 2001; Nelson and Cox, 2005). Oxidation of NEFA by hepatocytes produces acetyl CoA required for the tricarboxylic acid (TCA) cycle (Eaton et al. 1996; Sugden et al., 2001). When there is scarcity of glucose the quantity of NEFA around calving used to produce energy can often overwhelm the TCA cycle and trigger conversion of acetyl CoA into ketone bodies and TAG, leading to ketosis and fatty liver (Sugden et al., 2001). High blood BHBA and NEFA are indicators of cows undergoing a NEB (Nonnecke et al., 2003), and also

indicators of ketosis and fatty liver (Työppönen and Kauppinen, 1980). BHBA in early lactation can be used as a fuel source for multiple organs including mammary gland and brain; however, excessive ketone bodies have negative effects on cow health and productivity (Ingvartsen, 2006). According to Oetzel (2007) the cut off values for subclinical ketosis is when blood BHBA concentrations exceeds 1,200 μM, and >3000 μM for clinical ketosis.

Previous studies reported serum BHBA concentrations to be within normal range at -8, -4, and week of diagnosis of disease for metritis, retained placenta, mastitis, and lameness (Dervishi et al., 2015; Dervishi et al., 2016a; Dervishi et al., 2016b; Zhang et al., 2015). Zhang et al. (2016) did observe significant increases of serum BHBA concentrations in dairy cows diagnosed with ketosis starting at -4 weeks prior to parturition, but were within normal ranges. During the week of diagnosis for ketosis in the same study the BHBA concentrations were found to be above diagnosis levels. In the current study, all cows prior to dry off were within the normal ranges for BHBA concentrations for all cows that were diagnosed with diseases postpartum except for the low SCC cows diagnosed with metritis where their mean BHBA was found to be slightly above subclinical levels. According to Galvo et al. (2010), greater NEFA and BHBA during prepartum period have been associated with higher incidence of metritis and endometritis, possibly through implications on neutrophil functions.

The BHBA concentrations of healthy cows were significantly greater compared to the sick groups except for ketosis. In humans undergoing a fasting state or intense exercise the liver converts NEFA into BHBA as an alternative energy source (Berg et al., 2012). In the current study, the majority of cows sampled were fed once (in the morning) every 24 hours, and samples were collected prior to and during feeding time. It is possible that cows were in a fasting state during sampling time resulting in BHBA production by the liver for energy. However, a study by Nyman et al. (2008) found elevated BHBA was associated with decreased SCC in dairy cows. The authors speculate that elevated BHBA may be from the rumen due to increased fermentable carbohydrates around calving, which could have also been the case in the current study. More research is required on this matter to provide further explanations on the elevated BHBA levels in healthy cows.

Interestingly, the high SCC group of cows diagnosed with ketosis exhibited the greatest serum BHBA concentrations at +1 week compared to the low SCC cows whose BHBA levels were around subclinical diagnosis. Similarly, the high SCC group demonstrated greater serum BHBA concentrations at +2 weeks while the low SCC group was just above subclinical concentrations

for ketosis. In the previous chapter we reported that cows with high SCC prior to being dried off had increased odds of ketosis post-partum. Indeed, the results from this study support our previous hypothesis. At +1 week, the high SCC group of cows diagnosed with ketosis exhibited higher serum concentrations of BHBA and there was a strong positive correlation between SCC prior to dry off with BHBA concentrations at +1 week, for high SCC cows.

Several authors have indicated that elevated levels of BHBA impair the immune functions within the mammary gland decreasing udder defence capabilities (Kremer et al., 1993; Suriyasathaporn et al., 1999). It is speculated that endotoxin translocation from the mammary gland of cows with high SCC may be a contributing factor to elevated BHBA concentrations in those cows, impairing immune functions in various organs and increasing susceptibility to disease. Additionally, increased NEFA concentrations are known to impair the immune system as a result of intense lipolysis (Rukkwamsuk et al., 1999).

Increased lactate concentrations were found to be significantly higher for metritis at prior to dry off for both sick groups, and there was a tendency for the HD lameness group to have greater serum lactate. Interestingly, both the LD and HD group, diagnosed with metritis post-partum, demonstrated increased serum lactate where the LD group was greater. While the difference between the groups was not significant, the HD groups of cows for mastitis and retained placenta had numerically greater concentrations of lactate prior to dry off, while for ketosis, the LD group exhibited numerically greater lactate concentrations compared to the HD group. Reports have described a close relationship between milk SCC and elevated lactate concentration in the milk of ruminants making lactate a useful indicator of udder health (Davis et al., 2004). Furthermore, metabolic diseases in dairy cows including mastitis have been found in association to lactate concentrations in the blood (Davis et al., 2004). Moreover, increased lactate concentrations have occurred during endotoxemia or sepsis (Meszaroset al., 1987). Various tissues use lactate as an energy source including the liver and kidney (Bakker et al., 2013). During glycolysis and oxidative phosphorylation, lactate is crucial for adenosine-triphosphate (ATP) generation. Lactate is also important during inflammatory conditions (Bakker et al., 2013); however, pro-inflammatory cytokines including TNF-α and IL-1 have been shown to increase glycolysis but decrease the oxidation of glucose in the tricarboxylic cycle contributing to the increase in lactate concentrations (Taylor et al., 1988). In addition, lactate has shown to play a role in neutrophil adhesion to the vascular endothelium through decrease in intracellular pH, reduced L-selectin expression, increase

L-selectin shedding mechanism, and increased CD11b expression of bovine neutrophils (Kaba et al., 2008; Concha et al., 2014; Alarcón et al., 2017). Alarcón et al. (2017) observed elevation in the neutrophil extracellular trap (NET) and NETosis when bovine neutrophils were exposed to 5 mM D(-) lactic acid suggesting lactic acids role in the pro-inflammatory response.

Endotoxin in the systemic circulation can stimulate lactate dehydrogenase, an enzyme known for conversion of pyruvate into lactate, which increases lactate concentrations within the plasma (Block et al., 1985). Steiger et al. (1999) had previously shown that LPS infusion increased plasma concentrations of lactate in cattle. Interestingly, immune cells including monocytes/macrophages, T cells, and B cells have been identified as lactate sources where the release of lactate decreases the motility, killing capability, effector function, and supresses the inflammasome thereby easing inflammation (Fischer et al., 2007; Goetz et al., 2011; Haas et al., 2015).

Previous data generated by our team where elevations in serum lactate concentrations at -8 and -4 weeks prior to parturition in cows diagnosed with retained placenta, ketosis, mastitis, lameness, and metritis (Dervishi et al., 2015; Dervishi et al., 2016a; Dervishi et al., 2016b; Zhang et al., 2015; Zhang et al., 2016). From the same studies, lactate concentrations had remained higher during the week of diagnosis for ketosis and retained placenta, and at +4 weeks after parturition for mastitis and retained placenta. In the current study, we observed increased lactate concentrations at +1 week were greater in the LD group for mastitis, metritis, ketosis, and lameness while retained placenta cows had similar mean lactate concentrations between the LD and HD group. Lactate concentrations for the LD group remained greater at +2 weeks for mastitis and metritis. Retained placenta and ketosis lactate concentrations at +2 weeks were almost similar between the LD and HD groups. Lactate in the HD group for lameness at +2 weeks was observed to be greater.

Strong correlations between SCC and serum lactate were observed by Zhang et al. (2015; 2016) at -8, -4, week of diagnosis, and +4 weeks relative to parturition in cows diagnosed with lameness and ketosis. In the current study, there was a positive correlation between serum lactate concentrations for the HD group at +1 week to the SCC prior to dry off for cows diagnosed with lameness. The LD group diagnosed with metritis demonstrated a positive correlation between serum lactate concentrations at +1 week to SCC in the milk prior to dry off.

Generally, cholesterol concentrations in the serum of dairy cows decrease prior to parturition and slowly increase post-partum (Quiroz-Rocha et al., 2009). Reports have been conflicting whether low or high cholesterol is associated with incidence of periparturient disease. Kaneene et al. (1997) found cows with lower cholesterol prepartum were at higher risk for incidence of retained placenta. Sepúlveda-Varas et al. (2015) had similar results in grazing dairy cows where low cholesterol was associated with greater risk of clinical metritis and multiple postpartum diseases. In contrast, a study by Quiroz-Rocha et al. (2009) found that incidence of retained placenta was greater in cows with increased cholesterol around calving. The decrease in serum cholesterol has been strongly linked to NEB after parturition (Ruegg et al., 1992; Kim et al., 2003). In the current study, lower cholesterol concentrations were found in the high SCC cows before being dried off except for retained placenta where the low SCC cows were lower. Both +1 and +2 weeks exhibited significant lower cholesterol concentrations in the high SCC cows. Cows diagnosed with retained placenta in the HD group did exhibit lower serum cholesterol at +1 week, which could be related to retained placenta incidence.

Similarly to glucose, little data have been published for cholesterol concentrations during lameness. Yaylak et al. (2009) found high cholesterol concentrations from 71-140 DIM, and low during the dry period and early lactation but there were no significant differences between lame and non-lame cows. In the current study, the HD group exhibited significantly lower cholesterol concentrations at all time points. Potentially, ET circulation of high SCC cows may be a contributor to the lowered serum cholesterol concentrations.

Previous studies on LPS administration subcutaneously had resulted in a decrease in plasma cholesterol during endotoxemia in humans (Fraunberger et al., 1999; Khovidhunkit et al., 2004). Other studies have also reported that lower cholesterol is associated with intravenous infusion of LPS (Zebeli et al., 2011b). In contrast, Iqbal et al. (2013) showed that repeated oral administration of LPS 2 weeks prior and 1 week after parturition increased plasma cholesterol concentrations. Cholesterol is a precursor for synthesis of bile acids, which are important for detoxification of ET through breakdown into nontoxic fragments to prevent its translocation into the systemic circulation (Bertok, 2004). Iqbal et al. (2013) concluded that greater cholesterol may provide better health status in dairy cows. The healthy cows in this study also exhibited higher concentrations of cholesterol in the serum throughout the study. Other reports have shown disruption of cholesterogenic genes by LPS, TNF-α, and IL-1 (Feingold et al., 1993; Memon et

al., 1997; Feingold et al., 1998). Translocation of endotoxins into circulatory system and elevations in pro-inflammatory cytokines could therefore be impacting cholesterol synthesis in the liver of high SCC cows, which could potentially be lowering their ability to synthesis bile acids and neutralize endotoxins. Additionally, the lowering of cholesterol synthesis by ET secretion could potentially be increasing susceptibility of high SCC cows to incidence of disease. We did not measure alterations in cytokines, bile acids, or LPS during this study. Based on our findings, further investigation is warranted to evaluate whether immune system of high SCC cows is altered before entering the dry period.

3.5 Conclusion

In summary, results from the study indicate that high SCC cows diagnosed with periparturient disease are undergoing alterations in serum metabolites before being dried off. Both the LD and HD groups for mastitis demonstrated lower glucose and cholesterol concentrations in comparions to HG cows. Moreover, the HD group had increased serum NEFA at +1 week postpartum. For metritis, the HD group had lowered serum glucose after parturition, and increased lactate concentrations although not significant from the LD group. The HD group with ketosis had low serum glucose compared to healthy cows, and lower serum cholesterol but no significant differences in other variables. However, cows with high SCC from prior to dry off demonstrated a strong positive correlation to serum BHBA concentrations at +1 week for ketosis. Cows with lameness demonstrated lowered serum glucose for both the sick groups compared to HG cows, and the HD group demonstrated significantly lower serum cholesterol compared to LD and HG. Lastly, healthy cows demonstrated greater concentrations of BHBA in the serum compared to the diseased groups with lameness, metritis, mastitis, and retained placenta, but not for ketosis. This may be related to BHBA coming from the rumen or the time of the day the cows were sampled during the study. More research is warranted in order to determine metabolic effects of high SCC cows before entering the dry period.

3.6. Acknowledgments

We acknowledge the financial support of the funding agency Alberta Livestock and Meat Agency Ltd. (ALMA) for this project. Dr. Burim N. Ametaj was the principal investigator of this

study who contributed to conceptualization and designing of this research work, as well as organizing and managing the entire study. Dr. Ametaj supervised all lab analyses, and contributed in writing, editing as well as in improving the quality of this manuscript. We acknowledge the assistance of Dr. Andre Luiz Gracias Dias, Dr. Erkan Pehlivan, and Eduardo Barahona for collection of samples. We also acknowledge the assistance of Suzanna M. Dunn and Eduardo Barahona for laboratory analyses of samples. We are also grateful to the technical staff of the Dairy Research and Technology Centre, University of Alberta, and the staff of Sunalta Ltd., Ponoka, Alberta for their help and care to the cows.

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Table 3-1. Somatic cell count concentrations between groups and diseases prior to dry off.

	Somatic cell counts (10^3 cells/ml)				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis		89.83	1,525.00	497.21	0.09
Metritis		65.17	559.83	85.12	<0.01
Retained placenta	73.33	52.17	782.33	202.62	0.04
Ketosis		110.33	1,423.00	473.96	<0.01
Lameness		67.00	375.00	25.91	<0.01

[1]Disease.
Mastitis: LD (n=6), HD (n=6)
Metritis: LD (n=6), HD (n=6)
Retained placenta: LD (n=6), HD (n=6)
Ketosis: LD (n=6), HD (n=6)
Lameness: LD (n=6), HD (n=6)
[2]Healthy group (n=6): cows that were low SCC and no incidence of disease throughout study period
[3]Low-disease: SCC < 200,000 cells/mL prior to dry off
[4]High-disease: SCC > 200,000 cells/mL prior to dry off
[5]Standard error of the mean
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 3-2. Alterations in glucose concentrations (mg/dL) between SCC groups at all sampling time points for cows diagnosed with periparturient disease.

	Prior to dry off				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	74.98[a]	37.59[b]	37.24[b]	3.25	<0.01
Metritis	74.98	71.90	59.12	5.92	0.17
Retained Placenta	74.98	64.71	61.17	5.93	0.26
Ketosis	74.98	67.07	76.90	4.00	0.22
Lameness	74.98	50.66	49.65	3.53	0.38
	+1 weeks				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	64.61[a]	34.23[b]	32.31[b]	2.51	<0.01
Metritis	64.61[a]	69.15[a]	45.21[b]	4.07	<0.01
Retained Placenta	64.61	64.47	50.36	5.89	0.19
Ketosis	64.61[a]	49.58[b]	54.51[b]	3.62	0.03
Lameness	64.61[a]	52.99[ab]	41.43[b]	5.05	0.02
	+2 weeks				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	59.02[a]	32.38[b]	42.71[a]	5.55	0.01
Metritis	59.02[a]	63.33[a]	47.10[b]	4.35	0.05
Retained Placenta	59.02	64.31	59.61	7.84	0.87
Ketosis	59.02	47.78	58.30	4.86	0.22
Lameness	59.02	48.31	50.03	4.91	0.28

[1]Disease.
Mastitis: LD (n=6), HD (n=6)
Metritis: LD (n=6), HD (n=6)
Retained placenta: LD (n=6), HD (n=6)
Ketosis: LD (n=6), HD (n=6)
Lameness: LD (n=6), HD (n=6)
[2]Healthy group (n=6): cows that were low SCC and no incidence of disease throughout study period
[3]Low-disease: SCC < 200,000 cells/mL prior to dry off
[4]High-disease: SCC > 200,000 cells/mL prior to dry off
[5]Standard error of the mean
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 3-3. Alterations in cholesterol concentrations (mg/dL) between SCC groups at all sampling time points for cows diagnosed with periparturient disease.

	Prior to dry off				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	160.67[a]	131.80[b]	93.20[c]	7.20	<0.01
Metritis	160.67	127.80	127.64	13.18	0.51
Retained Placenta	160.67[a]	162.62[ab]	179.36[b]	29.67	0.04
Ketosis	160.67[a]	145.09[a]	113.62[b]	12.99	0.06
Lameness	160.67[a]	126.93[b]	86.20[c]	8.31	<0.01
	+1				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	103.05[a]	78.66[b]	60.29[c]	5.83	<0.01
Metritis	103.05	83.75	81.64	6.95	0.48
Retained Placenta	103.05	108.39	82.93	17.01	0.20
Ketosis	103.05[a]	73.50[b]	67.71[b]	8.03	0.02
Lameness	103.05[a]	73.34[b]	43.79[c]	5.87	<0.01
	+2				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	124.26[a]	103.05[b]	74.38[c]	7.25	<0.01
Metritis	124.26	111.89	104.47	9.86	0.22
Retained Placenta	124.26	109.40	119.98	23.51	0.84
Ketosis	124.26[a]	100.71[ab]	76.01[b]	12.50	0.05
Lameness	124.26[a]	82.55[b]	54.23[c]	7.24	<0.01

[1]Disease.
Mastitis: LD (n=6), HD (n=6)
Metritis: LD (n=6), HD (n=6)
Retained placenta: LD (n=6), HD (n=6)
Ketosis: LD (n=6), HD (n=6)
Lameness: LD (n=6), HD (n=6)
[2]Healthy group (n=6): cows that were low SCC and no incidence of disease throughout study period
[3]Low-disease: SCC < 200,000 cells/mL prior to dry off
[4]High-disease: SCC > 200,000 cells/mL prior to dry off
[5]Standard error of the mean
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 3-4. Difference non-esterified fatty acids (NEFA) concentrations (μM) between SCC groups at all sampling time points for cows diagnosed with periparturient disease.

	Prior to dry off				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	398.00	447.33	323.67	85.56	0.60
Metritis	398.00	362.00	321.50	66.82	0.72
Retained Placenta	398.00	560.67	478.50	77.84	0.36
Ketosis	398.00	514.17	470.33	65.05	0.46
Lameness	398.00	682.83	563.67	101.06	0.17
	+1 weeks				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	1,205[a]	1,294[a]	2,010[b]	213.73	0.03
Metritis	1,205	1,399	1,354	284.25	0.88
Retained Placenta	1,205	950.83	1,420	237.01	0.40
Ketosis	1,205	1,511	1,949	293.91	0.23
Lameness	1,205	1,553	1,134	195.08	0.29
	+2 weeks				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	1,407	1,106	1,108	281.27	0.69
Metritis	1,407	1,171	1,154	273.11	0.77
Retained Placenta	1,407	734.33	1,274	281.79	0.23
Ketosis	1,407	1,546	1,981	331.64	0.46
Lameness	1,407	1,125	1,316	295.32	0.79

[1]Disease.
Mastitis: LD (n=6), HD (n=6)
Metritis: LD (n=6), HD (n=6)
Retained placenta: LD (n=6), HD (n=6)
Ketosis: LD (n=6), HD (n=6)
Lameness: LD (n=6), HD (n=6)
[2]Healthy group (n=6): cows that were low SCC and no incidence of disease throughout study period
[3]Low-disease: SCC < 200,000 cells/mL prior to dry off
[4]High-disease: SCC > 200,000 cells/mL prior to dry off
[5]Standard error of the mean
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 3-5. Difference in lactate concentrations (μM) between SCC groups at all sampling time points for cows diagnosed with periparturient disease.

	Prior to dry off				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	4,846	7,658	8,026	1,483	0.28
Metritis	4,846[a]	12,528[b]	10,375[b]	1,320	<0.01
Retained Placenta	4,846	7,530	8,231	2,095	0.28
Ketosis	4,846	7,295	4,327	1,103	0.16
Lameness	4,846[a]	6,864[ab]	8,474[b]	1,033	0.07
	+1 weeks				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	5,278	6,824	4,652	1,025	0.33
Metritis	5,278[a]	8,688[b]	7,662[a]	978	0.07
Retained Placenta	5,278[a]	4,593[a]	4,594[a]	1,284	0.01
Ketosis	5,278	4,386	3,258	1,053	0.42
Lameness	5,278	4,928	5,498	941	0.91
	+2 weeks				
Disease[1]	HG[2]	LD[3]	HD[4]	SEM[5]	*P*-value
Mastitis	5,029[ab]	6,683[a]	3,868[b]	733	0.05
Metritis	5,029[a]	10,027[b]	6,727[a]	1,089	0.02
Retained Placenta	5,029	5,979	5,928	1,455	0.23
Ketosis	5,029	3,838	3,469	940	0.49
Lameness	5,029	5,296	7,170	1,375	0.50

[1]Disease.
Mastitis: LD (n=6), HD (n=6)
Metritis: LD (n=6), HD (n=6)
Retained placenta: LD (n=6), HD (n=6)
Ketosis: LD (n=6), HD (n=6)
Lameness: LD (n=6), HD (n=6)
[2]Healthy group (n=6): cows that were low SCC and no incidence of disease throughout study period
[3]Low-disease: SCC < 200,000 cells/mL prior to dry off
[4]High-disease: SCC > 200,000 cells/mL prior to dry off
[5]Standard error of the mean
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 3-6. Alterations in β-hydroxybutyric acid (BHBA) concentrations (μM) between SCC groups at all sampling time points for cows diagnosed with periparturient disease.

	Prior to dry off					
Disease[1]	HG[2]	SEM[3]	LD[4]	HD[5]	SEM[6]	*P*-value
Mastitis	1,242	190.88	925.00	691.67	174.25	0.14
Metritis	1,242	213.21	1,415	826.67	194.63	0.12
Retained Placenta	1,242[a]	135.86	750.00[b]	808.33[b]	124.02	0.04
Ketosis	1,242[a]	128.45	746.67[b]	601.67[b]	117.26	<0.01
Lameness	1,242	176.65	793.33	973.33	161.26	0.21
	+1 weeks					
Disease[1]	HG[2]	SEM[3]	LD[4]	HD[5]	SEM[6]	*P*-value
Mastitis	1,084[a]	81.78	785.00[b]	793.33[b]	74.66	0.03
Metritis	1,084	114.53	976.67	865.00	104.55	0.39
Retained Placenta	1,084[a]	93.65	596.67[b]	801.67[b]	85.49	<0.01
Ketosis	1,084	480.86	1,282	1,823	438.96	0.51
Lameness	1,084[a]	98.54	511.67[b]	686.67[b]	89.96	<0.01
	+2 weeks					
Disease[1]	HG[2]	SEM[3]	LD[4]	HD[5]	SEM[6]	*P*-value
Mastitis	1,216[a]	158.33	868.33[ab]	630.00[b]	144.54	0.05
Metritis	1,216[a]	158.86	1,142[a]	750.00[b]	145.02	0.09
Retained Placenta	1,216[a]	115.80	515.00[b]	750.00[b]	105.71	<0.01
Ketosis	1,216	384.12	1,457	1,683	350.65	0.68
Lameness	1,216[a]	140.45	593.33[b]	725.00[b]	128.21	0.01

[1]Disease.
Mastitis: LD (n=6), HD (n=6)
Metritis: LD (n=6), HD (n=6)
Retained placenta: LD (n=6), HD (n=6)
Ketosis: LD (n=6), HD (n=6)
Lameness: LD (n=6), HD (n=6)
[2]Healthy group (n=6): cows that were low SCC and no incidence of disease throughout study period
[3]Standard error of the mean for the healthy group
[4]Low-disease: SCC < 200,000 cells/mL prior to dry off
[5]High-disease: SCC > 200,000 cells/mL prior to dry off
[6]Standard error of the mean for the LD and HD groups
[a-c]Numbers with different superscripts with difference $P < 0.05$

Table 3-7. Correlation of SCC prior to dry off to concentration of serum variables at prior to dry off for different periparturient diseases for healthy group (HG), low disease (LD), and high disease (HD) groups.

			Healthy[1]		Low-Disease[2]		High-Disease[3]	
Disease		Item	r	*P*-value	r	*P*-value	r	*P*-value
Mastitis	SCC[4]	Glucose	0.26	0.62	0.31	0.54	-0.48	0.33
		Cholesterol	-0.78	0.07	0.49	0.33	-0.69	0.13
		NEFA	0.77	0.07	0.41	0.42	-0.35	0.50
		Lactate	0.41	0.41	-0.001	0.99	-0.11	0.83
		BHBA	0.22	0.68	-0.79	0.06	0.03	0.95
Metritis	SCC[4]	Glucose			0.14	0.80	0.28	0.59
		Cholesterol			-0.97	<0.01	0.55	0.26
		NEFA			0.55	0.26	-0.76	0.08
		Lactate			0.58	0.23	-0.17	0.75
		BHBA			-0.41	0.41	0.67	0.15
Retained placenta	SCC[4]	Glucose			0.11	0.84	0.55	0.26
		Cholesterol			-0.16	0.77	0.31	0.55
		NEFA			-0.46	0.36	-0.05	0.92
		Lactate			-0.44	0.38	-0.22	0.68
		BHBA			0.30	0.57	-0.47	0.35
Ketosis	SCC[4]	Glucose			0.48	0.34	0.60	0.20
		Cholesterol			-0.37	0.47	-0.76	0.08
		NEFA			0.19	0.72	-0.78	0.07
		Lactate			0.22	0.68	-0.07	0.89
		BHBA			-0.58	0.22	0.53	0.28
Lameness	SCC[4]	Glucose			-0.06	0.91	-0.23	0.66
		Cholesterol			0.27	0.60	-0.53	0.28
		NEFA			0.46	0.36	0.27	0.60
		Lactate			-0.44	0.38	0.18	0.73
		BHBA			-0.19	0.72	0.49	0.32

[1]Concentrations of serum variables were used from healthy cows at prior to dry off.
[2]Concentrations of serum variables were used from low-disease cows at +1 weeks relative to parturition.
[3]Concentrations of serum variables were used from high-disease cows at +2 weeks relative to parturition.
[4]Milk somatic cell counts were used from both healthy, low-disease, and high disease cows from each sampling point.
[5]Same healthy cows used for comparisons for all the diseases.

Table 3-8. Correlation of SCC prior to dry off to concentration of serum variables at +1 week postpartum for different periparturient diseases for healthy group (HG), low disease (LD), and high disease (HD) groups.

			Healthy[1,5]		Low-Disease[2]		High-Disease[3]	
Disease		Item	r	*P*-value	r	*P*-value	r	*P*-value
Mastitis	SCC[4]	Glucose	0.67	0.15	-0.76	0.08	-0.20	0.71
		Cholesterol	0.77	0.07	0.15	0.77	-0.31	0.55
		NEFA	0.37	0.46	0.51	0.30	0.17	0.74
		Lactate	0.67	0.15	-0.06	0.91	0.15	0.78
		BHBA	-0.48	0.34	0.56	0.25	0.33	0.53
Metritis	SCC[4]	Glucose			0.66	0.15	0.01	0.98
		Cholesterol			0.29	0.57	-0.75	0.08
		NEFA			0.35	0.49	0.71	0.11
		Lactate			0.47	0.34	0.47	0.35
		BHBA			0.03	0.95	0.95	<0.01
Retained placenta	SCC[4]	Glucose			0.84	0.04	0.17	0.75
		Cholesterol			-0.50	0.32	0.06	0.91
		NEFA			0.03	0.96	-0.42	0.41
		Lactate			0.15	0.77	-0.46	0.35
		BHBA			0.71	0.11	-0.82	0.04
Ketosis	SCC[4]	Glucose			-0.27	0.60	-0.61	0.20
		Cholesterol			-0.49	0.33	0.63	0.18
		NEFA			0.50	0.31	0.73	0.10
		Lactate			0.52	0.29	-0.05	0.92
		BHBA			0.08	0.88	0.91	0.01
Lameness	SCC[4]	Glucose			-0.56	0.25	0.32	0.54
		Cholesterol			-0.77	0.07	0.45	0.37
		NEFA			0.26	0.61	0.39	0.44
		Lactate			0.24	0.64	0.73	0.10
		BHBA			-0.64	0.17	0.22	0.67

[1]Concentrations of serum variables were used from healthy cows at prior to dry off.
[2]Concentrations of serum variables were used from low-disease cows at +1 weeks relative to parturition.
[3]Concentrations of serum variables were used from high-disease cows at +2 weeks relative to parturition.
[4]Milk somatic cell counts were used from both healthy, low-disease, and high disease cows from each sampling point.
[5]Same healthy cows used for comparisons for all the diseases.

Table 3-9. Correlation of SCC prior to dry off to concentration of serum variables at +2 weeks postpartum for different periparturient diseases for healthy group (HG), low disease (LD), and high disease (HD) groups.

			Healthy[1,5]		Low-Disease[2]		High-Disease[3]	
Disease		Item	r	*P*-value	r	*P*-value	r	*P*-value
Mastitis	SCC[4]	Glucose	-0.35	0.49	0.37	0.47	0.85	0.03
		Cholesterol	-0.26	0.62	0.74	0.09	-0.13	0.80
		NEFA	-0.30	0.56	0.41	0.42	-0.17	0.75
		Lactate	0.68	0.14	0.07	0.89	0.18	0.73
		BHBA	0.27	0.61	-0.43	0.38	-0.34	0.51
Metritis	SCC[4]	Glucose			0.70	0.12	-0.15	0.77
		Cholesterol			0.35	0.49	-0.21	0.68
		NEFA			0.69	0.13	-0.43	0.40
		Lactate			0.74	0.09	0.33	0.53
		BHBA			-0.16	0.77	-0.47	0.35
Retained placenta	SCC[4]	Glucose			0.50	0.31	-0.06	0.91
		Cholesterol			-0.45	0.37	-0.09	0.87
		NEFA			-0.24	0.65	-0.23	0.66
		Lactate			-0.34	0.52	-0.42	0.40
		BHBA			0.49	0.32	-0.18	0.74
Ketosis	SCC[4]	Glucose			-0.20	0.70	0.65	0.16
		Cholesterol			-0.49	0.33	-0.38	0.46
		NEFA			0.40	0.43	-0.02	0.97
		Lactate			-0.54	0.27	0.28	0.59
		BHBA			0.46	0.36	0.03	0.95
Lameness	SCC[4]	Glucose			-0.31	0.55	0.47	0.35
		Cholesterol			-0.62	0.19	0.35	0.49
		NEFA			-0.54	0.27	-0.30	0.56
		Lactate			-0.46	0.35	0.62	0.19
		BHBA			0.41	0.42	-0.13	0.80

[1]Concentrations of serum variables were used from healthy cows at prior to dry off.
[2]Concentrations of serum variables were used from low-disease cows at +1 weeks relative to parturition.
[3]Concentrations of serum variables were used from high-disease cows at +2 weeks relative to parturition.
[4]Milk somatic cell counts were used from both healthy, low-disease, and high disease cows from each sampling point.
[5]Same healthy cows used for comparisons for all the diseases.

Chapter 4 Overall Discussions

4.1 High somatic cell counts prior to dry off are associated with higher incidence of periparturient disease

The main hypothesis of this study was to determine whether high milk SCC in dairy cows prior to being dried off were related to the incidence of periparturient diseases. Indeed, results showed a relationship of high SCC prior to dry off to the incidence of ketosis, retained placenta, metritis, and lameness, but not to mastitis after parturition potentially due to incidence of new intramammary infections (IMI) during the dry period. To our best knowledge, this is the first study to determine a relationship of high SCC before dry off to the likelihood of periparturient diseases.

While the dry period for dairy cows is beneficial with regards to milk production and animal wellbeing, the incidence of periparturient diseases is the highest immediately following parturition, as a result of cows being in a state of immunosuppression. The elevation of SCC in the milk above 200,000 cells/mL is an indication of bacterial infection and inflammation within the mammary gland (Madouasse et al., 2010). During the transition into lactation dairy cows undergo physiological changes and dietary shifts to high grains which increase their susceptibility to periparturient disease following parturition. In addition, increased exposure to bacterial endotoxins (ET) can also facilitate a cow's likeliness of developing disease (Mallard et al., 1998; Ametaj et al., 2010). There is mounting evidence indicating ET translocation from the mammary gland, reproductive tract, or the rumen into systemic circulation and triggering an overall inflammatory state (Eckel and Ametaj, 2016). Figure 4-1 summarizes the potential role of ET translocation from the mammary gland of high SCC cows prior to dry off into systemic circulation, affecting various regions of the cow including the legs, liver, reproductive tract, and mammary gland while enhancing pro-inflammatory cytokine secretion, altering neutrophil recruitment, altering lipid metabolism within the liver all of which increase susceptibility to mastitis, metritis, retained placenta, ketosis, and lameness after parturition. In addition, Figure 4-1 also describes the contribution of ET from rumen and reproductive tract within bovine systemic circulation although the focus is on the mammary gland.

The primary cause for the increase in SCC in the mammary gland is bacterial infection of the udder. Endotoxins (ET), such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA), released from Gram-negative and Gram-positive bacteria, respectively, are able to translocate into

the systemic circulation and trigger multiple metabolic and immune-related responses (Wardenburg et al., 2006). Immune cells moving into the mammary gland during infection (especially neutrophils) are actively involved in killing the intruding bacteria, a process which is associated with the release of proinflammatory cytokines like tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) (Rainard and Riollet, 2006). The role of ET was originally thought to be limited to elevation of pro-inflammatory cytokine responses in the blood but not to translocation into systemic circulation (Hoeben et al., 2000). However, several studies have demonstrated increased plasma concentrations of LPS in dairy cows during naturally-occurring and induced mastitis models (Hakogi et al., 1989; Dosogne et al., 2002). The method in which ET could be translocating into systemic blood is by paracellular transport (Rainard and Riollet, 2006; Mani et al., 2012) or transcellular transport (Ghoshal et al., 2009). In addition to ET translocation, pro-inflammatory cytokine expression is of greater magnitude under the influence of ET (Baumann and Gauldie, 1994).

Previously, our team demonstrated alterations in innate immune reactants and serum metabolites related to carbohydrate and lipid metabolism as early as 2 months prior to parturition and diagnosis of disease including upregulation of TNF-α and IL-1 (Dervishi et al., 2015; Zhang et al., 2015; Dervishi et al., 2016a, Dervishi et al., 2016b, Zhang et al., 2016). Results from those previous studies suggested presence of an inflammatory state in dairy cows at the beginning of the dry off period that could potentially increase their susceptibility to incidence of periparturient disease. TNF-α and IL-1 play major roles in activation of the immune response including neutrophil recruitment and enhancement of neutrophil activity, and mediating the acute phase response from the liver (Alnakip et al., 2014).

The odds ratio generated from our data found that high SCC cows had a 166% increased likeliness in the incidence of ketosis after parturition. Zebeli et al. (2011) reported that increasing parenteral doses of LPS was associated with increased blood concentrations of BHBA. Zhang et al. (2016) also suggested that ET insults could be a potential factor in causing an elevation of BHBA. Therefore, we believe that ET arising from the mammary gland and entering into systemic circulation of high SCC cows before dry off might induce increases in BHBA levels, and contributing to higher incidence of ketosis.

The odds ratio for high SCC cows incidence of lameness, retained placenta or metritis, were found to be >1 meaning that high SCC were likely to have higher incidence rates of those

diseases. However, the p-values were found not to be statistically significant indicating other factors may also have contributed to the incidence of metritis, retained placenta, and lameness in cows with high SCC. When compared to cows with low SCC at dry off their odds ratio were <1 meaning the likelihood of being diagnosed with disease after parturition based on SCC was less likely to occur. This increased incidence of periparturient disease could again be related to ET insults from the mammary gland prior to dry off in cows with high SCC. Increased doses of LPS have been related to lesions within the corium and epidermis of the hoof leading to lameness (Boosman et al., 1991). For retained placenta, it has been suggested that intermittent parental administration of LPS around calving was associated with increased incidence of retained placenta (Zebeli et al., 2011).

While the likeliness in the incidence of periparturient disease was found to be higher in cows with high SCC prior to dry off, we still observed low SCC cows being diagnosed with periparturient disease. Dry cow therapy (DCT) treatment include antibiotics of choice for dairy producers when drying cows off to reduce the incidence of new intramammary infections (IMI) from occurring during the dry period (Jones, 2009). However, it has been indicated that DCT is not entirely effective at lowering the incidence of new IMI developing during the dry period (Oliver and Sordillo, 1988). We did not analyze microbiota composition in the udders of low and high SCC cows for this study, however this could be a possibility for low SCC cows becoming sick.

4.2 Higher somatic cell counts prior to dry off are associated with alterations in milk composition

The second objective of this study was to determine if milk composition and milk production were altered in cows with high SCC prior to dry off and diagnosed with periparturient disease after parturition. Indeed, high SCC cows diagnosed with disease showed alterations in the concentrations of lactose and protein in the milk and the fat:protein ratio (FPR) before and after parturition. Figure 4-1 summarizes the effects of an IMI and bacterial ET on milk composition.

An alteration in milk composition is one of the various outcomes when there is an infection of the mammary gland (Roberson, 2012). Following parturition, cows with high SCC prior to dry off and diagnosed with retained placenta exhibited greater SCC, while cows diagnosed with metritis, lameness, and ketosis had a decrease in SCC after parturition.

The decline in lactose concentrations has been known to occur during IMI of dairy cows. Serum glucose concentrations were lower for the high SCC group prior to dry off for all diseases except for ketosis where it was found to be greater than the low SCC cows. The lowered lactose reflects the lowered glucose concentrations of high SCC cows prior to being dried off. Pro-inflammatory cytokines are known to be upregulated during inflammation including TNF-α, which has been found to cause degradation of glucose transporter-1 (GLUT-1), and down regulation of genes for lactose synthesis (Kobayashi et al., 2016). Endotoxin induces greater expression of pro-inflammatory cytokines (Baumann and Gauldie, 1994); and potentially high SCC cows with lowered glucose and lactose concentrations may be related to ET enhancing cytokine expression. Studies have also reported suppression of lactose-synthesis related genes including α-lactalbumin gene with increased concentrations of TNF-α indicating inflammation can suppress lactose synthesis (Kobayashi et al., 2016). Mammary gland infections also result in an influx of leukocytes into the mammary gland, and often lactose declines as a result of leakage from the mammary gland, degradation by bacteria, and damage to the alveoli of the mammary gland (Audlist et al., 1995; Auldist and Hubble, 1998).

During IMI the concentration of total protein in the milk tends to increase as a result of an influx of immune-related proteins including immunoglobulins and serum amlbumin that are important for combatting infection, while milk proteins tend to decrease during infection (Shuster et al., 1991; Auldist et al., 1995; Auldist and Hubble, 1998). Cows with high SCC prior to dry off exhibited an increase in milk protein concentrations, specifically for cows affected by lameness and tendency for retained placenta. Although blood-borne proteins increase during infection, there is a decrease in milk proteins including whey proteins (e.g. α-lactalbumin and β-lactoglobulin) and casein. The decrease in milk proteins during infection can be attributed to proteases from bacteria, protein leakage from the mammary gland, and damage to the alveoli thus decreasing milk protein synthesis (Auldist and Hubble, 1998). Reports in rats found TNF-α had decreased gene expression coding for β-casein (Shea-eton et al., 2001); therefore, upregulation of pro-inflammatory cytokines could inhibit casein synthesis of high SCC cows. Furthermore, low milk protein has been strongly associated with negative energy balance (NEB) in dairy cows (Fahey, 2008). Negative energy balance is commonly linked to ketosis, and is was observed in high SCC cows diagnosed with ketosis with lower milk protein at +1 and +2 weeks indicating negative energy balance. In addition, the FPR has been used as a diagnostic measure for ketosis where a FPR >1.5 suggests clinical

ketosis (Heuer et al., 1999). High SCC cows diagnosed with ketosis exhibited greater FPR at +2 weeks compared to the low SCC and HG, while the other diseases showed the FPR to be within normal range.

Alterations in total fat content were observed in cows with high SCC prior to dry off that were diagnosed with metritis, but not after parturition. Mastitis, retained placenta, ketosis, and lameness did not exhibit alterations in fat concentration, milk urea nitrogen, or total solids at all sampling points.

4.3 Higher somatic cell counts prior to dry off are associated with lower milk production

Another objective of this study was to determine whether milk production for the first 60 days in milk (DIM) was impacted by high SCC cows prior to dry off diagnosed with periparturient disease. Indeed, results showed milk production of cows with high SCC prior to parturition had lower milk production compared to cows with low SCC prior to parturition and diagnosed with disease, and those that were healthy prior to dry off. Figure 4-1 describes the decrease in milk production as a result of ET from the mammary gland.

The main goal of the dry off period in dairy cows is to allow mammary gland enough time to regenerate milk-secreting tissue that was lost from the previous lactation (Sjaastad et al., 2016). However, our findings, for cows with high SCC before dry off, suggests presence of inflammation within the mammary prior to dry off potentially affecting milk production in the following lactation.

A problematic issue regarding IMI and other metabolic diseases is their impact on milk production, which results in losses for dairy producers. The decrease in milk production is attributed to reduced synthesis and secretion of milk components in the mammary gland during infection. Lactose functions as the predominant osmotoic regulator of milk volume; and the lowering of lactose in the milk leads to lowered milk production as well (Auldist and Hubble, 1998). In this study, cows with high SCC prior to dry off had a decrease in lactose content compared to the other groups, but also had lower total milk yield for the first 60 DIM. The most significant decrease in milk production was observed in cows with high SCC prior to dry off and diagnosed with retained placenta, mastitis, and ketosis. High SCC are suggestive of presence of infection and inflammation in the udder. This assumes an infection by Gram-negative bacteria that when killed by the host's immune cells release large amounts of endotoxin. Potential translocation

of endotoxin and proinflammatory cytokines from the mammary into the systemic circulation affect secretion of prolactin production (Mandrup-Poulsen et al., 1995; Turnbull and River, 1999). Prolactin is known to stimulate milk synthesis.

Based on these findings, daily milk losses to dairy producers per 100 cows that are dried off with high SCC can be up to 1,286 kg daily when compared to cows with low SCC and healthy ones. Total milk losses for 60 DIM for cows with high SCC before dry off and diagnosed with disease can reach as high as 77,160 kg per 100 cows. Comparatively cows with low SCC and diagnosed with disease, in 100 cows could produce 719 kg less a day. For 60 DIM, total milk losses of low SCC cows diagnosed with disease can be up to 43,140 kg per 100 cows per 60 DIM.

4.4 Metabolic alterations associated with high somatic cells around calving

Another hypothesis of this study was to determine whether cows with high SCC before entering the dry period will exhibit alterations in the serum metabolites before dry off and during the first 2 weeks following parturition when diagnosed with disease.

Concentrations of NEFA in the serum were significantly greater in high SCC cows with mastitis compared to the low SCC and healthy groups. Although the increase in NEFA concentrations around calving and then decreasing following parturition have been well established (Underwood, 1998); enhanced NEFA concentrations after parturition is related to NEB (Drackley, 2000). Endotoxins, potentially arising from the mammary gland (Eckel and Ametaj, 2016), trigger expression of TNF-α and IL-1 causing down regulation of acetyl-CoA synthase (Baumann and Gauldie, 1994; Weiner et al., 1991), lowering the concentrations of glucose and increasing those of NEFA, which was observed in the mastitis group. Furthermore, oxidation of NEFA yielding triacylglycerols (TAG) can be maximized in which the liver will switch to producing ketone bodies, mainly BHBA from NEFA (Spain and Scheer, 2001; Nelson and Cox, 2005). We observed greater serum concentrations of NEFA and BHBA, and lower glucose of the high SCC cows diagnosed with ketosis after parturition. In addition, cows diagnosed with lameness also exhibited lowered glucose especially at +1 week, in of high SCC cows.

Concentration of BHBA was within normal ranges for all diseases except for ketosis, and low SCC cows diagnosed with metritis exhibited elevated BHBA concentrations prior to dry off. In addition, a positive correlation for ketosis was found between serum concentrations of BHBA and NEFA at +1 week to the SCC of the high SCC group prior to dry off. Galvo et al. (2010) has

indicated that prepartum elevation of BHBA and NEFA were associated with increased risk of metritis and endometritis as a result of decreased neutrophil functions. Interestingly, Minuti et al. (2017) observed alterations in the liver transcriptome with intramammary infusion of LPS in dairy cows. They found intramammary infusion of LPS triggered upregulation of cytokines including IL-6, IL-8, leukemia inhibitory factor (LIF), and thymic stromal lymphopoietin (TSLP) in the mammary transcriptome, and were linked to seven transcription regulators in the liver. The authors reported that mammary gland responded to intrammmary infusion of LPS through activation of several recognition pathways like NOD-like receptor signaling, toll-like receptor signaling, RIG-like receptor signaling, and the apoptosis pathways. In the liver, fatty acid elongation in the mitochondria was inhibited along with activation of the p53 pathway, suggesting that liver responded to mammary gland LPS to maintain homeostasis (Minute et al., 2017).

The current study found serum cholesterol to be greater in healthy cows whereas in cows with high SCC prior to dry off serum cholesterol was at the lowest concentration after parturition. Cows diagnosed with lameness and mastitis and with high SCC cows prior to dry off exhibited the lowest serum cholesterol prior to dry off, suggesting that those cows were at an unhealthy state. There is scarcity of research regarding glucose and cholesterol concentrations during lameness, although the onset of a stressor such as lameness has been related to an elevation in glucose (Blebuyck, 1990). Subcutaneous administration of LPS in humans has been reported to be associated with lower concentrations of cholesterol in the plasma (Fraunberger et al., 1999; Khovidhunkit et al., 2004). Cholesterol's role during endotoxemia is that it serves as precursor to synthesis of bile acid, important in detoxifying intestinal ET (Bertok, 2004). Iqbal et al. (2013) also suggested that high concentrations of cholesterol in the blood might reflect better health status of the dairy cows.

Finally, concentrations of lactate in the serum were found to be high prior to dry off in both groups of cows with low and high SCC and diagnosed with metritis. In addition, there was a tendency for cows with high SCC prior to dry off and diagnosed with lameness to have greater lactate in serum prior to dry off. Concentrations of lactate have been a useful indicator in assessing udder health and metabolic status of dairy cows with disease (Davis et al., 2004), and in a state of endotoxemia (Meszaroset al., 1987). Elevations of lactate have been known to occur with the increase of pro-inflammatory cytokines contributing to elevated glycolysis and decreased rate of glucose oxidation (Taylor et al., 1988). Concentrations of lactate are increased during ET insults

where ET increases lactate dehydrogenase to convert pyruvate into lactate (Block et al., 1985). Previously lactate concentrations were shown to increase prepartum and postpartum in cows diagnosed with periparturient disease (Dervishi et al., 2015; Dervishi et al., 2016a; Dervishi et al., 2016b; Zhang et al., 2015; Zhang et al., 2016). Zhang et al. (2015; 2016) also observed strong correlations between serum lactate at various time points with SCC after parturition. We correlated SCC prior to dry off to lactate concentrations, and observed strong correlation of lactate at +1 week with high SCC cows diagnosed with lameness. However, low SCC cows prior to dry off and diagnosed with metritis had also greater lactate concentrations.

4.5 Future implications of high somatic cell count before dry off

Based on our findings, more investigation is warranted to develop a better understanding of the impact of high SCC cows before entering the dry period on health status and productivity of dairy cows. More specifically, we need to address the question whether inflammation is following dairy cows into the dry off period and whether ET is translocaed from the mammary gland into the systemic circulation in cows with high SCC. Results from this study indicate that even cows with low SCC are still likely to be affected by periparturient disease; however, their incidence is lower compared to cows with high SCC. This means that if we can lower the number of SCC before cows are dried off, then we could potentially lower the incidence rate of periparturient diseases, specifically ketosis, retained placenta, metritis, and lameness. Mastitis incidence may be a result of various factors including the incidence of new infections during the dry period, suppression of the immune system around parturition, or incidence of other diseases leading to supressed immunity and increased likelihood of mastitis incidence.

Developing a better understanding of the pathophysiology of high SCC cows prior to entering the dry off period may provide insights into the health issues arising following parturition. In addition, more research is warranted to understand the metabotype and genotype differences between cows with high and low SCC that became sick after calving compared to the healthy cows. Alterations in the immune system and metabolic pathways may be a contributing factor to disease incidence. Potentially, infectious pathogens and their endotoxins originating from the mammary gland of high SCC may be increasing the likeliness of disease incidence. However, this still does not provide explanation as to why low SCC cows also became sick after calving. It is possible that the causolgy of disease includes other than inflammation of the mammary gland and

certainly what happens to the udder during the dry period. Investigation of cows during the dry period may be required in order to develop an understanding of how low SCC as well as high SCC cows affect the overall health status of dairy cows.

For dairy producers, determining SCC in the milk before dry off may be a new routine to mamanging cows during the dry off. Identification of cows with high SCC cows prior to dry off and application of specific treatments to lower the number of SCC may provide long-term benefits not only on cow health and welfare, but also in milk production and farm profitability. As we demonstrated in the second chapter, high SCC cows diagnosed with periparturient disease have a large decrease in milk production compared to cows with low SCC that also were sick, and those that were healthy.

While routine testing of SCC in dairy herds of Canada is common; it may be beneficial to take SCC testing a step further by determining SCC in the milk roughly 1-2 weeks before cows are dried off. This means that dairy producers may require further planning in advance exactly when their cows are going to be dried off. Additionally, monitoring dry cows during the dry period may also be required in order to provide a better transition into the lactational cycle. Lastly, controlling high SCC animals before entering the dry period, which could potentially decrease the incidence of disease after parturition, could also potentially decrease farm labor during the transition period including administration of treatments and veterinary bills. This would therefore decrease farm costs to treat sick animals, which could be economically beneficial to producers.

4.6 Overall conclusions

Taken together, the data obtained from this study demonstrated that high SCC in the milk prior to dry off are related to greater incidence of periparturient diseases. Most notably the incidence of ketosis was greater in cows with high SCC prior to dry off. Moreover, high SCC were associated with increased likeliness of metritis, retained placenta, and lameness compared to cows with low SCC prior to dry off. Additionally, milk composition showed to be altered in cows with high SCC specifically lactose, which is important for regulation of milk volume and therefore contributes to lower milk production observed. This study demonstrated significant milk losses during the first 60 DIM in cows with high SCC suggesting that dairy farmers could improve milk production through implementation of a better monitoring and proactive lowering of SCC prior to dry off. Serum variables such as glucose and cholesterol were also shown to be altered in cows

with high SCC, potentially from ET translocation from the mammary gland before dry off. However, further research is warranted regarding high SCC cows before dry off, endotoxin translocation, and incidence of periparturient diseases.

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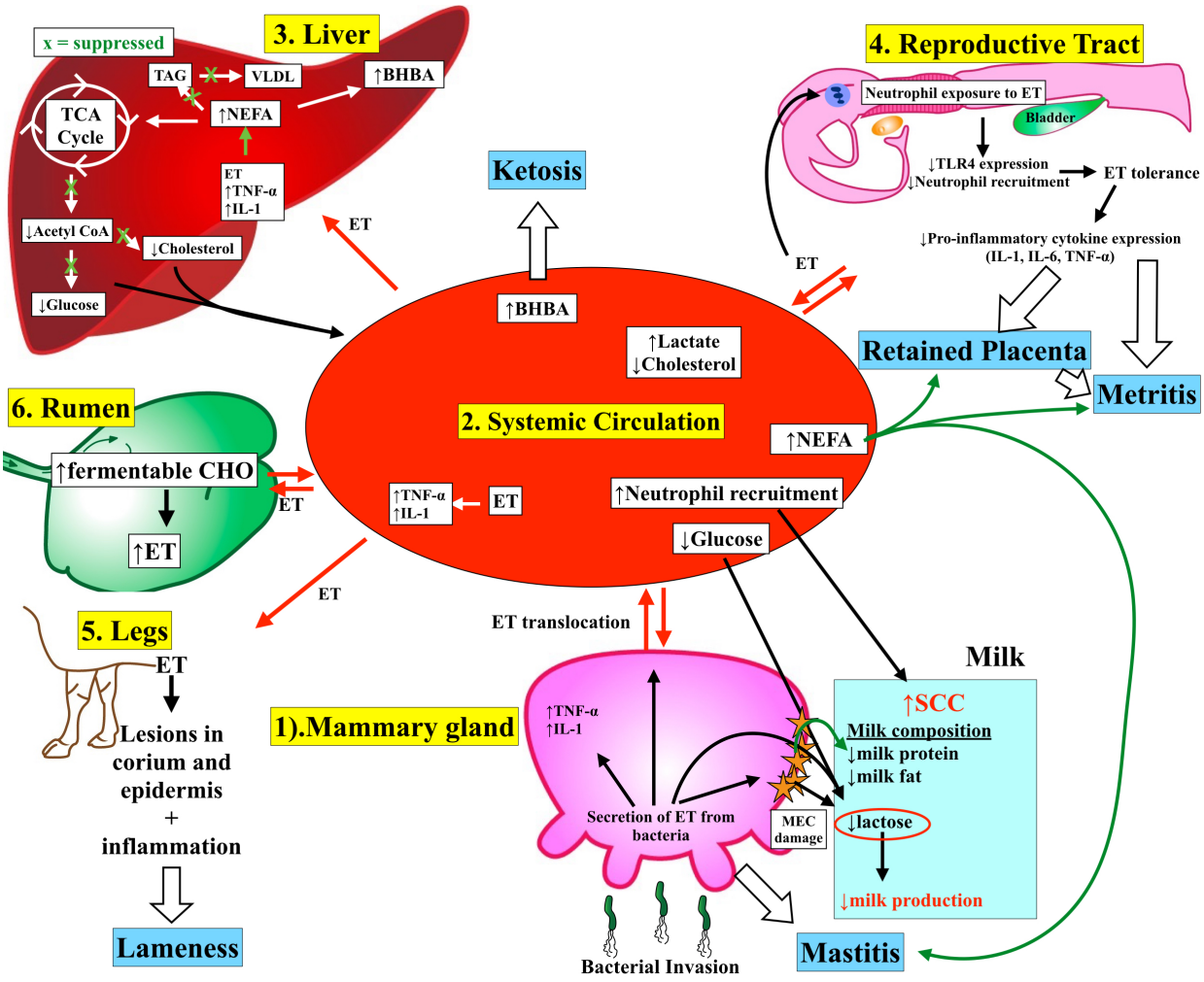

Figure 4-1. Interrelationships among somatic cell counts (SCC) prior to parturition and different potential causal factors in the etiopathology of periparturient diseases of transition dairy cows. Udder bacterial infection and their endotoxins (ET) as well as proinflammatory cytokines released during infection are involved in multiple alterations in the overall metabolism and disease in multiple organs including liver, reproductive tract, mammary gland and feet. There are 3 sources of ET in dairy cows including mammary gland, rumen, and reproductive tract. 1) In the mammary gland, bacterial infection and release of ET triggers release of pro-inflammatory cytokines like TNF-α, IL-1, damages mammary epithelial cells (MEC), and lower milk lactose synthesis and increase immune-related proteins. Decreased lactose in the milk is a result of bacteria using lactose and ET effects on glucose metabolism. Enhanced neutrophil recruitment to the mammary gland is reflected by an elevation in SCC in the milk. Mastitis is a result of bacterial invasion and increased SCC in mammary gland. In addition, elevated NEFA in blood increase susceptibility to mastitis.

2) ET translocation and enhanced pro-inflammatory cytokines (TNF-α, and IL-1) from the mammary gland translocation into the systemic circulation affecting various organs of the cow. 3) ET translocation from systemic circulation into the liver along with enhanced TNF-α and IL-1, results in impaired oxidation of NEFA into acetyl CoA through the TCA cycle, thereby, decreasing glucose and cholesterol synthesis. In addition, NEFA oxidation and utilization for production of TAG and VLDL is impaired under the influence of ET and pro-inflammatory cytokines. The increase in NEFA abundance and conversion of NEFA into BHBA results in increased BHBA in the blood circulation which triggers the state of ketosis. 4) In the reproductive tract, neutrophil exposure to ET leads to dysfunction of neutrophils and decreased TLR4 expression. Dysfunctional neutrophils are unable to migrate towards reproductive tract resulting in exacerbation of infection leading to retained placenta and metritis. In addition, metritis incidence may be a consequence of retained placenta. 5) Endotoxin also affects the health of feets of the cows through lesions in the corium and epidermis along with presence of inflammation leading to lameness. 6) Increased fermentable carbohydrates from dietary changes immediately after parturition enhances ruminal free ET and its translocation into systemic circulation.

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APPENDIX

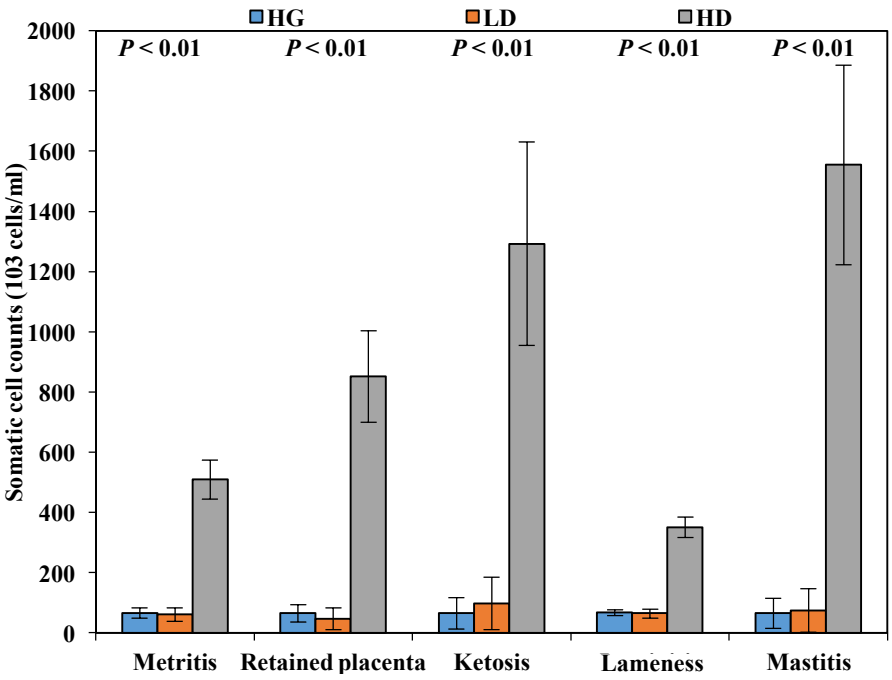
Appendix A: Other frequency data

Table A-1. Frequency of cows diagnosed with disease for parity

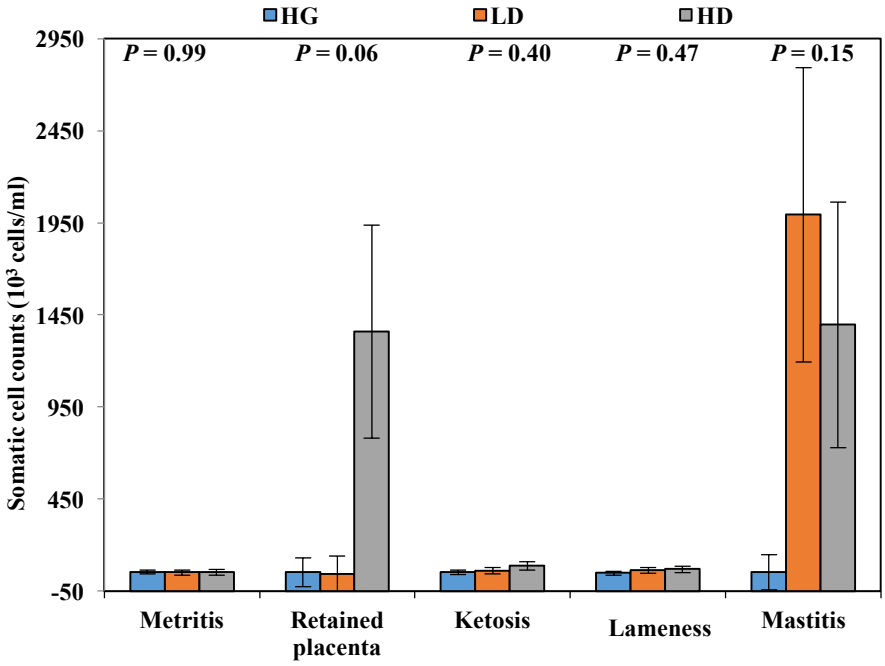
Disease	Parity	
	Primiparous	Multiparous
Metritis	22 (37.3)	37 (62.7)
Retained Placenta	8 (40.0)	12 (60.0)
Mastitis	13 (31.7)	28 (68.3)
Ketosis	11 (24.4)	34 (75.6)
Laminitis	4 (18.2)	18 (81.8)
Healthy	22 (71.0)	9 (29.0)

Appendix B: Other milk composition data

A)



B)



C)

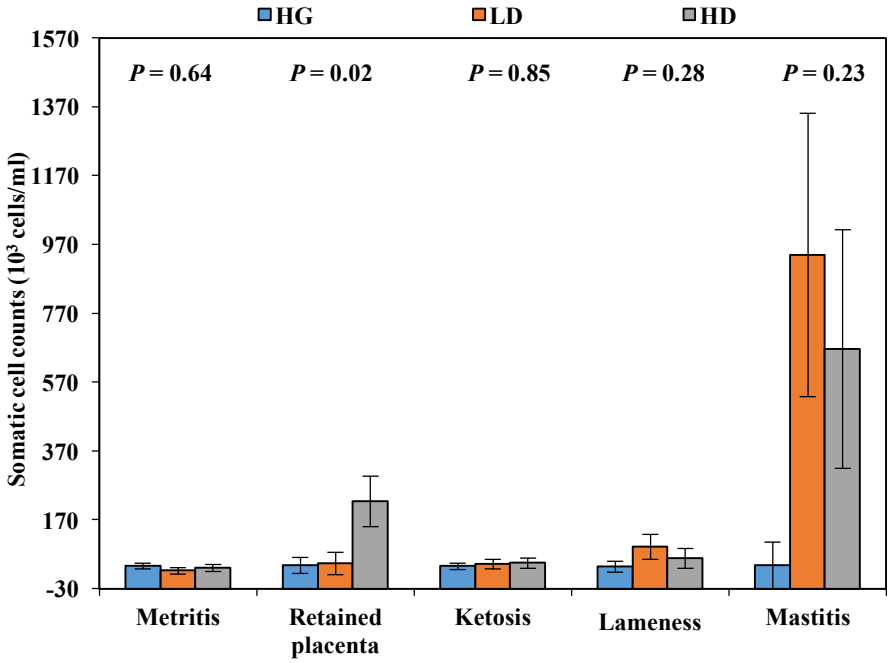
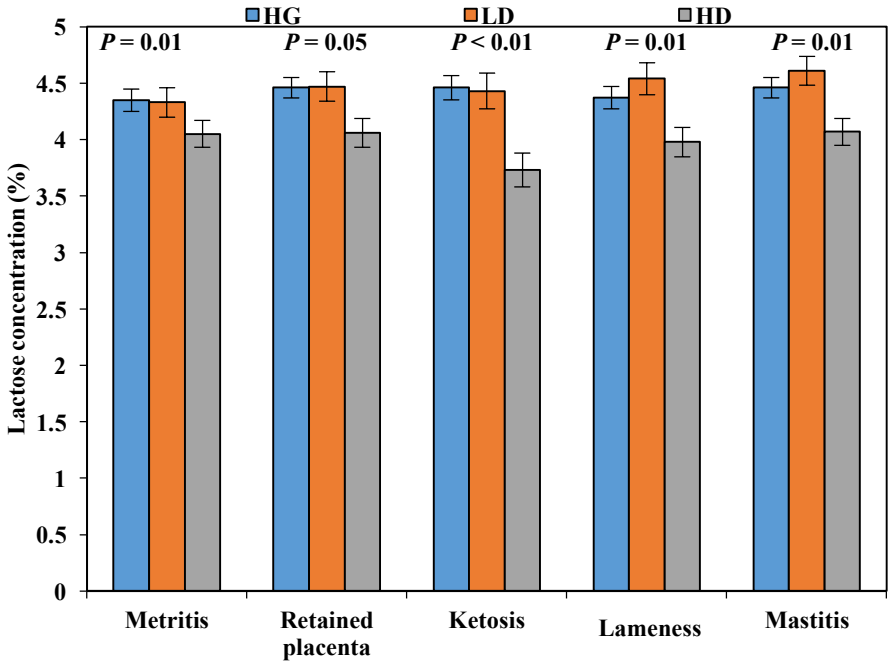
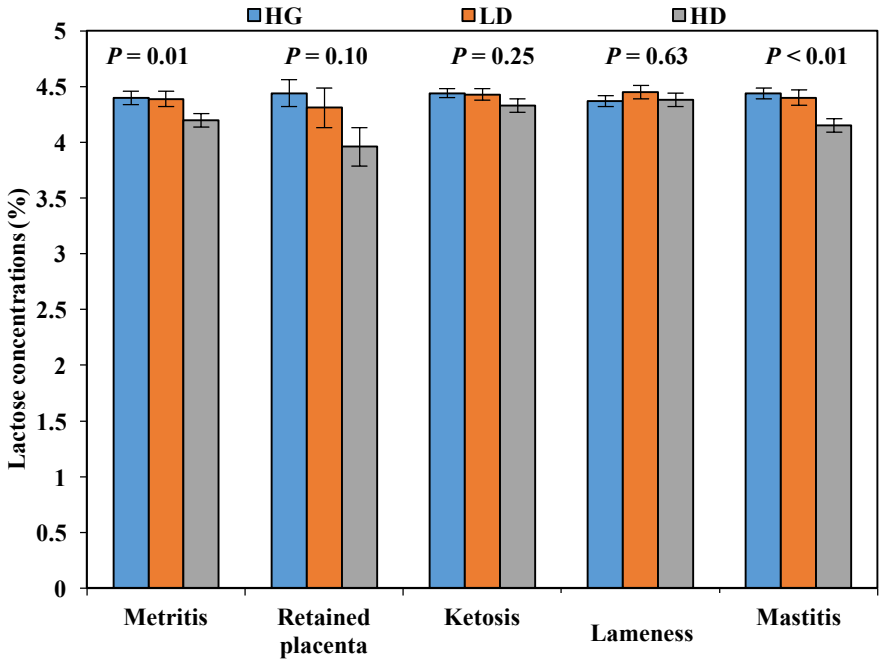


Figure B-1. Comparisons of somatic cell counts (10^3 cells/mL) between healthy group (HG), low-disease (LD), and high disease (HD) for cows diagnosed with metritis, retained placenta, ketosis, lameness, and mastitis at A) Prior to dry off (~1 week before date of dry off); B) +1 weeks after parturition; C) +2 weeks after parturition.

A)


HG
LD
HD
P = 0.01
P = 0.05
P < 0.01
P = 0.01
P = 0.01
Lactose concentration (%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Metritis
Retained placenta
Ketosis
Lameness
Mastitis

B)


HG
LD
HD
P = 0.01
P = 0.10
P = 0.25
P = 0.63
P < 0.01
Lactose concentrations (%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Metritis
Retained placenta
Ketosis
Lameness
Mastitis

C)

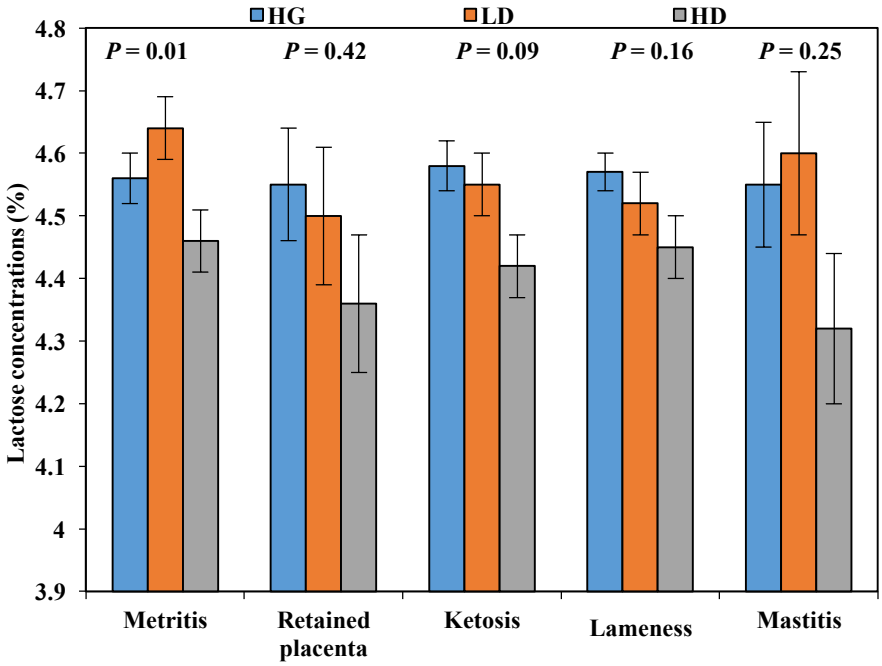
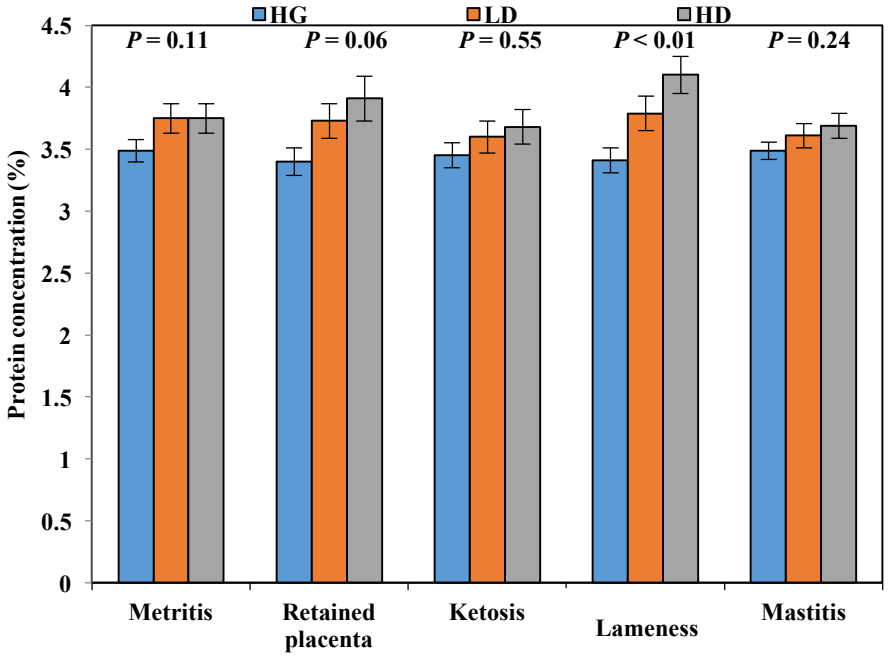
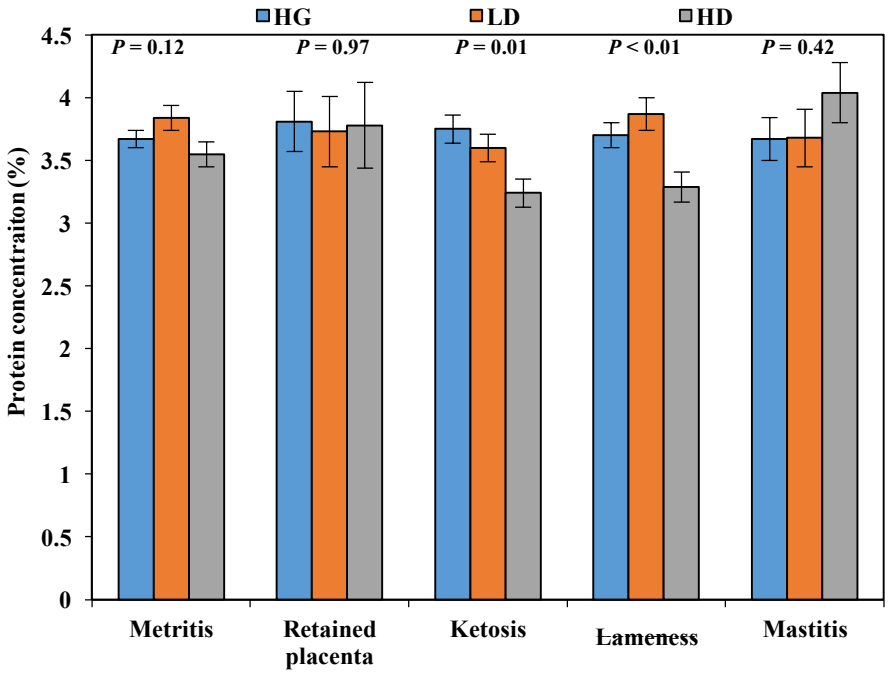


Figure B-2. Comparisons of lactose concentrations (%) between healthy group (HG), low-disease (LD), and high disease (HD) for cows diagnosed with metritis, retained placenta, ketosis, lameness, and mastitis at A) Prior to dry off (~1 week before date of dry off); B) +1 weeks after parturition; C) +2 weeks after parturition.

A)


HG
LD
HD
P = 0.11
P = 0.06
P = 0.55
P < 0.01
P = 0.24
Protein concentration (%)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Metritis
Retained placenta
Ketosis
Lameness
Mastitis

B)


HG
LD
HD
P = 0.12
P = 0.97
P = 0.01
P < 0.01
P = 0.42
Protein concentraiton (%)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Metritis
Retained placenta
Ketosis
Lameness
Mastitis

C)

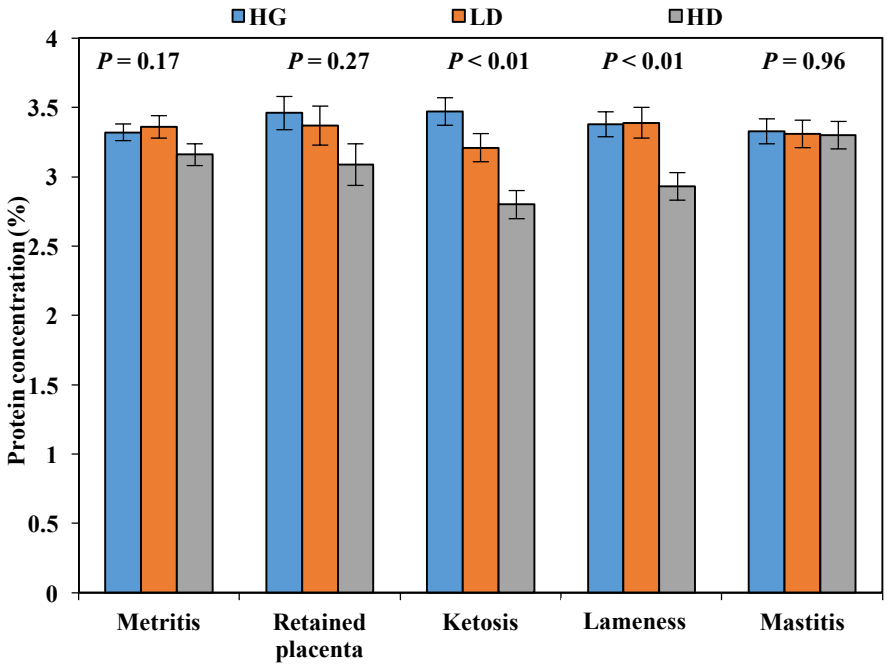


Figure B-3. Comparisons of protein concentrations (%) between healthy group (HG), low-disease (LD), and high disease (HD) for cows diagnosed with metritis, retained placenta, ketosis, lameness, and mastitis at A) Prior to dry off (~1 week before date of dry off); B) +1 weeks after parturition; C) +2 weeks after parturition.

Appendix C: Milk composition data throughout experimental period

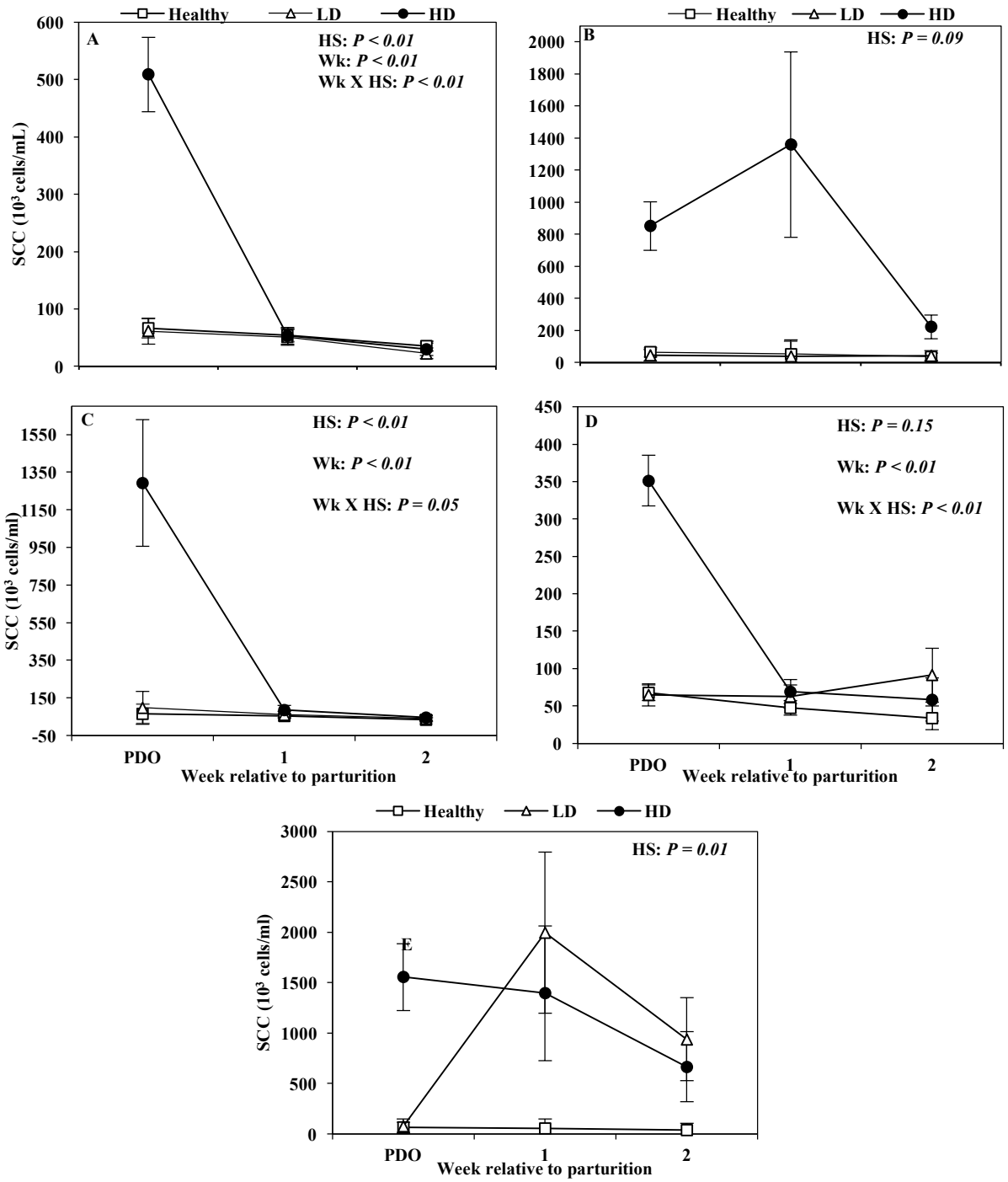


Figure C-1. Concentrations of somatic cell counts for a) metritis, B) retained placenta, C) ketosis, D) lameness, and E) mastitis between healthy, low-disease, and high-disease (LSM±SEM; HS = effect of health status; Wk = effect of week; Wk x HS = week x health status interaction).

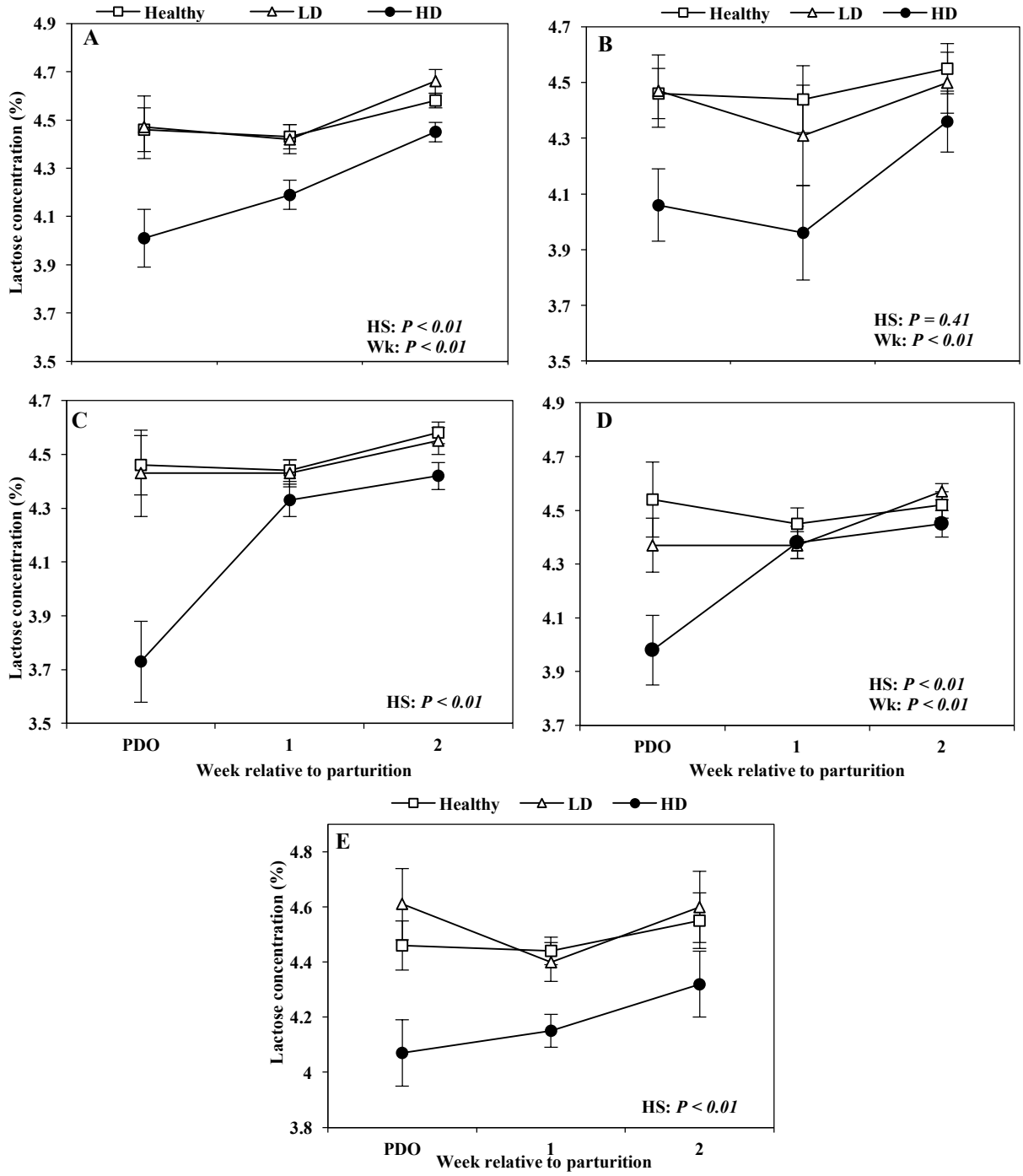


Figure C-2. Concentrations of lactose for a) metritis, B) retained placenta, C) ketosis, D) lameness, and E) mastitis between healthy, low-disease, and high-disease (LSM±SEM; HS = effect of health status; Wk = effect of week).

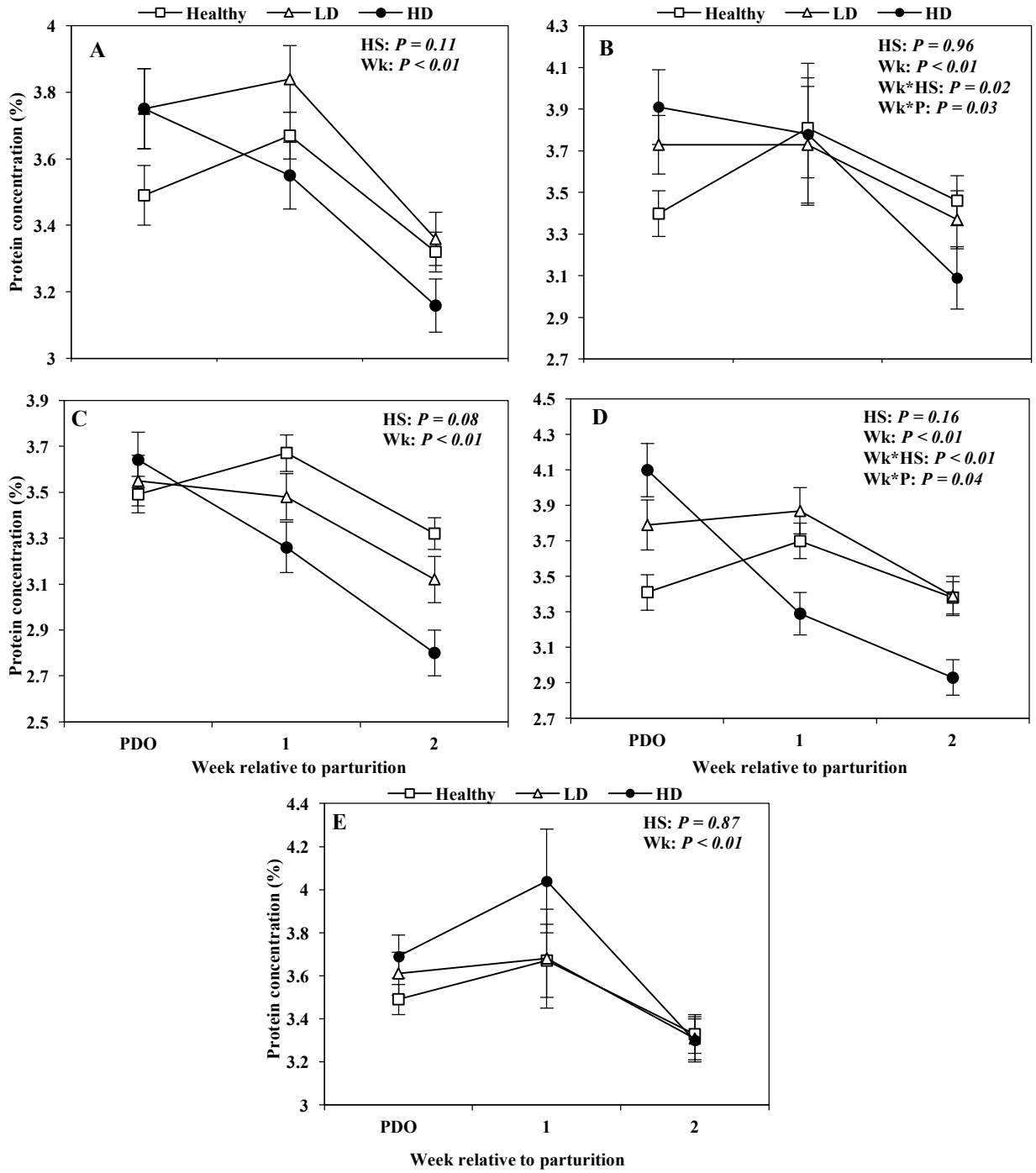


Figure C-3. Concentrations of protein for a) metritis, B) retained placenta, C) ketosis, D) lameness, and E) mastitis between healthy, low-disease, and high-disease (LSM±SEM; HS = effect of health status; Wk = effect of week; Wk*P = effect of sampling week and parity interaction).

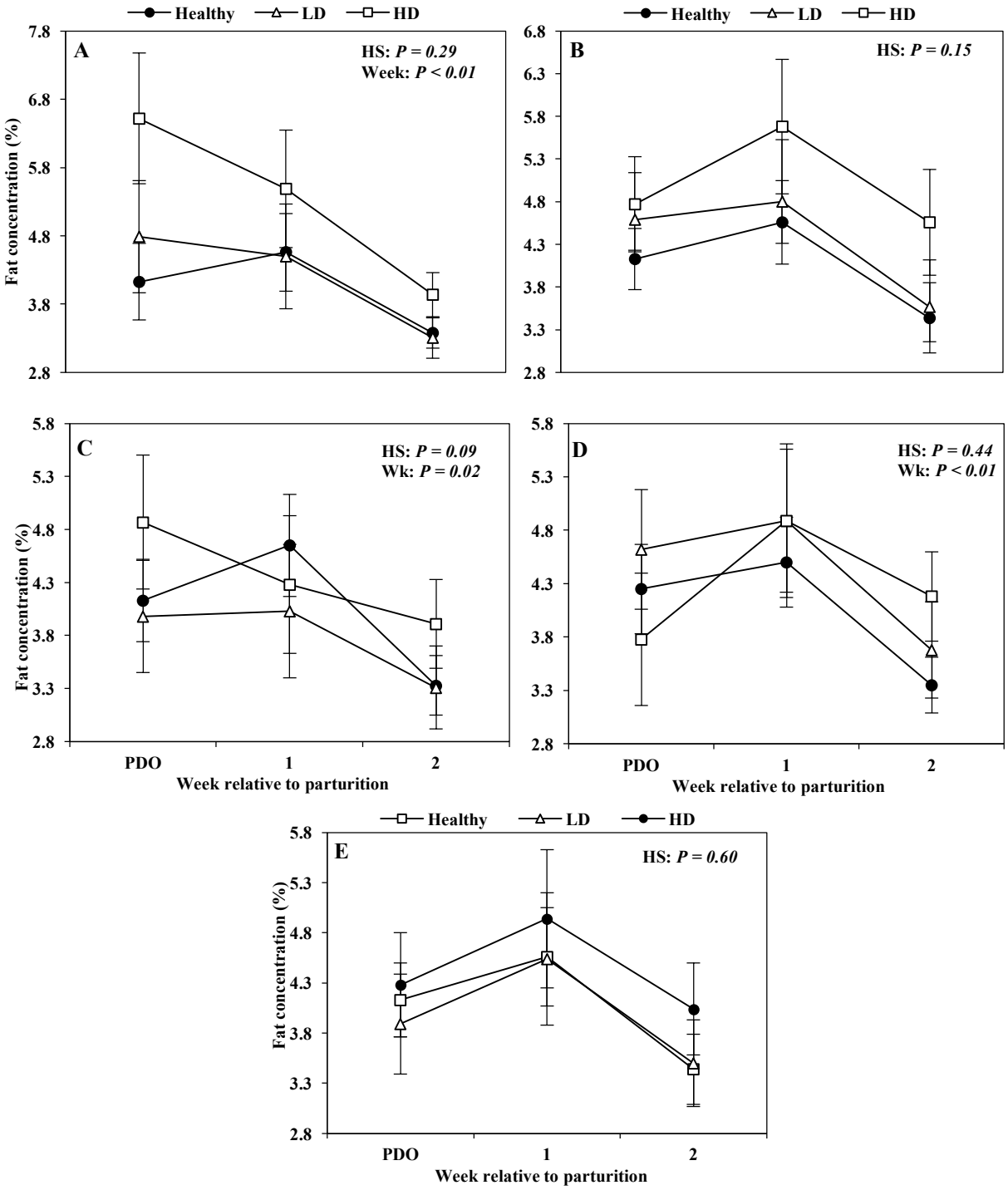


Figure C-4. Concentrations of fat for a) metritis, B) retained placenta, C) ketosis, D) lameness, and E) mastitis between healthy, low-disease, and high-disease (LSM±SEM; HS = effect of health status; Wk = effect of week).

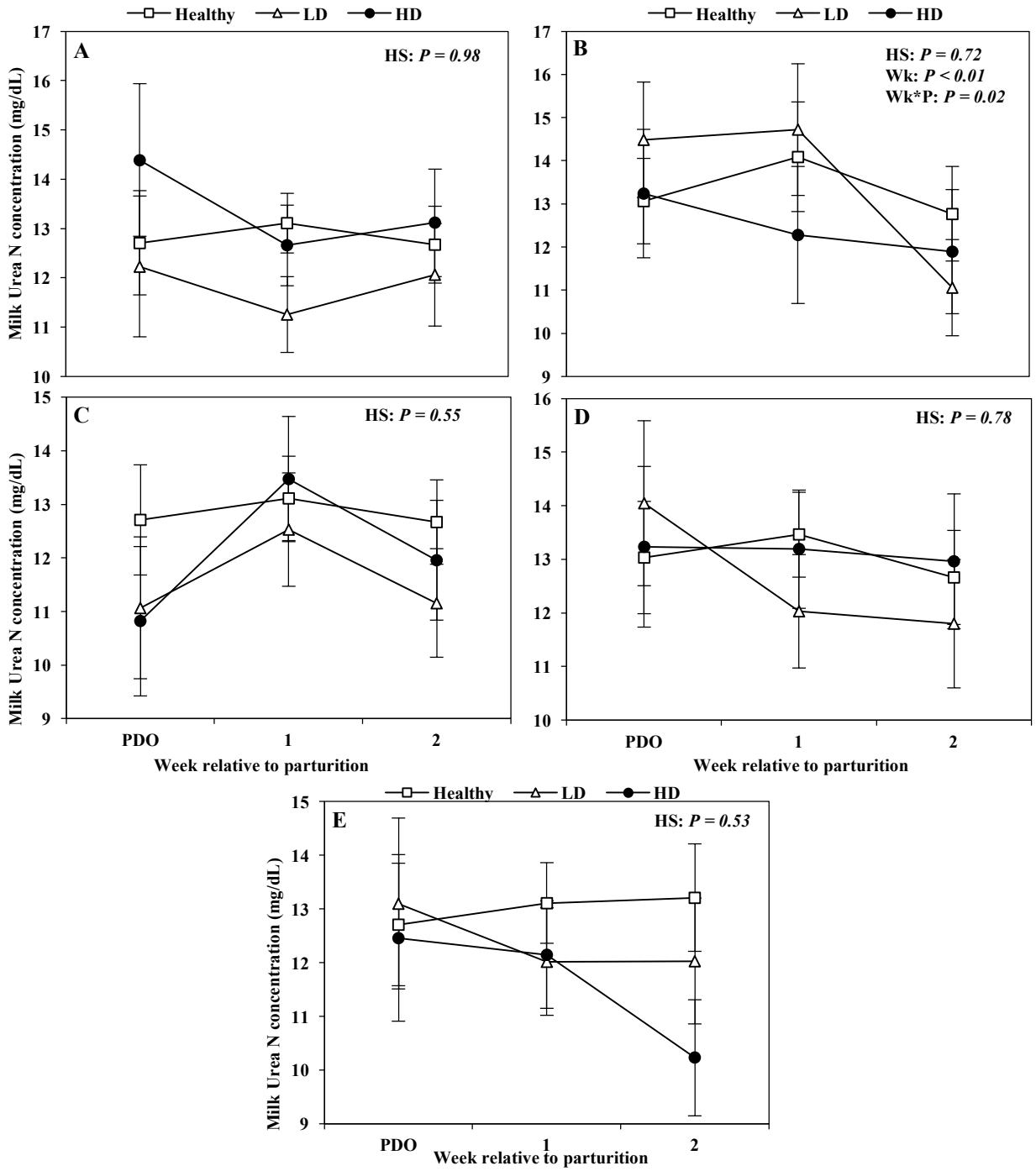


Figure C-5. Concentrations of milk urea nitrogen (Milk urea N) for a) metritis, B) retained placenta, C) ketosis, D) lameness, and E) mastitis between healthy, low-disease, and high-disease (LSM±SEM; HS = effect of health status; Wk = effect of week; Wk*P = effect of week * parity interaction).

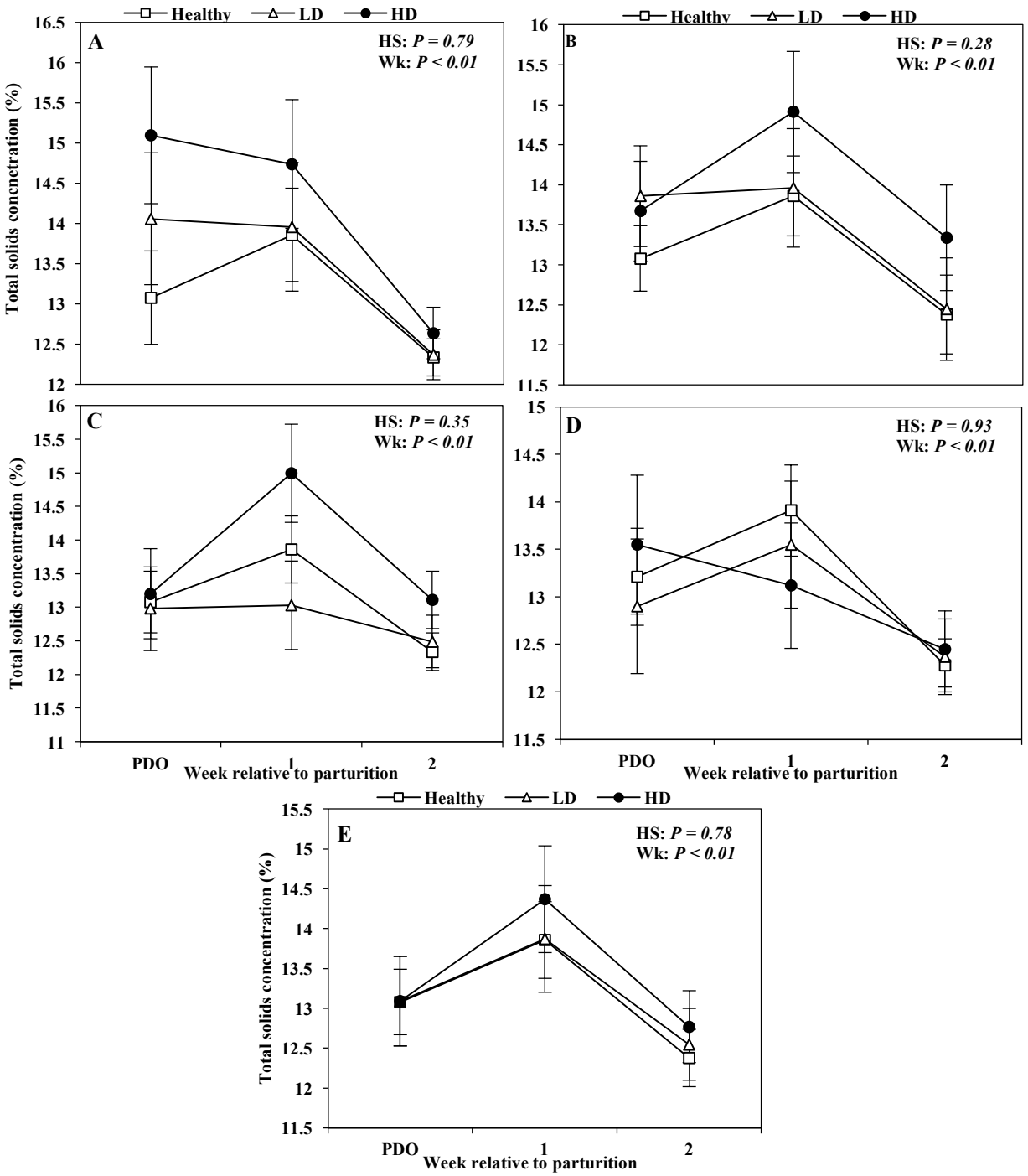


Figure C-6. Concentrations of total solids for a) metritis, B) retained placenta, C) ketosis, D) lameness, and E) mastitis between healthy, low-disease, and high-disease (LSM±SEM; HS = effect of health status; Wk = effect of week).