The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and reducing dietary starch content after calving on the immune response and nutrient digestibility in transition dairy cows

by

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Abstract

The transition period is a challenging time for dairy cows because they experience reduced immune system function, increased risk of sub-acute rumen acidosis, and inflammation. The combination of these can lead to increased susceptibility to disease and reduced productivity. Additionally, incidence of sub-acute rumen acidosis is associated with reduced nutrient digestibility. The objective of this thesis was to evaluate the effects of supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) and decreasing dietary starch content on gene expression in rumen papillae, serum concentrations of acute phase proteins, indicators of oxidative stress in plasma, adaptive immune response, and nutrient digestibility in transition dairy cows. Holstein dairy cows (n = 38) were fed a *Saccharomyces cerevisiae* fermentation product (SCFP, NutriTek®, Diamond V, Cedar Rapids, IA) during the transition period. Four weeks before calving, cows were fed a common close up diet containing 13% starch with or without SCFP. For 3 weeks after calving, cows were fed high or low starch (HS vs. LS; 27 and 21%, respectively) diets with or without SCFP (CON). Animals were assigned to one of 4 treatments (CON+HS, CON+LS, SCFP+HS, SCFP+LS). After week 3, all animals received high starch diets with or without SCFP. Rumen papillae samples were collected at d -10 relative to expected calving date and d 21 ± 3 after calving and analyzed for mRNA abundance using qRT-PCR. Acute phase protein concentrations in serum and indicators of oxidative stress in plasma were measured on d -10 relative to expected calving date and on d 1, 7, 21, and 42 ± 3. An ovalbumin challenge to measure adaptive immune response was conducted on d 7 and 21 ± 3 after calving. Nutrient digestibility was measured on d 7 and 21 ± 3 after calving. There was no interaction between starch content and SCFP treatments on mRNA abundance, acute phase response, adaptive immunity, or nutrient digestibility. There was an interaction between dietary

starch content and SCFP on indices of oxidative stress. Supplementation of SCFP tended to reduce plasma concentrations of total antioxidant capacity on d 1 and 7 after calving regardless of starch content of fresh diets, and d 21 after calving when low starch diets were fed. Similarly, regardless of starch content of fresh diets, SCFP supplementation increased plasma concentrations of malondialdehyde on d 21 after calving as compared to diets without SCFP, indicating that animals fed SCFP experienced greater oxidative stress. However, SCFP supplementation reduced serum concentrations of haptoglobin on d 7 after calving, indicating reduced systemic inflammation. Feeding a low starch diet after calving increased apparent total tract neutral detergent fibre digestibility on d 7 after calving and reduced mRNA abundance of interleukin receptor associated kinase-1in rumen tissue on d 21 after calving, suggesting reduced immune activation in rumen tissue. There was no effect of treatment on adaptive immunity. These results indicate that supplementing SCFP through the transition period and feeding a low starch diet after calving may reduce inflammation.

Preface

The research project, which this thesis is a part of, received research ethics approval from the University of Alberta Animal Care and Use Committee for Livestock and cattle were cared for according to the guidelines of the Canadian Council on Animal Care.

Caroline Knoblock and Weina Shi conducted all aspects of the study presented in this thesis. Yanhong Chen assisted with data analysis presented in chapter 3. Dr. Masahito Oba provided advice on protocols, data analysis and the final versions of chapter 2 and 3. Chapter 1 and 4 are the author's original work.

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List of Abbreviations

ADY: active dry yeast

APR: acute phase response

APP: acute phase proteins

BCS: body condition score

BHB: β-hydroxybutyrate

CLDN1: claudin 1

CLDN4: claudin 4

DDG: dried distillers grain

DM: dry matter

DMI: dry matter intake

Hp: haptoglobin

Ig: immunoglobulin

IgA: immunoglobulin A

IgG: immunoglobulin g

IL-1β: interleukin-1 beta

IL-6: interleukin-6

iNDF: indigestible NDF

IRAK1: interleukin-1 receptor associated kinase 1

LAI: liver activity index

LPS: lipopolysaccharide

MDA: malondialdehyde

NDF: neutral detergent fibre

NEB: negative energy balance

NF-κβ: nuclear factor-kappa beta

OCLN: occluding

OM: organic matter

PCR: polymerase chain reaction

peNDF: physically effective NDF

qRT-PCR: quantitative real-time PCR

ROM: reactive oxygen metabolites

SAA: serum amyloid-A

SARA: subacute rumen acidosis

SCFP: *Saccharomyces cerevisiae* fermentation product

TAOC: total antioxidant capacity

TBARS: thiobarbituric acid reactive substances

TJP1: tight junction protein 1

TLR2: toll-like receptor 2

TLR4: toll-like receptor 4

TMR: total mixed ration

TNF-α: tumor necrosis factor-alpha

VFA: volatile fatty acids

Chapter 1: Literature Review

1.1 Introduction

The transition period is defined as the three weeks before calving to three weeks after calving, and is often a challenge for cows as they face diet changes, a drop in dry matter intake (DMI), and the stress of calving (Drackley, 1999). When the cow is expending more energy than is being consumed she is in a state of negative energy balance (NEB). Most cows are in a state of NEB early postpartum as their milk production increases before their dry matter intake increases to fulfill the energy demand (Drackley, 1999). Lipolysis, the breakdown of adipose tissue, is increased to provide energy substrates such as free fatty acids. Free fatty acids are used by tissues and in the liver for energy production (Grummer, 1993). McArt et al. (2013) reviewed the prevalence of high free fatty acid concentrations (greater than 0.7 mMeq/L) and the effect on the cow. Increased free fatty acid concentrations were associated with health disorders such as displaced abomasum and metritis. Other health disorders of the transition dairy cow are fatty liver, displaced abomasum, milk fever, retained placenta, and metritis, which are associated with NEB (Esposito et al., 2014).

Transition cows are at risk for sub-acute rumen acidosis (SARA) because of the change in diet from pre-calving to post-calving and the lack of capability of the rumen to adapt to this change quickly (Krause and Oetzel, 2006). Sub-acute rumen acidosis can result in depressed DMI, milk fat depression, reduced nutrient digestibility, and immune system activation (Plaizier et al., 2008; Zebeli et al., 2010).

Around calving, the immune system response is depressed, increasing the risk of bacterial infection (Mallard et al., 1998). While the immune response is depressed, dairy cows face risk of uterus and udder infections. Metritis is due to bacterial invasion of the uterus, and a depressed immune system is implicated as a cause of metritis because of the inability to defend against bacteria (LeBlanc, 2008). Mastitis is a production problem in all stages of lactation, and a depressed immune system is also implicated as a cause of mastitis during early lactation (Mulligan and Doherty, 2008). Both diseases require a healthy immune response to recognize and clear pathogens.

While cows are at risk for SARA, they are also facing a depressed immune system (Mallard et al., 1998). There are multiple dietary strategies we can employ to reduce the risk of SARA, and reduce activation of the immune system. In this literature review I will discuss the immune response, the impact of SARA on the immune response, the effects of SCFP supplementation, and dietary strategies to reduce the incidence of SARA.

1.2 Immune System

While the immune system's response to pathogens is important, excessive immune system activation is undesirable. Immune cells utilize glucose (Calder et al., 2007), as does cellular proliferation (Vander Heiden et al., 2009). Glucose is needed to produce milk lactose, and inflammation decreases the amount of glucose available to the animal and may decrease milk yield, and there is evidence of a relationship between inflammation and milk yield. Bertoni et al., (2008) placed cows in quartiles (upper, intermediate upper, intermediate lower, and lower) based on liver activity index (LAI). The mean blood concentration of negative acute phase proteins synthesized by the liver, with a higher LAI representing higher activity of negative acute phase proteins, determined LAI. Negative acute phase proteins are anti-inflammatory and decrease during acute inflammation (Gabay and Kushner, 1999). Therefore, the upper quartile represented a reduced inflammatory response. Upper quartile cows had higher milk yield (40.8 kg/d) at 28 d post-partum than all other quartiles; the lower quartile group had the lowest milk yield (34.1 kg/d). Haptoglobin is released at the onset of an immune response and is used as an indicator of inflammation (Theilgaard-Möonch et al., 2006, Ceciliani et al., 2012). Cows in the lowest quartile had the highest concentrations of haptoglobin (Hp) one week post-calving.

Kvidera et al. (2017) provided evidence of the relationship between immune system activation and reductions in milk yield due to glucose use by the immune system. Kvidera et al. (2017) administered endotoxin, a potent immune system activator, and monitored blood glucose using a euglycemic clamp. In this experiment, milk yield was measured over 720 minutes for three groups of cows: control (saline infused), LPS-C (endotoxin administered), and LPS-eu (endotoxin administered and glucose infused). Glucose was infused to the LPS-eu group at a rate to maintain euglycemia. As expected, milk yield was reduced in the endotoxin administered

groups (~80% reduction compared to control). Milk yield between LPS-C and LPS-eu groups did not differ. In LPS-eu cows, the infused glucose was not used for milk production as might be expected, but continued to be re-directed elsewhere, presumably towards the immune system. Amount of glucose infused and that spared from lactose synthesis (as compared to control group) were used to estimate glucose use of the immune system. Over 720 minutes 1,092 g glucose was used by the immune system. While this amount is an approximation, it indicates that the immune system uses a large amount of glucose during periods of activation.

1.2.1 Innate Immune Response

There are two distinct categories of the immune response: innate and adaptive. The innate response is the first reactor to a pathogen. Innate immune cells recognize invading cells through pattern-recognition receptors, such as toll-like receptors, and activate an immune response by releasing cytokines (O'Neill, 2002). These cytokines attract more immune cells to the area as well as stimulate a systemic whole body response by activating the acute phase protein response, or more simply, the acute phase response (APR).

1.2.1.1 Acute Phase Response

The APR is an important immune system activator and a crucial part of the innate immune response. Acute phase proteins (APP) are proteins primarily synthesized in the liver. There are two main types: positive and negative APP. Positive APP increase during an inflammatory response and make up most of the APP, while negative APP decrease during an inflammatory response. Serum amyloid-A (SAA) and haptoglobin (Hp) are two positive acute proteins. Both are activated by cytokines interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor – alpha (TNF-α) (Gruys et al., 2005). Haptoglobin binds hemoglobin in order to limit the capacity to release oxidants such as nitric oxide, and in this way Hp has an antioxidant role (Edwards et al., 1989). Haptoglobin also binds immune cell surfaces to activate cytokine release (Galicia and Ceuppens, 2011). Haptoglobin is anti-inflammatory but also stimulates an immune response. Serum amyloid-A production is predominately stimulated by IL-1β (Smith and McDonald, 1992). Serum amyloid-A stimulates immune cells (monocytes, macrophages, neutrophils) to produce cytokines such as IL-1β, IL-6, and TNF-α, and chemokines (De Buck et al., 2016). These cells recruit more immune cells to the area and stimulate a systemic immune

response. Serum amyloid-A rises rapidly in the first 24 hours post stimulation and, without additional stimulation, falls rapidly within 24 hours (Wang et al., 2001b). Unlike SAA, concentrations of Hp remain relatively constant after immune system activation. The half-life of Hp is around five days (Gruys et al., 2005). Acute phase proteins have long been used as indicators of acute and sub-acute inflammation in dairy cattle (Alsemgeest et al., 1994).

1.2.1.2 Oxidative Burst

Oxidative burst is the production of reactive oxygen metabolites (ROM) by neutrophils and macrophages to defend against a pathogen and is a normal response to kill a pathogen (Valko et al., 2007). Oxidative burst is an important and necessary aspect of the innate immune response. However, when there is an imbalance between oxidants and the antioxidants to neutralize them, oxidative stress occurs (Brenneisen et al., 2005). Reactive oxygen metabolites include H_2O_2, or hydroperoxide, and can be used as a measure of oxidative stress. Plasma ROM are markers of free radical production and oxidation (Miller et al., 1993). Without an increase in the body's antioxidants, ROM continue to activate an inflammation cascade (Schreck et al., 1991). Malondialdehyde (MDA) is used as a marker of lipid peroxidation, or the breakdown of lipids, such as the phospholipid by-layer of cell membranes, by free radicals (Armstrong and Browne, 1994). MDA increases after calving (Bernabucci et al., 2005, Bouwstra et al., 2008). Total antioxidant capacity (TAOC) is a measure of the capability of antioxidants present in plasma to neutralize oxidants (Ghiselli et al., 2000). The measurement can be used as a sum of all antioxidants and their interactions. In heifers, TAOC decreased one week before calving and returned to original concentrations one week after calving (Turk et al., 2013). The oxidative stress response of the transition cow is complex and involves many interactions.

1.2.2 Adaptive Immune Response

While the innate immune response is non-specific, the adaptive immune response is specialized against known invaders. White blood cells, or lymphocytes, are cells involved in the adaptive immune response. There are two types of lymphocytes, T-cells and B-cells. These lymphocytes have different functions in the adaptive immune response, two categories known as cellular and humoral immunity.

Innate immune cells present cell surface proteins, or antigens, of invading cells to a matching antibody on naïve B and T cells in order to activate cell maturation. Antibodies are an important component of the immune response. Adaptive immune cells recognize a secondary infection by attachment of antibodies to antigens. The response is much faster and more efficient than the innate response. Antibodies decrease the threshold of invading cell presence needed to stimulate an immune response (Carter et al., 1988).

1.2.2.1 Cell-mediated Immunity

Cell-mediated immunity is dependent upon T-cell recognition of pathogens or infected cells. Upon secondary infection, the antibody present on T-cells activates the cell to release cytokines to attract immune cells such as macrophages and neutrophils, and T-cells also attach to the pathogen or infected cell, and initiate cell death through apoptosis (Herberman and Holden, 1978). There are two subclasses of T-cells: CD4+ T-helper cell and CD8+ T-helper cells. The first subclass, CD4+ T helper cells, differentiate into further T-helper cell subsets that have multiple functions including activating innate immune cells and B-cells. In addition, the T-helper cells play a role in suppressing immune activation (Luckheeram et al., 2012). The other main class of T-cells, CD8+ T-helper cells, mainly act to clear pathogens by killing infected cells through apoptosis (Zhang and Bevan, 2011).

1.2.2.2 Humoral Immunity

Certain subsets of mature B-cells produce antibodies, or immunoglobulin (Ig) (Banchereau and Steinman, 1998). Immunoglobulins are present on B-cells, and certain Ig classes circulate in the blood stream or the mucosa of the digestive tract. Immunoglobulin M (IgM) is the first class of Ig to be present on B-cell surfaces and switches classes upon stimulation by immune cells. Immunoglobulin A (IgA) is present in the mucosa of the digestive tract. The most common Ig is Immunoglobulin G (IgG). Other classes of Ig include IgE and IgD. Immunoglobulin G dissociates from the B-cell and freely floats through blood stream or lymph. When IgG comes in contact with an antigen it binds to the antigen and coats the cell in antibody. One end of the antibody is the same regardless of the antigen present on the other side, and is referred to as the constant end. The constant end of the antibody is recognized by neutrophils, which marks the

cell for destruction. B-cells and antibodies are involved in humoral immunity, because antibodies are present in the blood stream or lymph (Merlo and Mandik-Nayak, 2013).

An ovalbumin challenge measures the ability of the animal to produce antibodies and is used across science applications, and in many species such as mice, guinea pigs, dogs, and cattle. The ovalbumin challenge is the most common technique used to measure the adaptive immune response in lactating dairy cows (Wagter et al. 2000, 2003, Yuan et al. 2015, Silva et al., 2015). Ovalbumin is used as an antigen to induce an immune response. An adjuvant is used to enhance the animals' immune response to the antigen (Cox and Coulter, 1997). Ovalbumin and an adjuvant are dissolved into solution and injected at least twice over multiple time points to stimulate innate and adaptive immune responses. Mallard et al. (1997) introduced the protocol often used in dairy cattle literature; they injected the ovalbumin solution at -8 weeks and -3 weeks relative to calving and measured IgG concentration (optical density) at calving, week three after calving, and week six after calving, but the time points used in literature vary depending on the objective of the experiment.

An increase in IgG in response to ovalbumin challenge is beneficial to the cow. The ovalbumin challenge measures the health of the humoral immune system and the ability to handle a stressor. Individual animal IgG response to an ovalbumin challenge has been used to rank cows as high, medium, or low responders (Wagter et al., 2000). An increased animal IgG response to an ovalbumin challenge has been associated with better health outcomes and increased milk yield (Thompson–Crispi et al., 2013, Wagter et al., 2003).

1.2.3 Challenges During the Transition Period

Antioxidants, which are needed to neutralize oxidants and prevent oxidative stress, may be limited during the transition period (Bernabucci et al., 2005). Cows often experience oxidative stress because of decreased antioxidants and increased oxidants (Castillo et al., 2006; Bernabucci et al., 2005; Bouwstra et al., 2008). Bernabucci et al. (2005) found an association between body condition score (BCS) and oxidative status. Although all cows, regardless of BCS, experienced oxidative stress after calving, cows with higher BCS had higher concentrations of ROM and TBARS and lower concentrations of antioxidants.

Sub-acute rumen acidosis is defined as the incidence of depressed rumen pH (typically less than 5.8, though the definition differs from one study to another) for three or more hours a day (Plaizier et al., 2009). The consequences of SARA include reduced DMI, milk fat depression, lameness, and inflammation (Plaizier et al., 2009). Plaizier et al. (2009) also reviewed the acute phase response to SARA and found that SAA and Hp are increased, potentially due to free lipopolysaccharides (LPS) in rumen fluid that may cross the rumen epithelium into the blood stream. During the transition period, cows are moved from a high fibre, low concentrate diet to a low fibre, high concentrate diet, and this can pose a challenge to the rumen environment and increase the risk of SARA (Zebeli et al., 2010).

Lipopolysaccharides, also called endotoxin, are found on the outer membrane of most gram-negative bacteria such as *E. coli* and *Salmonella*. An increase of free rumen LPS is evidence of an increase of gram-negative bacteria (Wells and Russell, 1996). Rumen adaptation to a high starch diet increases gram-negative cell replication and increases the concentration of bacteria in the rumen (Gozho et al., 2006). Lipopolysaccharides are released by gram-negative bacteria and activate an immune response in the rumen epithelium by binding to TLR4 (Ulevitch and Tobias, 1995). Activation of TLR4 by LPS releases inflammatory cytokines such as IL-6, IL-1β, and TNF-α into the blood through activation of the nuclear factor-kappa beta (NF-κB) pathway (Wang et al., 2014). An increase in circulating LPS is followed by an increase in haptoglobin and serum amyloid A, whose expression was activated by cytokines (Dong et al., 2011; Khafipour et al., 2009b). Post-calving cows are fed a high concentrate, highly digestible diet. This diet is different from the low concentrate, high forage close-up diet as it contains more concentrate and less forage. This abrupt change poses a challenge to the rumen microbial environment and may induce SARA. These changes in the rumen environment pose a challenge to the transition cow by activation of an immune response. Reducing starch content of fresh cow diets may reduce risk of acidosis, which is a potential cause of immune system activation (Zebeli et al., 2010).

Toll-like receptor 4 is present in rumen papillae (Trevisi et al., 2014), and other immune function related genes are also present, such as toll-like receptor 2 and Iterleukin-1 receptor-associated kinase 1 (IRAK1), and expression of these genes has been shown to change during the transition

period (Minuti et al., 2015). Interleukin-1 receptor-associated kinase 1 is a part of the innate immune responses and is activated by LPS stimulation of TLR4 and stimulates the activation of the NF-κB pathway by the binding of IL-1 to the IRAK1 protein (Gottipati et al., 2008). Through this pathway, IL-1 activates NF-κB and causes the transcription of genes for cytokines such as IL-6, IL-1β, and TNF-α (Wolf et al., 2001). However, NF-κB can be stimulated by IL-1 through another pathway with or without binding to IRAK, although this pathway takes longer to activate (Auron, 1998). While TLR4 is primarily activated by LPS, TLR2 is activated by a multitude of cell wall molecules from gram-positive bacteria to yeast cell walls (Medzhitov, 2001). Toll-like receptor 4 activates NF-κB through IRAK, but TLR4 can also activate NF-κB independently of IRAK, albeit as a delayed response compared to IRAK mediated activation (Kawai et al., 1999).

Khafipour et al. (2009b) induced SARA (pH < 5.6 for 180 min/day) by rapidly increasing highly fermentable carbohydrates by feeding wheat-barley pellets. Rumen fluid and blood LPS concentrations increased during the SARA challenge. Conversely, in Khafipour et al. (2009a) cows were fed 50% concentrate and 50% chopped alfalfa hay, and SARA was induced by gradually replacing alfalfa hay with alfalfa pellets in treatment cows. Lipopolysaccharide concentrations in rumen fluid increased, but serum LPS, SAA, and Hp concentrations did not differ from control cows. Depending on the feed source, induction of SARA had different effects on acute phase response and LPS concentration in the blood. However, these two studies also varied in SARA induction protocol and the diet fed before inducing SARA. The diet fed during grain-induced SARA had greater starch content than control, while the diet fed for alfalfa-induced SARA had the same starch content of the control diet. Khafipour et al., (2009a) suggest that the increase of starch from the first study contributed to the translocation of LPS as there was a lack of LPS in the blood during alfalfa induced SARA, where starch content between diets did not change. They suggest that, because of hindgut fermentation, LPS may translocate across the large intestinal wall rather than through the rumen.

Tight junctions are an important component of the barrier between the rumen and the blood system. It has been suggested that LPS can translocate across the rumen epithelium into the blood (Emmanuel et al., 2007). Minuti et al. (2015) reported decreased tight junction gene expression of occludin and tight junction protein 1 (TJP1) after calving. Occludin (OCLN) is a

protein that acts as the link of tight junction proteins, and the decreased expression of occludin is associated with decreased barrier function (Feldman et al., 2005). Tight junction protein-1 is an anchor inside the cell of tight junctions and is bound to occludin and claudins (Fanning et al., 1998). Tight junction protein-1 is also known as ZO-1. Expression of other tight junction genes such as claudin-1 (CLDN1) and claudin-4 (CLDN4) were not changed during the periparturient period (Minuti et al., 2015); however these genes are another important component of the tight junction structure. Claudin-1 and CLDN4 also bind to TJP1 and, like OCLN, are physical links between cells (Findley and Koval, 2009).

Minuti et al. (2015) found that both TLR4 and IRAK1 expression in rumen epithelium were reduced after calving. Chen et al. (2012) found that acidosis susceptible steers had decreased expression of TLR4 in rumen papillae compared with acidosis resistant steers. However, TLR4 gene expression of late-lactation dairy cows did not differ between animals at low or high risk for acidosis (Gao and Oba, 2016). The epithelium may adapt to an increase of LPS by reducing expression of TLR in order to prevent over stimulation of an immune response (Abreu, 2010). This is known as endotoxin tolerance. One mechanism of endotoxin tolerance is reduced expression of TLR4 protein on surface of cells (Nomura et al, 2000). Multiple studies have found decreased expression of TLR4 in rumen papillae during periods of SARA, but have not found the consequence of this decreased expression and endotoxin tolerance. Dias et al. (2018,b) found increased TLR4 expression when cows were receiving a low starch diet, compared to a high starch diet.

1.3 Nutrient Digestibility

Apparent total tract nutrient digestibility is a way to quantify the digested fraction of a diet or forage. This information is important for diet formulation because the same feedstuff with similar chemical composition could be more or less digestible, making the nutrients more or less available for digestion and absorption.

1.3.1 Importance of Increasing Nutrient Digestibility

In a meta-analysis of 54 studies with a total of 1,942 observations, de Souza et al. (2018) used dry matter intake, diet composition, and cow body weight to create a model to predict nutrient

digestibility. They found that increasing dietary starch content decreases neutral detergent fibre (NDF) digestibility and that NDF digestibility decreases as DMI increases. This result agrees with a meta-analysis by Ferraretto et al. (2013). A mechanism may be the effect of physical fill of forages on satiety (Allen, 1996).

A low starch diet had greater NDF digestibility three weeks after calving compared to a high starch diet, but had no effect on starch digestibility (McCarthey et al., 2015b). Sun and Oba (2014) fed a low starch diet containing wheat dried distiller grain (DDG), or a high starch diet containing barley grain, and found decreased starch digestibility when feeding the low starch diet, and no effect of diet on NDF digestibility. With decreasing dietary starch content and increasing NDF content, total tract starch digestibility increased (Gencoglu et al., 2010), or did not differ compared to a high starch, decreased NDF diet (Voelker and Allen 2003, McCarthy et al., 2013, Dias et al., 2018,b). Total tract NDF digestibility increased when feeding a low starch diet (Voelker and Allen, 2003, Gencoglu et al., 2010, McCarthy et al., 2013, Dias et al., 2018,b), but did not differ between low or high starch diets (Oba and Allen, 2003a). The effects of dietary starch content on nutrient digestibility are variable, and may depend on factors such as physical characteristics of feedstuffs and NDF content.

Apparent total tract digestibility is also related to how the animal digests feedstuffs. Studies cited above fed different feedstuffs, including corn grain with different digestibility values (Oba and Allen, 2003a), or feedstuff used to replace starch, such as beet pulp (Voelker and Allen, 2003), or wheat DDG (Sun and Oba, 2014). The effects of starch content on total tract nutrient digestibility vary, depending on multiple factors such as NDF content, feed types, and digestion rate of feedstuffs. The effect of starch content on NDF digestibility can be attributed to rumen pH and the effects on growth of cellulolytic bacteria (Ferraretto et al., 2013). Decreased rumen pH leads to reductions in cellulolytic bacteria that are necessary for fibre digestion (Mouriño et al., 2001). de Souza et al. (2018) found that with increased dietary starch content, starch digestibility decreased, and attributed this effect to increased passage rate. Digestion rate of NDF was increased due to a low starch diet because of higher rumen pH (Oba and Allen, 2003a; Oba and Allen, 2003b). Oba and Allen (2003a,b) fed different amounts of dietary NDF and did not find differences in total tract NDF digestibility due to dietary starch content.

1.3.2 Measurement of Nutrient Digestibility

Apparent total tract nutrient digestibility is a measure of the amount of nutrient that disappeared from the total tract. The measure is apparent because the portion of the digestive tract where nutrients were digested cannot be determined.

Equation to estimate apparent total tract nutrient digestibility:

(nutrient intake, kg/d – nutrient output, kg/d) / nutrient intake, kg/d x 100 = apparent total tract nutrient digestibility, %

In order to determine apparent total tract nutrient digestibility, fecal output of each nutrient needs to be determined. To determine fecal output of individual nutrients, daily fecal output needs to be measured or calculated. Indigestible NDF (iNDF) is used as an internal marker to estimate daily fecal output using the assumption that intake of iNDF = output of iNDF. Other methods to determine fecal output include dosing external markers such as chromium oxide and total fecal collection. Total fecal collection is intensive and requires specialized animal housing. Estimating fecal output has been accepted as an alternative to total fecal collection. When dosing with chromium oxide, the animal is administered a capsule containing a measured amount chromium oxide. Fecal grab samples are taken in intervals to calculate recovery of ingested marker.

Several methods are available to determine iNDF fraction of feces and feeds. Indigestible NDF is the proportion of digested NDF and undigested NDF. Two common methods to determine iNDF are *in vitro* digestion and *in situ* digestion. *In vitro* digestion is a process in the lab that mimics the rumen environment in a test tube. Samples are placed in a flask and rumen fluid and other nutrients are placed in the flask under CO_2 and are incubated for 120 or 240 h. *In situ* digestion occurs in the rumen of a ruminally cannulated animal. Small nylon bags with a pore size of 50 µm containing feed or fecal samples are sealed and placed in a small laundry bag, or similar bag, and incubated in the rumen. Incubation time varies from lab to lab, but recent studies have determined that 288 h incubation is the most accurate incubation time because it returned the lowest, and most accurate, iNDF value (Krizsan and Huhtanen, 2013; Bender et al., 2016).

Following digestion of feeds and feces, by either *in vitro* or *in situ* digestion, the sample bags or contents of flask are boiled in neutral detergent solution following protocol by Van Soest et al. (1991) to determine NDF content. The remaining NDF is considered indigestible NDF. Bender et al. (2016) compared *in vitro* and *in situ* methods for determining iNDF fraction and found that results from both methods were comparable and not significantly different.

Equation to determine fecal output in kg DM/d:

iNDF ingested, kg/d / iNDF in feces, % = fecal output, kg DM/d

1.4 Yeast Product Supplementation

1.4.1 Active Dry Yeast

Active dry yeast (ADY) contains viable *Saccharomyces cerevisiae* yeast cells that are collected from a growth medium and dried. There are many positive effects of supplementing an active dry yeast product such as increased dry matter intake, milk production, and rumen population of fibrolytic bacteria (Chaucheyras-Durand et al., 2008). Wholt et al. (1998) supplemented the diet of transition cows with an active dried yeast product. The milk yield during 12-18 wk in lactation was higher for supplemented cows than for control cows (43.4 vs. 38.2 kg/d). Dry matter intake was increased for cows fed the yeast supplement (24.9 vs. 23.6 kg/d). Active dry yeast is proposed to have an effect on digestion in the rumen by increasing the population of fibrolytic bacteria with a positive effect on fibre degradation (Chaucheyras-Durand et al., 2008). Active dry yeast had a positive effect on fibrolytic bacterial population in the rumen in a study by AlZahal et al. (2017). Sixteen ruminally cannulated cows were fed a control or ADY supplemented diet, and ADY supplemented group had an increase of fibrolytic bacterial populations. An increase of fibrolytic bacteria did not have a positive effect on digestion in a study by Doreau and Juoany (1998). Apparent total tract nutrient digestibility did not differ between ADY and control treatments.

A potential problem associated with feeding ADY is yeast cell viability. Active dry yeast products are recommended to be stored at 4°C, but on farm this is not practical. At elevated heat

(40°C) viability of yeast cells decreased by 90% within the first month of storage for most ADY products (Sullivan and Bradford, 2011). This temperature is not likely to be reached on most Canadian farms, and even many North American farms, but it is an example of the variability in quality of ADY products.

1.4.2 *Saccharomyces cerevisiae* Fermentation Product

Another commercially available products using *Saccharomyces cerevisiae* yeast are *Saccharomyces cerevisiae* fermentation products. The *Saccharomyces cerevisiae* fermentation product (SCFP) contains the growth medium where the yeast was grown including vitamins and antioxidants, and contains no viable yeast cells.

1.4.2.1 In vitro studies

Allen and Ying (2012) found that supplementation of yeast culture decreased ruminal starch digestion rates, but found no effect of SCFP on milk yield, milk components, or DMI. Plata et al. (1994) found an increase of NDF digestibility with yeast culture supplementation. Mullins et al. (2013) found that yeast culture increased the abundance of a hemicellulose fermenter, and Plata et al. (1994) saw an increase in rumen protozoa populations. An increase in abundance of hemicellulose fermenters and rumen protozoa populations may explain the increase in NDF digestibility because of the total increase in cellulose digesting bacteria or protozoa.

In vitro studies have shown positive effects of SCFP on rumen fermentation variables. Callaway and Martin (1997) grew rumen bacteria on plates containing yeast culture and found that lactate utilizing bacteria produced more acetate and propionate. Miller-Webster (2002) used a continuous culture fermenter and fed TMR with or without SCFP for 10 days. Addition of *Saccharomyces cerevisiae* fermentation products significantly increased total volatile fatty acid (VFA) and propionate production and decreased acetate production. Dry matter digestion tended to increase with SCFP.

The benefits of feeding a yeast culture supplement on rumen fermentation are inconsistent among studies. Multiple studies found no difference in VFA concentrations between treatments (Hristov et al., 2010; Li et al., 2016; Plata et al., 1994; Roa et al., 1996; Yoon and Stern, 1996)

while other studies found a tendency for increased propionate or decreased acetate proportions in rumen fluid (Erasmus et al., 2005 and Harrison et al., 1988, respectively). While there are varying effects of SCFP on rumen pH and VFA concentrations, there is also evidence it stabilizes rumen pH. Harrison et al. (1988) and Li et al. (2016) ran homogeneity of variance tests and found that SCFP supplemented groups had reduced variation of rumen pH than non-supplemented groups, even though there was no difference in mean pH between groups. *Saccharomyces cerevisiae* fermentation products have also increased the production of propionate without decreasing rumen pH (Erasmus et al., 2005; tendency). Feeding SCFP may modulate the immune response and lead to a more stable, less variable rumen environment.

There are several possible mechanisms to ascertain why SCFP supplementation may modulate the immune response, and these mechanisms were found through multiple in vitro studies by Jensen et al. (2008). Addition of SCFP to several in vitro assays has protected cells from oxidative damage, reduced reactive oxygen species (ROS) formation in cells, activated natural killer cells, and increased B-cell activation. The protection from oxidative damage and reduction of ROS formation is possibly attributed to the ability of the antioxidants in SCFP to penetrate cells and neutralize oxidants within the cells. The activation of natural killer cells may be related to β-glucans from the yeast cell walls. Similarly, the activation of B-cells to produce antibody may also be due to the effects of β-glucans. Both results may be related to the role β-glucans play in activating the immune system from the GIT. These actions may be a result of the antioxidants, polyphenols, and β-glucans present in SCFP.

Table 1.1 A summary of effects of *Saccharomyces cerevisiae* fermentation products on *in vivo* rumen fermentation.

Study	Rumen pH[a]	VFA, molar proportion[a]	NDF digestibility[a]	Bacterial populations[a]
Allen and Ying 2012	-	-	No difference	-
Erasmus et al. 2005	No difference	↑ propionate[t]	-	-
Harrison et al. 1988	↓	↓ acetate ↑ propionate	No difference	↑ cellulolytic bacteria
Hippen et al., 2010	No difference	No difference	-	-
Hristov et al. 2010	No difference	No difference	No difference	-
Li et al. 2016	No difference	No difference	-	-
Plata et al. 1994	No difference	No difference	↑	↑protozoa
Roa et al. 1996	No difference	No difference	No difference	-
Yoon and Stern 1996	No difference	No difference	No difference	↑ proteolytic bacteria
Mullins et al. 2013	-	-	-	↑hemicellulose fermenter
Dias et al., 2018,b	↓ Min below pH<6.0	↓ acetate:propionate	No difference	-

[a]Effects of SCFP supplementation vs. no SCFP supplementation; All differences significant ($P \leq 0.05$) unless otherwise noted as a tendency([t]) where $P > 0.05 \leq 0.10$.

1.4.2.2 Chickens

Briefly, yeast culture products had positive effects on bacterial shedding and immune status of chickens, steers, and calves. Supplementation of yeast culture products reduced bacterial shedding in broilers (Feye et al. 2016b), feedlot steers (Feye et al. 2016a), and dairy calves (Magalhães et al. 2008). Antibiotic resistance and intestinal colonization of *Salmonella* was reduced in all three studies when a *Saccharomyces cerevisiae* fermentation product was fed.

Feye et al. (2016b) conducted a study on broiler chickens. One hundred fifty day old broiler chicks were enrolled and underwent a *Salmonella* challenge. Birds were orally inoculated with *Salmonella* on d 2 (2×10^8 CFU), 9 (4×10^8 CFU), and 16 (8×10^8 CFU). Following successful inoculation, birds were assigned to treatment or control group based on body weights and salmonella fecal load. Fecal samples were assessed for *Salmonella* load. Birds were euthanized on day 49 and a portion of the large intestine was dissected. Birds fed a *Saccharomyces cerevisiae* fermentation product experienced reduced *Salmonella* shedding in feces as well as reduced *Salmonella* load in the large intestine. This indicates that SCFP fed birds experienced reduced *Salmonella* attachment in the large intestine. Additionally, SCFP fed birds were 0.3 kg heavier on d49 of life, and the end of the study, than control birds (3.5 vs. 3.2 kg, respectfully).

1.4.2.3 Steers and Pre-weaned Calves

Feye et al. (2016a) conducted a study on feedlot heifers. Heifers (n = 1,495) were assigned to two groups upon arrival at a feedlot and fed an industry standard diet with or without SCFP. Heifers were slaughtered between 125 and 146 d of the study. There was a decrease in *Salmonella* load in feces and decreased lymph node infiltration of *Salmonella* in SCFP fed heifers, and a fewer number of heifers fed SCFP had *Salmonella* present in feces and lymph nodes. The virulence of *Salmonella* was significantly reduced in SCFP supplemented heifers. Supplementing SCFP reduced virulence and the ability of *Salmonella* to infiltrate intestinal tract cells.

Brewer et al. (2014) conducted a study on forty Holstein calves, 32 female and 8 male. Calves were assigned to a control group receiving only milk replacer or a treatment group receiving milk replacer with an SCFP additive and a gelatin capsule of SCFP administered daily. On day

14 of life calves were inoculated with *Salmonella* administered by gel capsule. Calves were fecal scored with a higher score referring to a greater extent of diarrhea, and *Salmonella* colonies were quantified from fecal samples. Calves were euthanized on day 21 post inoculation, and a portion of the distal ileum was removed to measure *Salmonella* colonization. Control calves had higher fecal scores and increased presence of diarrhea than treatment calves. Fecal shedding of *Salmonella* was significantly lower in treatment calves on day 6 post inoculation, and on day 3 post inoculation 56% of control calves and 10% of treatment calves were shedding *Salmonella*. *Salmonella* colonization the ilium of was significantly increased in control calves. Treatment calves had a higher percent growth from day 0 to day 35 of calf life than control (23.8% vs. 16.7%, respectively).

As shown in the three previous studies (Feye et al., 2016a,b; Brewer et al., 2014), administration of SCFP reduced pathogen shedding and load of *Salmonella* in feces. Reduced shedding reduces the potential for *Salmonella* infection in animals that come in contact with the shedding animal, as well as reduces risk of meat being contaminated by *Salmonella* at slaughter. *Salmonella* shedding was reduced by reduced adherence to the intestine (Feye et al., 2016b; Brewer et al., 2014). *Salmonella* were unable to adhere and thus multiply in the intestine. One possible explanation is that SCFP increases butyrate (Possemiers et al., 2013), which in turn reduces the virulence of *Salmonella* and its ability to adhere to the intestine (Durant et al. 2000).

1.4.2.4 Dairy Cows

There have been inconsistent reports about the effect of SCFP on DMI, milk yield, and milk components. Dann et al. (1999) reported an increase in DMI the week before calving as well as d 1 to 42 after calving when feeding SCFP. Longuski et al. (2008) fed a diet with a mix of dried ground corn and soybean meal with a top-dressed SCFP for 25 d, and conducted a high moisture corn challenge the last two days of the treatment period (d 26-28). There was no interaction between SCFP and dried ground corn. The high moisture corn challenge had negative effects on milk yield, dry matter intake, and fat yield for unsupplemented cows. Cows supplemented with SCFP increased dry matter intake, milk yield, and fat yield during the high moisture corn challenge. Hippen et al. (2009), Bruno et al. (2009), and Zhu et al. (2016) reported an increase in milk yield with no effect on DMI. Harris et al. (1990) and Wang et al. (2001a) reported an

increase of milk fat yield with no effect on DMI or milk yield. While there are inconsistent reports for the effects of yeast culture, a meta-analysis of 36 individual studies by Poppy et al. (2012) found an overall positive effect of yeast culture supplementation on energy corrected milk, milk fat yield, and early lactation DMI (1.65 kg/d, 0.06 kg/d, 0.62 kg/d for cows < 70 DIM, respectively).

Supplementation of a yeast culture product is shown to increase milk fat (Harris et al. 1990, Zhang et al. 2013, Wang et al. 2001a), but a response is most dramatic during a 'challenge' such as a HMC challenge or a SARA challenge. "Challenge diets" are a diet change towards a highly fermentable starch source such as high moisture corn (Longuski et al., 2009) or low fibre, high concentrate meal to induce SARA (Li et al. 2016, Dias et al., 2018a,b). Rather than increasing milk fat yield (kg), the yeast culture product prevented a drop in milk fat (Longuski et al., 2009) or had a less severe drop (Li et al., 2016). There are no differences in molar acetate concentration between treatments, which is a precursor for milk fat (Baumann and Griinari, 2003). This suggests that feeding a low fibre, high concentrate diet to drive milk production can be supplemented with SCFP to prevent milk fat depression.

Table 1.2 A summary of effects of *Saccharomyces cerevisiae* fermentation product supplementation on the milk fat response in dairy cows.

Study		Dietary NDF, %	Milk fat, %		FCM		Ruminal Acetate Concentration[g]	
			CON[e]	SCFP[f]	CON	SCFP	CON	SCFP
Allen and Ying 2012		28	3.49	3.59	34.8	36.1	80.8	82.2
Bruno et al. 2009		36	3.6	3.5	-	-	-	-
Erasmus et al. 2005		31	3.7	3.8	-	-	65.9	68
Harris et al. 1990		-	3.27	3.41*	29.9	31.4*	-	-
Hippen et al. 2010	DDG[a]	31	3.07	3.00	38.4	39.0	61.0	60.1
	No DDG	29	3.23	3.21	40.8	42.7	61.1	62.4
Hristov et al. 2010		31	3.48	3.27	-	-	69.2	68.4
Li et al. 2016		37.2	3.25	3.24	-	-	63.8	65.5
	SARA[b]	25.9	2.71	2.92[t]	-	-	55.2	54.5
Longuski et al. 2009		24.6	3.34	3.32	41.6	41.0	65.4	66.1
	HMC[c]	23.9	3.03	3.31[t]	39.8	43.0*	66.3	67.6
Wang et al. 2001a	FNDF[d] 17%	35	3.36	3.53	42.5	41.2	-	-
	FNDF 21%	30	3.33	3.48[t]	40.4	45.1	-	-
Zhang et al. 2013	Farm1	39	3.12	3.24	18.1	19.5*	-	-
	Farm2	29	2.79	3.11*	15.9	18.3*	-	-
Dias et al.,		38, 32.9	3.77	3.86	40.3	42.5*	75.3	80.3

2018a

[a]DDG = dried distiller's grains; [b] SARA = SARA challenge; [c]HMC = high moisture corn; [d] FNDF = forage NDF; [e]CON = no SCFP supplementation; [f]SCFP = SCFP supplementation; * significant difference, $p \leq 0.05$; [t] tendency, $0.05 \geq p \leq 0.10$; [g]molar proportion

The effect of SCFP on DMI, milk yield, and ruminal fermentation has been extensively study, but there is a lack of research into the effect of SCFP on the immune response in dairy cattle. Of studies evaluating the immune response, there has been little or no difference between supplemented and control groups on immune response indicators even when there were positive effects on production and health. A study by Magalhães et al. (2008) fed yeast culture in grain to calves d 1 to 70 from birth. There were no differences in grain intake and body weight throughout the experiment. An ovalbumin challenge at d3, 21, and 42 resulted in no difference in serum IgG optical density between groups. The only significant difference found was a reduction in mortality for SCFP supplemented calves. Zaworski et al. (2014) supplemented SCFP to dairy cows for 28 d pre and post calving. Supplemented cows had increased milk production (40.7 kg/d vs. 36.1), but immune response indicators such as Hp, IgG, IgA, and IgM were not affected by SCFP supplementation. Supplemented cows had lower concentrations of SAA one week before calving and higher concentrations one day post calving. While SCFP supplementation had no effect on adaptive immunity in calves (Magalhães et al., 2008), it modulated the immune response in transition dairy cows (Zaworski et al., 2014).

Zaworski et al. (2014) measured the effects of SCFP on the immune response during the transition period, and other studies have conducted an acidosis challenge to measure the effects of SCFP supplementation on the immune response to SARA. Because of the effects of SCFP supplementation on modulating rumen fermentation (Li et al., 2016; Erasmus et al., 2005; Harrison et al., 1988), SCFP can be expected to reduce the negative effects of the increase in rapidly fermentable carbohydrates on rumen fermentation (Longuski et al., 2009). Li et al. (2016) conducted a SARA challenge and found no difference in acute phase proteins or rumen LPS concentration during or following the challenge. However, Guo et al. (2017) also conducted a SARA challenge and found that SAA was not different between the time points before and during the challenge when cows were fed SCFP, while cows not supplemented with SCFP had

increased SAA during the challenge. These studies are evaluating SCFP during an experimentally induced challenge, but there is a lack of research into the effects of SCFP during the transition period.

1.5. Dietary Strategies During the Calving Transition

1.5.1 Close-up Period

During the close up period, the three weeks before calving6 , cows fed ad libitum for 150% of energy requirement had greater free fatty acid and BHB concentrations one week after calving (Janovick et al., 2011), and gene expression in liver, as suggested by the authors, showed a predisposition to fatty liver syndrome (Loor et al., 2006). Diets fed at (100%) or below (80%) requirement had better energy balance and decreased free fatty acid or BHB concentrations after calving than groups fed at 125 or 150% requirement (Janovick et al., 2011, Janovick and Drackley, 2010, Mann et al., 2015), or had no effect after calving (Dann et al., 2006, Salin et al., 2017, Winkelman et al., 2008). Cows fed at 150% of requirement pre-calving had reduced neutrophil activity than cows fed at 100% of requirement, indicating that overfed cows had reduced capability of their innate immune response (Graugnard et al., 2012). These results indicate that feeding a controlled energy close up diet that provides 100% of requirement has benefits on energy balance and immune function of the cow before and after calving.

1.5.2 Fresh Period

The aim of feeding strategies in the fresh period is to increase dry matter and energy intake in order to meet demands of milk production without compromising rumen and metabolic health. Several studies have explored feeding ‘high’ or ‘low’ starch diets for the first three weeks after calving. High starch ranged from 26.2-29.2 and low starch ranged from 17.8-21.5 (McCarthy et al., 2015a,b, Sun and Oba, 2014, Anderson et al., 2003). A high starch diet after calving led to better energy balance than a low starch diet with no differences in feed intake or milk yield (McCarthy et al., 2015b). High or low dietary starch content had no effect on milk yield, DMI, or energy balance (Rabelo et al., 2003). Anderson et al. (2003) fed diets differing in concentrate amount which resulted in diets with different starch contents found that the high concentrate diet increased DMI and milk yield. Barley grain was replaced with dried distillers grain (DDG) in order to reduce starch content, and the diet with DDG tended to increase free fatty acid

concentration after calving (Sun and Oba, 2014). Milk efficiency (Milk yield / DMI) was increased by a low starch diet (McCarthy et al., 2015a), but there were no milk yield differences between high or low starch diets fed in early lactation (McCarthy et al., 2015a, Sun and Oba, 2014). There was no difference in feed intake between high or low starch treatments for the first three weeks after calving (McCarthy et al., 2015a), but primiparous cows had greater feed intake when fed a low starch, greater NDF diet compared to a high starch, lower NDF diet (Sun and Oba, 2014). Yasui et al. (2016) found that a high starch diet fed after calving led to increased immune activation one week after calving. A high starch diet may lead to better energy balance after calving, but it has the potential to increase the risk of SARA and has been shown to negatively modulate the immune response.

1.6. Knowledge Gap

The response of certain aspects of the immune response to SCFP has not been studied; antioxidant status and the adaptive response. Additionally, the effect of starch on the adaptive response has not been explored. Important components of SCFP are vitamins and antioxidants, and there is a lack of research into the effects of SCFP supplementation on antioxidant status and oxidative stress in transition cows. Feeding antioxidants may have beneficial effects by reducing oxidative stress after calving (Bouwstra et al., 2008). An ovalbumin challenge measures the ability of the adaptive immune response to mount an antibody response. Ovalbumin challenges have been conducted during the transition period in previous research, but the effects of dietary starch content or SCFP supplementation on the adaptive immune response has not been explored. During the transition period cows move from a high forage, low concentrate diet to a low forage, high concentrate diet, and this change poses a challenge to the rumen environment. Feeding a low starch diet may reduce the impact of dietary changes, but the combination of a controlled energy close-up diet and a low starch fresh diet and the effect on digestibility needs to be explored. Supplementation of SCFP may reduce the impact of dietary changes on the rumen environment. *Saccharomyces cerevisiae* fermentation products have been fed with diets varying in starch content during the transition period, but there have not been studies into the interaction between dietary starch content and SCFP supplementation. There are positive effects of feeding a low starch diet after calving, and there are benefits to supplementing SCFP. There is the potential

that these two dietary strategies may interact to have a combined positive effect on dairy cow health and production after calving.

The overall hypothesis of this thesis is that supplementation of a *Saccharomyces cerevisiae* fermentation product and reducing starch content of the fresh diet will benefit the immune response and nutrient digestibility of transition dairy cows.

Chapter 2: The effects of decreasing dietary starch content and supplementation of a *Saccharomyces cerevisiae* fermentation product on apparent total tract nutrient digestibility in dairy cows during the first 3 weeks after calving

2.1 Introduction

Apparent total tract nutrient digestibility is an important measure to determine if a diet will be utilized more efficiently than another. Increasing apparent total tract nutrient digestibility may benefit the animal and producer by increasing the amount of nutrients absorbed from the diet, which may contribute to an increase in milk yield (Oba and Allen, 1999). Many studies have measured the effects of starch content on apparent total tract nutrient digestibility in mid-lactation, but few have measured apparent total tract nutrient digestibility in the fresh period. During the fresh period cows face reduced feed intake and dietary changes. It has become common to feed a low energy, high forage diet before calving in order to reduce metabolic disorders after calving (Janovick and Drackley, 2010; Janovick et al., 2011; Mann et al., 2015). However, a change from a high forage diet to a low forage, high concentrate diet poses a challenge to the rumen environment. It takes three to four weeks for the rumen to adapt to dietary changes (Bannick et al., 2012). A high concentrate diet has the potential to decrease pH and limit the growth and function of fibre utilizing bacteria (Zebeli et al., 2010, Shi and Weimer, 2002). During this adaptation period the rumen may not be able to handle the effects of a high concentrate diet on the rumen environment. A dietary strategy is to feed a low starch diet following calving to reduce the impact of dietary changes. Another dietary strategy is supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP). *Saccharomyces cerevisiae* fermentation products have positive effects on apparent total tract nutrient digestibility including increasing in situ neutral detergent fibre (NDF) digestibility and increasing apparent total tract dry matter (DM) digestibility (Plata et al., 1994; Allen and Ying, 2012). *Saccharomyces cerevisiae* fermentation products also reduce variability of pH and increase volatile fatty acid (VFA) production without decreasing pH (Erasmus et al., 2005; Li et al., 2016; Harrison et al., 1988). Feeding a low starch diet after calving may reduce the impact of post-calving dietary changes, and supplementation of SCFP may stabilize the changing rumen environment.

The hypothesis of this study is that decreasing dietary starch content and supplementing with SCFP will increase apparent total tract NDF, DM, organic matter (OM), and starch digestibility. Therefore the objective of this study was to evaluate the effects of a low starch diet and SCFP supplementation on apparent total tract OM, DM, NDF, and starch digestibility.

2.2 Materials and Methods

All experimental procedures used in this study were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP#1915) and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

2.2.1 Experimental Design, Animal Description, and Treatments

Thirty-eight Holstein dairy cows were assigned to one of two treatments at 28 d before expected calving date (CON: no SCFP supplementation, SCFP: SCFP supplementation). After calving cows were assigned to one of four treatments (LS: low starch content, HS: high starch content; CON+LS, SCFP+LS, CON+HS, SCFP+HS) in a 2 × 2 factorial arrangement of treatments. Treatments were balanced for parity and body condition score at dry off using a five-point scale (Table 2.1; Edmonson et al., 1989). Parity was not different between treatments, and averaged 1.97. Each treatment had 3 primiparous animals and 6-7 multiparous cows. Before calving, cows were fed a basal close up diet containing 13% starch and 1.43 Mcal/kg DM (Table 2.2 and 2.3). Feeding a low energy TMR ad libitum was accomplished by feeding 30% chopped barley straw to reduce the energy density of the diet. In the fresh diet, rolled barley was replaced with beet pulp to reduce the starch content for the low starch diet. Animals were housed in individual tie stalls and fed a total mixed ration (TMR) once daily at 0800 h accounting for 5 - 10% refusals. Cows were milked twice daily at 0330 and 1500 h. Animals were allowed outside to exercise three times a week for 3 h a day.

2.2.2 Apparent total tract nutrient digestibility

Apparent total tract digestibility of DM, OM, NDF, and starch was determined on week 1 and 3 after calving. Fecal grab samples were collected on d 7-9 and d 21-23 after calving every 9 h to obtain a representative sample accounting for every 3 h of a 24-h period. Fecal samples were

composited for each cow by combining approximately 100 g of feces from each time point. Samples of feed ingredients were collected weekly and were dried in a forced-air oven at 55°C for 48 h and feces for 72 h and ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas). Weekly feed ingredient samples were composed by load of feed delivered to the farm (barley silage, n = 9; concentrates, n = 3-6; alfalfa hay, n = 12) and analyzed for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), NDF (Van Soest et al., 1991), and starch (Hall, 2009), by Cumberland Valley Analytical Services (Hagerstown, MD).

Indigestible NDF was determined after 288 h of in situ digestion using a method adapted from Huhtanen et al. (1994), and used as an internal marker to estimate fecal output. Approximately 2 g of feed ingredients and feces were sealed in nitrogen-free 50-µm pore size nylon bags (R510, Ankom Technology, Macedon, NY) in quintuplicate. Nylon bags containing samples were placed in four small nylon laundry bags, placed into the rumen, and attached to cannula plug. The laundry bags were placed into the ventral sac of the rumen of a ruminally-cannulated dry cow fed a TMR containing 59.4% triticale silage, 25.5% barley straw, 4.24% rolled barley, 4.24% canola meal, and 5.94% barley malt sprouts with 50% dietary NDF content on a DM basis. Upon removal of the bags from cow, nylon bags were immediately placed in iced water and washed in cold water until water ran clear. Nylon bags were dried in a 55°C forced air oven for 24 h. Indigestible NDF of feed ingredients and feces was determined by boiling nylon bags in 300 mL neutral detergent solution (FND20C, Ankom Technology, Macedon, NY) with 0.5-g anhydrous sodium sulfite (Sigma-Aldrich, St. Louis, MO) per beaker for 1 h (Van Soest et al., 1991). Neutral detergent solution was poured out and bags were washed in hot water four times and then rinsed twice in acetone and dried in a fume hood for 12 h. Dried bags were placed into a 105° forced air oven for 12 h and hot weighed. Indigestible NDF fraction was determined by the following equation (all weights are dry matter):

((post incubation weight – nylon bag weight)/pre incubation sample weight) * 100.

Volatile fatty acid profile was measured using gas chromatography according to the method described by Khorasani et al. (1996). The pH measurement system was developed by Penner et

al. (2006). Rumen pH was measured every 30 s for a 3-d period in the ventral sac and daily values (minimum, mean, maximum, minutes pH < 5.8) were averaged for each period.

2.2.3 Calculations

Data was analyzed using the following equations. Indigestible NDF (iNDF) was used as a marker to determine fecal output:

iNDF intake (kg/d) /iNDF (%) of feces = fecal output (kg DM/d)

Apparent total-tract digestibility was calculated according to Maynard et al. (1979) as a percentage of intake. Apparent total tract nutrient digestibility of diets was determined by the following equation:

(nutrient intake (kg/d) – nutrient fecal output (kg/d)) / nutrient intake (kg/d) × 100 = apparent total tract nutrient digestibility (%)

2.2.4 Statistical Analysis

Data were analyzed using the FIT model procedure of JMP (version 13.1.0 SAS Institute Inc., Cary, North Carolina, USA) using the following model:

$$Y_{ij} = \mu + T_i + S_j + I_{ij} + e_{ij}$$

Where Y_{ij} is the dependent variable, μ is the overall mean, T_j is the fixed effect of SCFP supplementation, S_j is the fixed effect of starch content, I_{ij} is the interaction between SCFP supplementation and starch content, and e_{ij} is the residual. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

2.3 Results

There were no treatment effects of SCFP supplementation on apparent total tract DM, OM, or NDF digestibility (Table 2.4). There were no differences between starch or SCFP treatments in

apparent total tract OM or DM digestibility; however low starch diets increased apparent total tract NDF digestibility over high starch diets (40.7 vs. 35.3%, $P = 0.01$) on d 7.

There was an interaction between apparent total tract starch digestibility and parity ($P < 0.01$). A low starch diet increased apparent total tract starch digestibility (Table 2.5) for multiparous cows on d 7 (98.7 vs. 97.8%; $P = 0.01$) and d 21 (98.7 vs. 97.7%; $P < 0.01$). A low starch diet decreased apparent total tract starch digestibility for multiparous cows on d 7 (98.6 vs. 99.1%; $P = 0.04$) and d 21 (98.7 vs. 99.3%; $P = 0.04$).

In this study Shi et al. (2018b), measured daily mean rumen pH, maximum pH, minimum pH, duration under pH 5.8, acidosis index, and volatile fatty acid profile for 18 multiparous cows included in this data set. I tested correlations between apparent total tract NDF digestibility and these measures. On d 7, there were no correlations ($P > 0.05$) between apparent total tract NDF digestibility and rumen fermentation variables measured in the study, including mean rumen pH ($P = 0.90$, Figure 2.1), molar proportion of acetate in rumen fluid ($P = 0.22$, Figure 2.2), and molar proportion of propionate in rumen fluid ($P = 0.36$, Figure 2.3). On d 21 apparent total tract neutral detergent fibre digestibility was positively correlated to molar proportion of acetate in rumen fluid (r = 0.49; $P = 0.04$, Figure 2.4). I also tested correlations between apparent neutral detergent fibre digestibility and DMI for all cows on the current study (n = 38). Apparent total tract NDF digestibility on d 7 was not correlated with DMI ($P > 0.05$), but apparent total tract NDF digestibility on d 21 was positively correlated to DMI (r = 0.40; $P = 0.01$, Figure 2.5).

2.4 Discussion

2.4.1 Effects of SCFP

Saccharomyces cerevisiae fermentation product supplementation may increase mean pH (Plata et al., 1994), decrease time < pH 6.0 (Dias et al., 2018b), increase propionate concentration without increasing pH (Erasmus et al., 2005), and reduce variability of pH (Harrison et al., 1988; Li et al., 2016). By modulating the rumen environment in this way SCFP supplementation may allow for more rumen fermentation without a decrease in pH, and may increase nutrient digestibility. *Saccharomyces cerevisiae* fermentation product supplementation has increased the amount of cellulolytic bacteria (Harrison et al., 1988) and hemicellulose fermenters (Mullins et al., 2013).

Therefore, I expected SCFP to have a positive effect on apparent total tract nutrient digestibility. However, in this study SCFP had little effect on apparent total tract nutrient digestibility. There are several factors that affect nutrient digestibility, and among them are the rumen microbiome, pH, and physically effective NDF (peNDF). Rumen microbes and pH often interact; most fibre digesting microbes are most efficient at pH above 6.0, and digestion of fibre ceases at a pH of 5.3 (Russell et al., 2009). Physically effective NDF is the physical characteristics of fibre and its effects on chewing activity and rumination (Mertens, 1997). Increased peNDF has positive effects on NDF digestibility, and tended to increase total tract NDF digestibility over diets containing less peNDF (Yang and Beauchemin, 2005).

Plata et al. (1994) found an increase in in situ NDF digestibility due to SCFP supplementation, but found no difference in pH between diets. They reported an increase in NDF digestibility due to an increase in ruminal protozoa in the SCFP supplemented diets. A unique factor in the Plata et al. (1994) study was that diets contained a high percentage of oat straw to measure the effect of SCFP on low quality forages, and also had high peNDF. In the current study we did not measure rumen bacteria populations, and pH was not affected by starch or SCFP treatment (Shi et al., 2018b). Plata et al. (1994) is not comparable to the current study because I fed diets without wheat straw after calving. The diets I fed after calving would not have peNDF as high as Plata et al. (1994) because of the lack of wheat straw.

The results of Hristov et al. (2010) are comparable to results in the current study: they found no difference in pH or nutrient digestibility due to SCFP supplementation, and the pH and apparent total tract NDF digestibility % was similar to the low starch diets in the current study. In a study that found differences in pH due to SCFP supplementation, there were no differences between diets in nutrient digestibility. Dias et al. (2018b) observed an increase in mean daily pH when supplementing a high starch diet with SCFP, but found no difference in nutrient digestibility due to the addition of SCFP. *Saccharomyces cerevisiae* fermentation products have positive effects on nutrient digestibility, but these results occurred in studies that are not comparable to the current study.

2.4.2 Effects of dietary starch content

2.4.2.1 Apparent Total Tract Starch Digestibility

Even though there is a 5 point difference in starch content between low starch and high starch diets (21.6%, vs. 27%, respectively), I did not expect to see a difference in apparent total tract starch digestibility due to starch content of diets. The effect of starch content on starch digestibility varies from study to study, and may be dependent on factors other than starch content. Oba and Allen (2003) found that a high starch diet increased starch digestibility, while Gencoglu et al. (2010) and Fredin et al. (2015) found that low starch diets increased starch digestibility. Oba and Allen (2003a), Gencoglu et al. (2010), and Fredin et al. (2015) fed diets differing in starch content and NDF content such that low starch diets contained more NDF on a DM basis than high starch diets. All diets were corn and alfalfa silage based and fed dried ground corn grain. Starch content, NDF content, and dietary ingredients may have been the reason for differences in starch digestibility in these studies. Physical characteristics of diets are more responsible for differences in digestibility and passage rates rather than chemical characteristics of diets (Oba and Allen, 2003a). While there were differences in apparent total tract starch digestibility between treatments and parity interactions were significant, these differences are small and are unlikely to have biological implications.

2.4.2.2 Apparent Total Tract NDF Digestibility

I had expected to see a higher pH due to the low starch diet, but there was no difference in pH between treatments (Shi et al., 2018b). There was also no relationship between mean pH and apparent total tract NDF digestibility on d 7 (Figure 2.1). In theory, NDF digestibility and pH should be related because of the effect of pH on fibre digestion. Function of bacteria and cellulose degradation slows under pH 6.0 and ceases at pH 5.3 (Russell et al., 2009). Studies that measure pH and nutrient digestibility that report an increase in NDF digestibility due to starch content do not report an increase in pH (Fredin et al., 2015). An exception to this is Dias et al. (2018b). They reported an increase in daily mean pH due to a low starch diet (compared to a high starch diet) and also found increased apparent total tract NDF digestibility due to the low starch diet. It is important to note that in Dias et al. (2018b) NDF content was different between low starch and high starch diets, and this cannot be ruled out as a contributing factor to the differences in NDF digestibility between diets. Physical characteristics of diets were different;

diets differed in corn silage content (39.1 vs. 32.7). Physical characteristics of diet are responsible for digestion and passage rates, which has an effect on digestion and digestibility of the diets (Oba and Allen, 2003a). In the current study physical characteristics of diets, such as forage NDF content, NDF content, and particle size were similar, and were not a factor contributing to the increase in apparent total tract NDF digestibility.

On d 21, apparent total tract NDF digestibility was positively related to molar acetate proportion (Figure 2.4). Digestion of fibre results in the production of acetate (Sutton et al., 2003), therefore it is expected that NDF digestibility be related to molar proportion of acetate. Also on d 21, apparent total tract NDF digestibility was positively related to dry matter intake (Figure 2.5). As DMI increases, nutrient digestibility is typically reduced (de Souza et al., 2018). With increasing rumen fill, passage rate also increases (Dado and Allen, 1995), reducing the time feedstuffs spends in the rumen and therefore reducing digestion. However, increasing in vitro nutrient digestibility may also decrease rumen fill (Oba and Allen, 1999), and may allow for greater DMI, which may have happened in the current study.

I had expected a shift in ruminal fermentation between diets differing in starch content the first week after calving. The time period for ruminal adaptation to a diet is 21-28 days (Bannick et al., 2012). The change from the pre-partum to post-partum diets was less drastic for the low starch diet. Barley silage content of the diets were similar, but the high starch diet increased rolled barley from 7.46 % of DM to 18.1 % of DM, while the low starch diet increased to 8.92% of DM. The risk of low pH and change in rumen fermentation increases with increasing amount of rapidly fermentable carbohydrate in the diet (Dijkstra et al., 2012), and a decrease in pH reduces the growth of fibrolytic bacteria, bacteria responsible for the digestion of fibre (Shi and Weimer, 2002). An experiment measuring in situ NDF degradation before and during a SARA challenge found reduced fibre degradation due to the episode of SARA (Krajcarski-Hunt et al., 2002). In the current study there was no difference in pH (Shi et al., 2018b), and there was no relationship between apparent total tract NDF digestibility and mean pH on d 7 ($P = 0.90$, Figure 1). Additionally, there were no differences in VFA profile in the rumen between treatments (Shi et al., 2018b), and there were no relationships between apparent total tract NDF digestibility and acetate ($P = 0.22$, Figure 2) or propionate ($P = 0.36$, Figure 3) on d 7. In the current study

apparent total tract NDF digestibility results cannot be explained by rumen fermentation variables such as pH or VFA production.

Beet pulp was used to replace rolled barley in order to reduce the starch content and keep NDF content similar between low starch and high starch diets. The NDF in beet pulp is digested faster than forage NDF (Bhatti and Firkins, 1995). Voelker and Allen (2003) conducted a study that measured the effect of replacing highly digestible high moisture corn with beet pulp at 0, 6, 12, or 24 % inclusion rate. Beet pulp significantly increased total tract NDF digestibility; the effects were also linear. The authors suggest that the increased digestion rate of pdNDF will increase the digestion of NDF from sources other than beet pulp. There were no differences in apparent total tract digestibility of starch, OM, or DM. In the current study, beet pulp contributed to the differences in apparent total tract NDF digestibility between low and high starch diets on d 7 after calving.

There was no effect of dietary starch content on apparent total tract NDF digestibility at d 21 after calving. As has been discussed, it takes 21-28 d for the rumen to adapt to the fresh diet after calving, and the low starch diet may have been more similar to the close-up diet than the high starch diet. While there were no differences in rumen fermentation variables d 7 after calving (Shi et al., 2018b), increased apparent total tract NDF digestibility is an indicator of a difference in rumen fermentation between high starch and low starch diets one week after calving. Therefore, lack of significant difference in apparent total tract nutrient digestibility between low and high starch diets d 21 after calving suggests that by d 21 animals fed high starch diets were adapted to the diet.

2.5 Conclusion

There was no effect of dietary starch content or SCFP supplementation on apparent total tract OM or DM digestibility. Similarly, SCFP had little effect on apparent total tract starch digestibility, and differences in apparent total tract starch digestibility due to dietary starch content are not biologically relevant. Apparent total tract NDF digestibility was increased on d 7 by feeding a low starch diet, and SCFP had no effect on apparent total tract NDF digestibility. The inclusion of beet pulp in the low starch diets contributed to the increase in apparent total

tract NDF digestibility on d 7. A low starch diet has the potential to increase total tract NDF digestibility.

Table 2.1 Animal information at enrollment (28 days before expected calving date).

Item		Treatments			
		CON+LS	SCFP+LS	CON+HS	SCFP+HS
No. of animals		9	10	10	9
	Primiparous	3	3	3	3
	Multiparous	6	7	7	6
Average parity		2.00	2.00	1.90	1.89
Body Condition Score		3.31	3.25	3.35	3.29

Table 2.2 Feed ingredients of experimental diets.

Ingredient, % DM	Prepartum		Postpartum			
			Low starch		High starch	
	CON	SCFP	CON	SCFP	CON	SCFP
Barley silage	46.9	46.9	46.5	46.5	46.5	46.5
Alfalfa hay	—	—	2.77	2.77	2.77	2.77
Barley straw	29.1	29.1	—	—	—	—
Barley rolled	7.46	7.46	8.92	8.92	18.1	18.1
Corn gain, ground, dry	—	—	12.5	12.5	12.5	12.5
Canola meal mech. Extract	8.19	8.22	10.2	10.2	7.96	7.96
Soybean meal, Sol 44% CP	—	—	3.00	3.00	3.00	3.00
Corn gluten meal, dried	0.45	0.45	0.85	0.85	2.00	2.00
Amino Plus	1.81	1.81	2.00	2.00	2.00	2.00
Beet pulp	—	—	8.34	8.34	—	—
F-100, Dairy fat	—	—	1.10	1.10	1.10	1.10
Magnesium Oxide	0.35	0.34	0.18	0.18	0.18	0.18
Limestone	0.72	0.7	1.38	1.38	1.60	1.60
Salt	0.14	0.14	0.48	0.48	0.48	0.48
Magnesium Sulfate (H2O)	0.59	0.59	—	—	—	—
Potassium carbonate	—	—	—	—	0.09	0.09
Calcium Chloride	0.43	0.43	—	—	—	—
Calcium Sulfate	0.48	0.48	—	—	—	—
Vitamin premix	0.05	0.05	0.02	0.02	0.02	0.02
Trace mineral premix	0.01	0.01	0.01	0.01	0.01	0.01
Selenium	0.006	0.006	0.004	0.004	0.006	0.006
Nutri A-Z C	0.1	0.1	0.05	0.05	0.05	0.05
Biotin 2%	—	—	0.005	0.005	0.005	0.005

Zinpro 4 plex C	—	—	0.03	0.03	0.03	0.03
NutriTek Premix CON[1]	1.92		1.06	—	1.06	—
NutriTek Premix TRT[2]	—	1.92	—	1.06	—	1.06
Rumensin premix[3]	0.8	0.8	0.58	0.58	0.58	0.58
Urea	0.5	0.5	—	—	—	—

[1]Contains 99.0% of dry, ground corn and 1.0% of canola oil, providing 0 g/d of NutriTek.

[2]Contains 7.9% of NutriTek, 91.1% of dry, ground corn, and 1% of canola oil, providing 19 g/d of NutriTek based on expected dry matter intake of the cow (11kg/day prepartum; 20 kg/d postpartum).

[3]Contains 1.21% of Rumensin, 97.54% of dry, ground barley, and 1.25% of canola oil, providing 242 mg of monensin sodium activity for close-up cows at the amount of 22 mg/kg monensin sodium total diet dry matter and 320 mg of monensin sodium activity for lactating cows at the amount of 16 mg/kg monensin sodium total diet dry matter.

Table 2.3 Nutrient composition of experimental diets.

Nutrient composition, DM basis	Prepartum		Postpartum			
	CON	SCFP	Low starch		High starch	
			CON	SCFP	CON	SCFP
NDF, %	49.5	49.4	33.3	32.7	31.7	31.5
Forage NDF, %	45.1	45.1	24.0	24.0	24.0	24.0
ADF, %	31.6	31.5	20.9	20.3	19.3	19.2
NFC, %	25.6	25.6	39.3	41.3	41.1	42.8
CP, %	15.3	15.3	17.3	17.0	17.5	16.9
Starch, %	13.8	13.9	21.6	22.5	27.0	29.5
Fat, %	2.5	2.5	3.6	3.6	3.7	3.7
TDN, %	61.0	61.0	71.0	72.0	72.0	73.0
NE_L, Mcal/kg DM	1.43	1.43	1.60	1.62	1.63	1.64
Calcium, %	0.94	0.92	1.09	0.95	1.10	0.73
Phosphorus, %	0.33	0.33	0.42	0.42	0.43	0.42
Magnesium, %	0.43	0.43	0.33	0.31	0.30	0.28
Potassium, %	1.69	1.69	1.48	1.46	1.47	1.44
Sodium, %	0.19	0.19	0.32	0.28	0.29	0.24
Chloride, %	0.86	0.86	0.6	0.62	0.62	0.62
Sulfur, %	0.40	0.40	0.25	0.25	0.26	0.26
Cobalt, ppm	0.55	0.59	1.89	1.89	1.92	1.92
Copper, ppm	12.1	12.0	21.2	19.7	28.1	16.7
Iodine, ppm	0.59	0.59	0.66	0.66	0.66	0.66
Manganese, ppm	39.6	40.1	48.5	44.0	44.3	40.5
Selenium, ppm	0.27	0.27	0.27	0.26	0.28	0.28
Zinc, ppm	44.7	53.3	80.1	72.1	91.6	69.6

Vitamin A, 1000 IU/kg	30	30	10.6	10.6	10.6	10.6
Vitamin D, 1000 IU/kg	3.0	3.0	1.1	1.1	1.1	1.1
Vitamin E, IU/kg	99.8	99.8	35.1	35.1	35.1	35.1
DCAD, mEQ/kg	26	25	192	164	170	138
DM, %	50.2	50.2	50.2	49.9	50.2	50.1

Table 2.4 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and reducing dietary starch content on apparent total tract dry matter (DM), organic matter (OM), and neutral detergent fibre (NDF) digestibility d 7 and 21 after calving.

		Treatments[1]					*P*-value[2]		
		Low Starch		High Starch		SEM	S	SCFP	S*SCFP
Week	Item, %	CON	SCFP	CON	SCFP				
wk 1	DM	65.2	64.8	63.7	65.3	1.21	0.66	0.61	0.40
	OM	67.4	66.9	65.7	67.4	1.16	0.61	0.62	0.37
	NDF	41.9	39.4	33.7	36.8	2.06	0.01	0.89	0.18
wk 3	DM	65.3	64.4	64.8	65.5	1.39	0.83	0.96	0.57
	OM	66.9	66.6	66.6	67.5	1.28	0.81	0.82	0.61
	NDF	40.2	38.9	36.4	37.8	2.25	0.27	0.97	0.55

[1]Treatments: CON = TMR without SCFP; SCFP = TMR with SCFP; Low = low starch TMR; High = high starch TMR

[2]S = Starch content; SCFP = SCFP supplementation; S*SCFP = Interaction between starch and SCFP

Table 2.5 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and reducing dietary starch content on apparent total tract starch digestibility and parity d 7 and 21 after calving.

		Treatments[1]					*P*-value[2]		
		Low Starch		High Starch		SEM	S	SCFP	S*SCFP
Day, ± 3	Parity	CON	SCFP	CON	SCFP				
d 7	1	98.4	98.9	99.0	99.1	0.18	0.04	0.13	0.24
	2+	98.8[a]	98.5[a]	97.3[b]	98.3[a]	0.32	0.01	0.35	0.06
d 21	1	98.8	98.5	99.2	99.4	0.26	0.04	0.78	0.31
	2+	98.9	98.6	97.4	97.9	0.31	0.003	0.71	0.19

[1]Treatments: CON = TMR without SCFP; SCFP = TMR with SCFP; Low = low starch TMR; High = high starch TMR

[2]S = Starch content; SCFP = SCFP supplementation; S*SCFP = Interaction between starch and SCFP

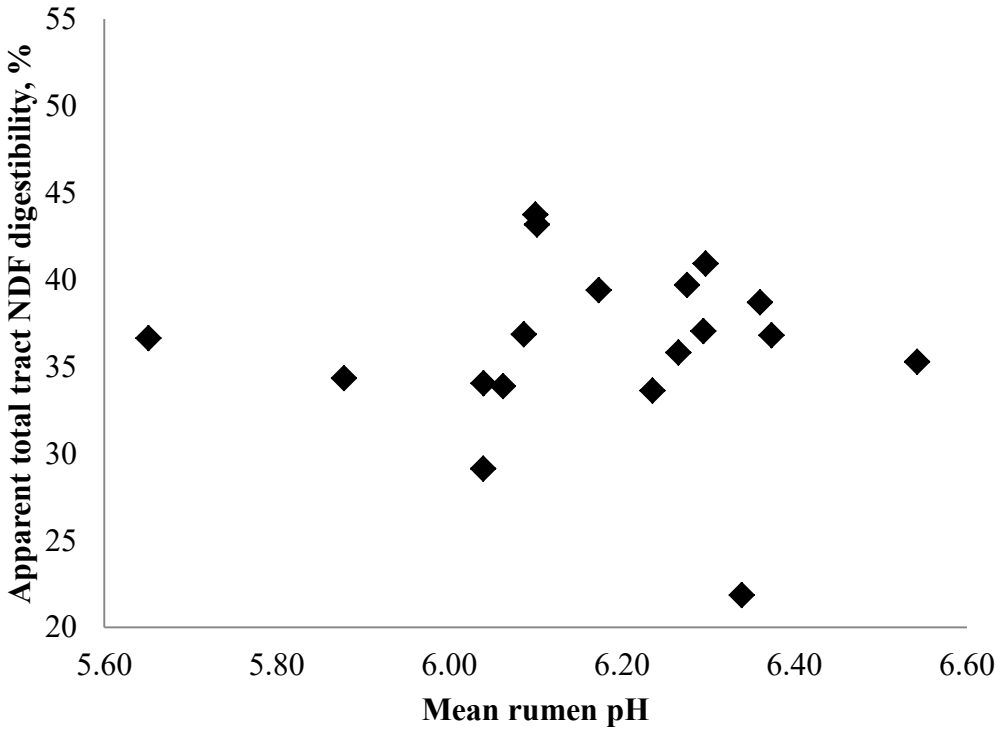

Figure 2.1 The relationship between apparent total tract neutral detergent fibre (NDF) digestibility and mean rumen pH on d 7 after calving, $P = 0.90$, r = 0.03.

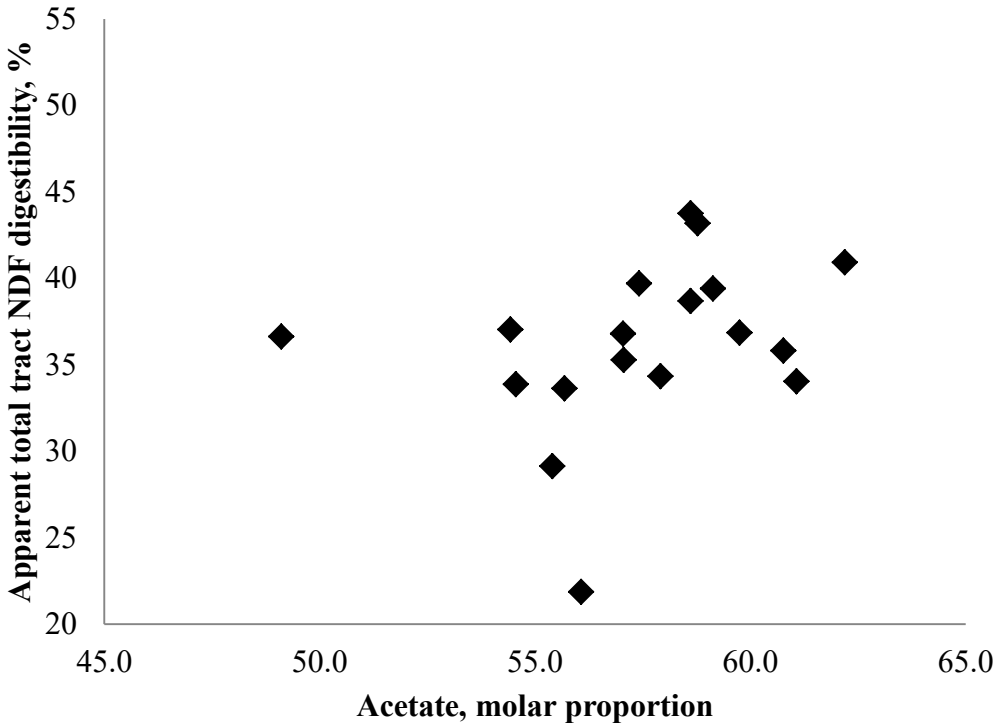

Figure 2.2 The relationship between apparent total tract neutral detergent fibre (NDF) digestibility and molar proportion of acetate on d 7 after calving, $P = 0.22$, r = 0.31.

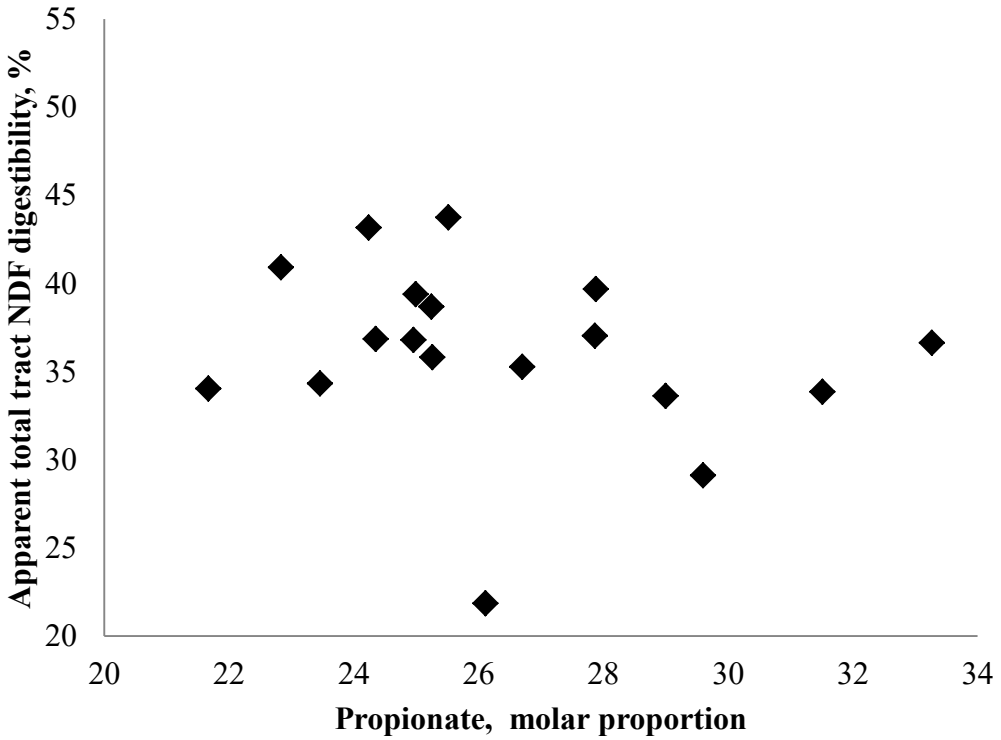

Figure 2.3 The relationship between apparent total tract neutral detergent fibre (NDF) digestibility and molar proportion of propionate on d 7 after calving, $P = 0.36$, r = 0.23.

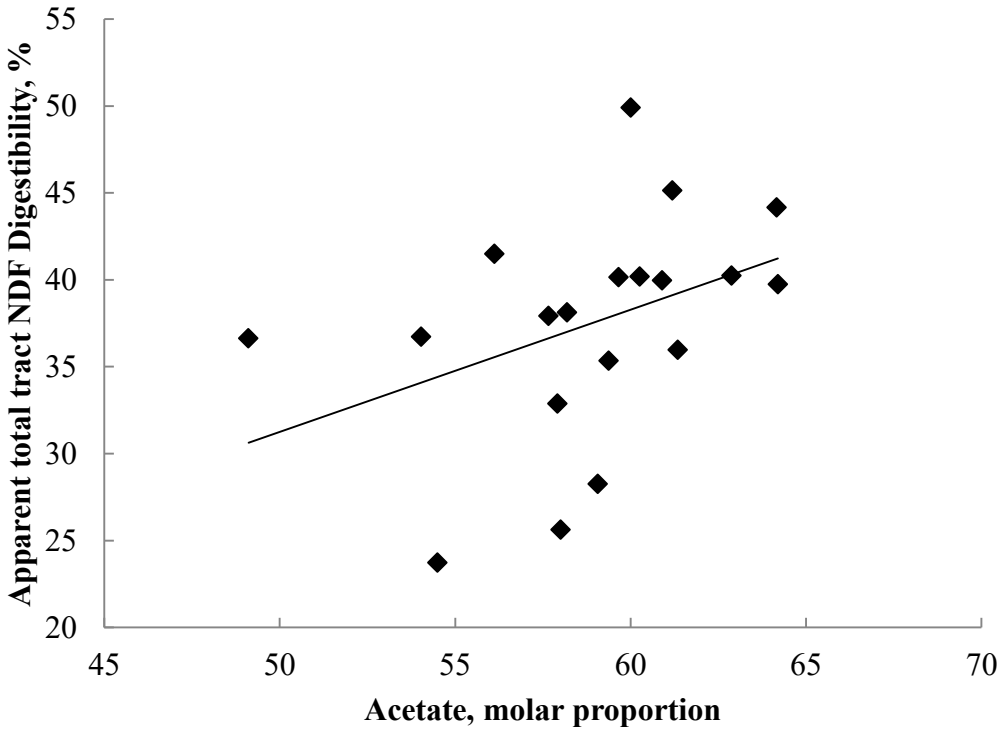

Figure 2.4 The relationship between apparent total tract neutral detergent fibre (NDF) digestibility and molar proportion of acetate on d 21 after calving, $P = 0.04$, r = 0.42.

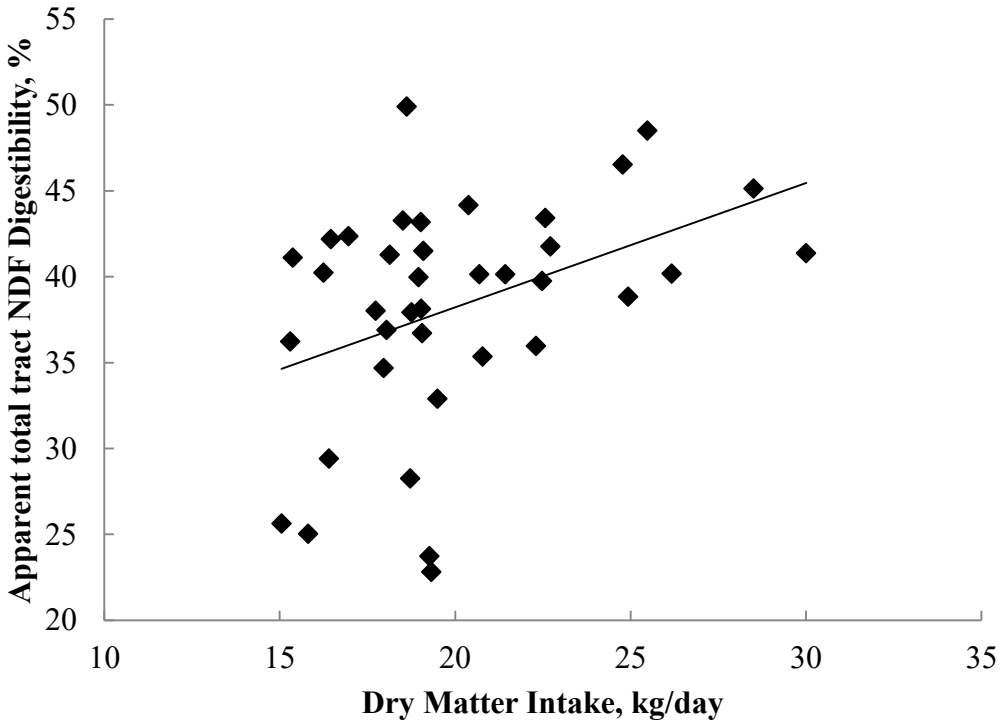

Figure 2.5 The relationship between apparent total tract neutral detergent fibre (NDF) digestibility with dry matter intake (kg/day) on d 21 after calving, $P = 0.01$, r = 0.40.

Chapter 3: The effects of reducing dietary starch content and supplementation of a *Saccharomyces cerevisiae* fermentation product on the immune response during the transition period in dairy cows

3.1 Introduction

During the transition period cows face depressed immunity and an inability to initiate a proper immune response (Mallard et al., 1998). An adequate immune response is necessary to defend against harmful pathogens that the animal faces after calving, such as bacterial infiltration into the uterus and mammary gland (LeBlanc, 2008; Mulligan and Doherty, 2008). Inflammation is an important physiological process during the transition period (Bradford et al., 2015). The onset of inflammation is critical to initiate parturition and expel the placenta (van Engelen et al., 2009; Boro et al., 2014). It is important that the animal initiate inflammation, but resolving the inflammation is also critical (Bradford et al., 2015). Continued unchecked inflammation has detrimental effects on cow health and production. Multiple studies have noted an association between inflammation and a reduction in milk yield (Bertoni et al., 2008; Bionaz et al., 2007; Yuan et al., 2013). Feeding a low energy, high forage diet before calving has been shown to improve metabolic status of the fresh cow by preventing an increase in body condition during the dry period (Janovick and Drackley, 2010; Janovick et al., 2011; Mann et al., 2015). However, a change from a high forage close-up diet to a high concentrate fresh diet increases the risk of sub-acute rumen acidosis (SARA; Zebeli et al., 2010). Subacute rumen acidosis is defined as periods of rumen pH less than 5.8 for three or more hours a day (Plaizier et al., 2009). Approximately 20% of transition dairy cows experience SARA due to the shift from a high forage, low concentrate close-up diet to a low forage, high concentrate fresh diet (Plaizier et al., 2009). Subacute rumen acidosis is associated with activation of the immune system, tight junction failure, and an increase in acute phase proteins serum amyloid-A (SAA) and haptoglobin (Hp) (Plaizier et al., 2009).

A *Saccharomyces cerevisiae* fermentation product (SCFP) is a dried feed product that contains multiple vitamins and antioxidants (Jensen et al., 2008). In addition to the antioxidants and vitamins found in SCFP, yeast cell wall components such as β-glucans may be present. β-glucans have been shown to modulate the immune response in humans as well as animals by priming the innate and adaptive immune response through activation of immune cells (Volman et

al., 2008; Li et al., 2005). In vitro, SCFP supplementation increased activation of the innate and adaptive immune response (Jensen et al., 2008). During the transition period it is important that the cow is able to mount an immune response before and immediately after parturition. Zaworski et al. (2014) found that SCFP supplementation decreased serum concentrations of serum amyloid-A (SAA) one day before calving and increased serum concentrations of SAA one day after calving, but was not different from control at other time points after calving. This suggests that SCFP was able to prime the immune response when it was beneficial to the animal. Milk production was also increased in SCFP supplemented cows, so there were no apparent negative effects of the enhanced immune response on production. Oxidative stress, the imbalance between oxidants and antioxidants, is common during the transition period and can have detrimental effects on cow health (Bernabucci et al., 2005; Bouwstra et al., 2008). The effects of the vitamins and antioxidants in SCFP have been explored in vitro, but the effects on the oxidative status of the animal have not been explored.

An additional proposed benefit of SCFP supplementation is the modulation of rumen fermentation to reduce the risk of SARA. *Saccharomyces cerevisiae* fermentation products reduce variability of pH and increase volatile fatty acid (VFA) production without decreasing pH, reducing the risk of developing SARA (Erasmus et al., 2005; Li et al., 2016; Harrison et al., 1988). There is work investigating the effect of SCFP supplementation of the incidence of SARA, but none has investigated the effect of SCFP supplementation on rumen tight junctions and immune activation in the rumen.

Feeding a low starch diet may reduce the incidence of SARA by reducing the impact of the dietary change, and reduce tight junction failure, immune system activation and an acute phase response (Zebeli et al., 2010; Plaizier et al., 2009). However, effects of dietary starch content on tight junctions and the immune system have not been extensively studied. Of the few studies that evaluated the effect of dietary starch content on the immune response, Yasui et al. (2016) found that feeding a low starch fresh diet reduced activation of macrophages at d 8 post calving. This effect was beneficial because between low and high starch diets there were no differences in incidence of cytological endometritis, which was the focus of the study. There were no

confounding effects of health events on immune activation. Additionally, a low starch diet fed after calving reduced serum Hp concentrations (McCarthy et al., 2015a).

During the transition period cows face not only a depressed innate immune system, but also a decreased activity of the adaptive immune system (Herr et al., 2011). It is important to increase the cow's ability to defend against pathogens. *Saccharomyces cerevisiae* fermentation products have increased B-cell activation in vitro and may have positive effects on the in vivo response to a challenge (Jensen et al., 2008). However, the benefits of reducing dietary starch content on the adaptive response in not known. There are multiple suggested benefits of SCFP supplementation and dietary starch content on the immune response during the transition period, but an interaction between these two factors needs to be explored. The hypothesis for this study is that reducing dietary starch content and supplementing with SCFP will reduce immune system activation after calving. The objectives of this study were to evaluate the effects of a low starch diet and SCFP supplementation on gene expression in rumen papillae, the acute phase response, oxidative status, and adaptive immunity in transition dairy cows.

3.2 Material and Methods

All experimental procedures used in this study were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP#1915) and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

3.2.1 Experimental Design, Animal Description, and Treatments

Thirty-eight Holstein dairy cows were enrolled into the study at 28 d ± 3 before expected calving date and were assigned to one of two treatments (CON: no SCFP supplementation, SCFP: SCFP supplementation). Treatments were balanced for parity and BCS at dry off (Table 3.1). Body condition score was determined on a 5-point scale (Edmonson et al., 1989). There was no difference in parity between treatments, and parity averaged 1.97. There were 3 primiparous animals per treatments and 6-7 multiparous cows per treatment. After calving cows were assigned to one of four treatments (LS: low starch content, HS: high starch content; CON+LS, SCFP+LS, CON+HS, SCFP+HS) in a 2 × 2 factorial arrangement of treatments. Animals continued with the same SCFP supplementation. From three weeks after calving to the end of the

experiment at six weeks after calving all cows received the high starch diet, continuing with the same SCFP supplementation. Before calving, cows were fed a basal close up diet containing 13% starch and 1.43 Mcal/kg DM (Table 3.2 and 3.3). Animals were fed the low energy TMR ad libitum by feeding 30% chopped barley straw to reduce energy density of the diet. After calving, rolled barley was replaced with beet pulp to reduce the starch content of the low starch diet while keeping NDF content similar. Animals were housed in individual tie stalls bedded with wood shaving and fed a TMR once daily at 0800 h accounting for 5 - 10% refusals. Cows were milked twice daily at 0330 and 1500 h. Animals were allowed to exercise outside three times a week for 3 h a day. Cows were monitored by staff and researchers daily and health events were recorded for the following: retained placenta (fetal membranes retained for greater than 24 h after calving), metritis (purulent vaginal discharge), mastitis (abnormal milk when fore stripping before milking; swollen, hot, and/or hard quarters), and displaced abomasum (LDA; diagnosed and surgically treated by veterinarian).

3.2.2 Plasma and Serum Sampling and Analysis

Approximately 15 mL of blood was taken from the coccygeal vessels on d -10 relative to expected calving date, on d 1 within twelve hours after calving, and on d 7, 21, and 42, ± 3 relative to calving date into tubes containing sodium heparin (Fisher Scientific Company, Nepean, ON, Canada) and a glass tube. Tubes containing sodium heparin were placed on ice and spun immediately after collection. Glass tubes were allowed to sit at room temperature for 2 h to coagulate. All samples were spun at 3,000 × *g* at 4 °C for 20 min. Plasma and serum samples were stored at -20 °C until time of analyses. Plasma samples were analyzed using commercial kits for concentrations of reactive oxygen metabolites (ROM; d-ROMs Test, MO003, Diacron Labs, Grosseto, Italy) and malondialdehyde (MDA; TBARS (TCA Method) Assay Kit, Item No. 700870, Cayman Chemical, Ann Arbor, Michigan, USA), and total antioxidant capacity (TOAC; Antioxidant Assay Kit, Item No. 709001, Cayman Chemical, Ann Arbor, Michigan, USA). Serum samples were analyzed using commercial kits for concentrations of serum amyloid A (SAA; Multispecies SAA ELISA kit, Catalog No. TP 802, Tridelta Development Ltd., Maynooth, Ireland) and haptoglobin (Hp; PHASE Haptoglobin Assay, Catalog NO. TP 801, Tridelta Development Ltd., Maynooth, Ireland).

3.2.3 Ovalbumin Challenge

An ovalbumin challenge was conducted on d 7 and 21, ± 3 relative to calving date. Cows were injected with 1 mL of an ovalbumin solution consisting of 1 mg ovalbumin (Cat. No. A5503, Sigma-Aldrich, Oakville, Ontario, Canada) and 0.25 mg of a Quil-A vaccine adjuvant (Brenntag, Frederikssund, Denmark) per 1 mL of phosphate-buffered saline (PBS; Cat. No. P3813, Sigma-Aldrich, Oakville, Ontario, Canada). Approximately 5 mL of blood was taken into glass tube on d 7 and 21 before the challenge, and on d 28 and 35. Tubes were allowed to sit at room temperature for 2 h to coagulate and spun at 3,000 × *g* for 20 min. Serum samples were stored at -20 °C until analysis. Serum samples were analyzed for IgG concentration using a commercial kit (Bethyl IgG ELISA Kit, Cat. No. E11-118, Bethyl Laboratories, Inc., Montgomery, Texas, USA).

3.2.4 Rumen Papillae Sampling

Rumen papillae samples were taken on d -10, ± 3 relative to expected calving date, and on d 21, ± 3 relative to calving date. Rumen was partially emptied through the rumen cannula into double walled trashcans to keep contents from cooling. The ventral sac was pulled through the cannula, and approximately 20 mg of papillae were cut from rumen epithelium and immediately placed into ice-cold 0.01 M PBS and rinsed three times to dislodge rumen content and placed into RNA*later* solution (Cat. No. AM7020, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Tubes were placed into -20 °C until analysis. Papillae were analyzed for mRNA abundance.

3.2.5 RNA Extraction

To extract RNA, papillae were cut into small pieces and homogenized with beads (CK 14 Precellys ® lysine) and 1 mL of TRIzol Reagent (Cat. No. 15596026, ThermoFisher, Waltham, Massachusetts, USA) using a Precyllys® 24 homogenizer (Bertin Technologies, Montigny, France) using two cycles of 30 s at 6200 rpm with a 10 s pause between cycles. Following homogenization, chloroform was added to separate RNA from DNA and protein. A high salt solution (1.2M NaAc, 0.8M NaCl) was added to precipitate RNA, and pellet was washed with 75% ethanol. Pellet was solubilized in nuclease-free water and 3M NaAc and 100% ethanol were

added to tube and allowed to incubate overnight at -20°C. The following day the RNA was centrifuged to create a pellet and washed in 75% ethanol twice to remove salt. The pellet was allowed to dry and dissolved in nuclease-free water. Samples were stored at -80°C. Quality and concentration of RNA were measured at absorbance 260 nm with a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). If RNA concentration was above 1,000 ng/µL samples were diluted using 25 µL original in 25 µL nuclease-free water to reduce concentration by half. Template RNA was added to nuclease-free water for a final amount of 16 µL. Single strand cDNA was generated using iScript™ Reverse Transcription Supermix for RT-qPCR (Cat. No. 1708841, Bio-Rad, Montreal, Quebec, Canada). Briefly, a master mix was prepared according to iScript protocol. Fifteen µl of master mix and 5 µl of template RNA were mixed in a tube. The sample mix was incubated in a thermal cycler (Veriti 96 Well Thermyl Cycler, Applied Biosystems, Foster City, CA, USA) using the following protocol: priming for 5 min at 25 °C, reverse transcription for 20 min at 46 °C and reverse transcriptase inactivation for 1 min at 95 °C. Samples of cDNA were diluted 20 × by mixing 3 µL cDNA in 57 µL nuclease-free water and stored at -80 °C until use.

3.2.6 mRNA Abundance

Primers were tested using quantitative real-time PCR (qRT-PCR) to ascertain presence in all samples. β-actin was used as a housekeeping gene after testing for presence and minimal fluctuation in all samples using qRT-PCR. Genes analyzed were toll-like receptor 4 (TLR4; Moyes et al., 2010), interleukin-1 receptor associated kinase 1 (IRAK1; Moyes et al., 2010), nuclear factor kappa-beta 1 (NFKB1; Moyes et al., 2014), occluding (OCLN; Minuti et al., 2015), claudin 1 (CLDN1; Minuti et al., 2015), claudin 4 (CLDN4; Malmuthuge et al., 2013), and tight junction protein 1 (TJP1; Minuti et al., 2015). Expression of genes was measured by qRT-PCR using gene specific primers (Table 3.4). The qRT-PCR was performed using StepOnePlus™ Real-Time PCR System (Applied Biosystems by Life Technologies, Foster City, CA, USA) using SYBR green. The reaction was 95 °C for 5 s then 40 cycles of 95 °C for 10 s followed by annealing/ extension for 30 s at 60 °C. Final melting curve stage was 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Fluorescence was measured at 0.1 °C intervals and the point at which the signal crossed the threshold was the threshold cross value (Ct). Relative transcript quantity as compared to a control was expressed as fold change. One cow fed a high starch fresh

diet without SCFP supplementation was chosen as the control. The average Ct of each sample was compared to the corresponding average Ct of the housekeeping gene by subtracting Ct of sample – Ct of housekeeping to calculate ΔCt. The ΔCt of the control was used as the reference value to calculate ΔΔCt by subtracting ΔCt of a sample – ΔCt of the control. Fold change was calculated as $2^{-\Delta\Delta Ct}$.

3.2.7 Statistical Analysis

Data for mRNA abundance were analyzed separately for d -10 and 21 relative to calving using the FIT model procedure of JMP (version 13.1.0 SAS Institute Inc., Cary, North Carolina, USA) using the following model:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + e_{ij},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the fixed effect of SCFP supplementation, S_j is the fixed effect of fresh diet starch content, TS_{ij} is the interaction of SCFP supplementation by fresh diet starch content, and e_{ijk} is the residual. Starch content of fresh diets and its interactions with other parameters were not included in the model for analysis of data from d -10 relative to calving. In addition, day effect (d -10 vs. 21) was evaluated for mRNA abundance data using the following model:

$$Y_{ij} = \mu + C_i + D_j + e_{ij},$$

where Y_{ij} is the dependent variable, μ is the overall mean, C_i is the random effect of cow, D_j is the fixed effect of day, and e_{ij} is the residual.

The other data were analyzed using the FIT model procedure of JMP (version 13.1.0 SAS Institute Inc., Cary, North Carolina, USA) using the following model:

$$Y_{ijk} = \mu + T_i + S_j + D_k + TS_{ij} + TD_{ik} + SD_{jk} + TSD_{ijk} + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, T_j is the fixed effect of SCFP supplementation, S_j is the fixed effect of fresh diet starch content, D_k is the fixed effect of day as a repeated measure, TS_{ij} is the interaction of SCFP supplementation by fresh diet starch content, TD_{ik} is the interaction of SCFP supplementation by day, SD_{jk} is the interaction of fresh diet starch content by day, TSD_{ijk} is the interaction of SCFP supplementation, fresh diet starch content, and day, and e_{ijk} is the residual. The data were also analyzed and presented separately for each day (e.g., d -10, 1, 7, 21, and 42). Starch content of fresh diets and its interactions with other parameters were not included in the model for analysis of data from d -10 and 1 relative to calving. Initially, treatment by parity (primiparous vs. multiparous cows) were evaluated all response variables. As significant interaction effects were noted for TAOC data, these data were presented separately for primiparous and multiparous cows. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

3.3 Results & Discussion

3.3.1 Gene Expression in Rumen Epithelium

3.3.1.1 Tight Junctions

There were no differences between treatments at d -10 and 21 for mRNA expression of tight junction genes (Table 3.5 & 3.6). There was also no effect of day on tight junction mRNA expression (Table 3.7). In the present study there were no differences in mean rumen pH between treatments on d 21 after calving, but there was a tendency for SCFP supplementation to reduce duration pH < 5.8 (Shi et al., 2018b). Despite this difference, there were no differences in tight junction gene expression due to SCFP supplementation or reducing dietary starch content.

The change from a high forage close-up diet to a high concentrate fresh diet increases the risk of SARA (Zebeli et al., 2010), and there is evidence that SARA can lead to tight junction failure (Plaizier et al., 2008; Steele et al., 2011). When tight junctions are compromised, microbes such as lipopolysaccharide (LPS) endotoxin can translocate into the blood stream and induce an immune response (Plaizier et al., 2008). The three tight junction protein mRNA abundance measured in the present study interact to form the tight junctions between cells. Tight junction protein 1 is on the intracellular side of the tight junction and interacts with OCLN, CLDN1, and CLDN4 to form a fence-type structure between the apical and basolateral sides of cells

(Schneeberger and Lynch, 2004). Multiple tight junction changes around calving have been reported. Minuti et al. (2015) reported decreased expression of OCLN and TJP1 10d after calving. Both genes are important components of the tight junction structure, and decreased expression is associated with reduced barrier function (Feldman et al., 2005; Fanning et al., 1998).

Steele et al. (2011) rapidly increased concentrate feeding to induce SARA and found that during the first week of concentrate feeding the animals experienced SARA. During this period rumen papillae showed evidence of disrupted tight junctions. As compared to Steele et al. (2011), rumen pH parameters in the present study did not decrease to the same extent (Shi et al., 2018b). The differences in rumen fermentation may not have been drastic enough to cause a difference in tight junction gene expression. Based on these results, I cannot determine if animals experienced tight junction failure.

3.3.1.2 Immune System Activation

There was an effect of day on mRNA expression of TLR4 and NFKB1 (Table 3.7). Relative mRNA abundance of TLR4 tended to decrease from d -10 to 21, and NFKB1 significantly decreased from d -10 to 21. Feeding a low starch diet significantly reduced relative mRNA abundance of IRAK1 in rumen tissue on week 3 after calving compared to a high starch diet (Table 3.6). There are multiple immune proteins present in the rumen epithelium including TLR4, IRAK1, and NFKB1 (Minuti et al., 2015). Lipopolysaccharide activates TLR4 (Ulevitch and Tobias, 1995). This activation leads to the phosphorylation of IRAK1 and the activation of the NFκ-β pathway (Gottipati et al., 2008). The NFκ-β pathway leads to the transcription of genes for cytokines such as IL-6, IL-1β, and TNF-α (Wolf et al., 2001). These cytokines are involved in recruiting immune cells and activating an acute phase response (Gruys et al., 2005).

These results are similar to Minuti et al. (2015). They found decreased expression of TLR4 in rumen epithelium after calving. The authors suggest this is a consequence of endotoxin tolerance, which is the adaptation of tissues to increased endotoxin exposure. Tissues reduce expression of TLR4 in order to prevent excessive immune activation due to continual exposure to LPS (West and Heagy, 2002). When excess endotoxins are present, tolerance to endotoxins

may develop. Endotoxin tolerance results in decreased expression of TLR4 on the cell surface (Abreu, 2010). Unlike TLR4, decreased mRNA abundance of IRAK1 is not indicative of endotoxin tolerance; there was no difference in mRNA abundance of IRAK1 between baseline and endotoxin tolerant macrophages (Medvedev et al., 2002). Therefore, reduced mRNA abundance of IRAK1 suggests reduced activation of the gene, and reduced immune system activation in rumen tissue three weeks after calving. This is beneficial to the animal because activation of the immune system is taxing. The immune system utilizes a substantial amount of glucose, directing glucose away from the mammary gland (Kvidera et al., 2017). Instead of partitioning glucose to the immune system, animas fed the low starch diet may have been able to use the glucose for other processes such as milk production.

During SARA LPS concentrations in rumen fluid are increased. Li et al. (2016) compared a SARA challenge diet to a control diet and found a greater concentration of LPS in rumen fluid 6 h after feeding when feeding the SARA challenge than the control diet (21,043 vs. 125,242). In the present study, feeding a high starch diet did not result in greater incidences of SARA than a low starch diet (Shi et al., 2018b). However, a high starch diet contains more grains compared with a low starch diet (18.1% vs. 8.9% rolled barley; HS and LS, respectively), which is often associated with SARA, and it can reasonably be speculated that animals fed a high starch diet had greater ruminal concentrations of LPS than cows fed low starch diets. Decreased concentrations of LPS in the rumen may have led to reduced activation of TLR4 and reduced phosphorylation of IRAK1. This may be associated with lower mRNA abundance of IRAK1 in rumen tissue of cattle fed low starch diets.

Toll-like receptor 4 is exclusively activated by LPS (Ulevitch and Tobias, 1995), but IRAK1 phosphorylation occurs through the activation of most toll-like receptors (Gottipati et al., 2008). There may have been increased activation of IRAK1 when feeding the high starch diets by any one of the toll-like receptors. Nonetheless, a low starch diet reduced mRNA expression of IRAK1 at week three after calving. All cows may have experienced a degree of endotoxin tolerance after calving, shown by a decrease in expression of TLR4 and NFKB1 from d -10 to 21 and no difference in expression between treatments before or after calving. However, cows fed

the low starch diets had decreased mRNA expression of IRAK1 and may have experienced reduced immune system activation in rumen tissue.

3.3.2 Acute Phase Response

Serum amyloid-A concentrations were not different between treatments (Table 3. 8), but there was a significant effect of day such that, on d 1 and 7, SAA concentrations in serum were greater than other time points (d -10, 21, and 42) (Figure 3.1). These results indicate that regardless of treatment all cows experienced inflammation during and after calving. In previous studies, SCFP supplementation has had an effect on serum concentrations of SAA. Zaworski et al. (2014) found that SCFP supplementation decreased serum concentrations of SAA one day before calving and increased serum concentrations of SAA one day after calving. At other time points after calving (d 3 and 7) there were no differences between SCFP supplemented groups and control groups. This suggests that SCFP supplementation has an effect on concentrations of SAA. During a SARA challenge it is common for serum concentrations of SAA to increase (Gozho et al., 2007; Khafipour et al., 2009b; Rodríguez-Lecompte et al., 2014; and Li et al., 2016). There are few studies that have measured the response of serum concentrations of SAA to dietary starch content in the absence of experimentally induced SARA. One study in mid-lactation dairy cows compared two different dietary starch contents (24.1 vs. 28.5; LS vs. HS, respectively). There were no differences between treatments for serum concentrations of SAA. However, the effects of SAA are short-lived; concentrations increase and fall rapidly within 24 h without additional stimulation (Wang et al., 2001b). Sampling for the current study may not have been frequent enough to detect a treatment effect of SCFP supplementation or dietary starch content on serum concentrations of SAA.

On d 7 after calving, supplementation of SCFP significantly reduced serum concentrations of Hp (Table 3.8). Animal response to treatment was affected by day; serum Hp concentrations were higher for CON cows on d 7 than at other time points (Figure 3.2). This suggests that cows not receiving SCFP had greater systemic inflammation one week after calving. For cows supplemented with SCFP, serum concentrations of Hp either did not rise after calving, or rose immediately after calving and returned to baseline concentration by d 7 after calving. Serum sampling may not have been frequent enough to allow us to detect an increase in serum Hp

concentrations when supplementing SCFP. Regardless, cows supplemented with SCFP had reduced Hp d 7 after calving and experienced decreased systemic inflammation, which may have been associated with an increase in DMI. Systemic inflammation results in the symptoms of sickness, which includes fever, depression, and depressed feed intake (Gruys et al., 2005). The initiation of these symptoms is controlled by the same cytokines that induce an acute phase response (Gruys et al., 2005). In a companion paper for the present study, Shi et al. (2018a) reported that SCFP supplementation increased DMI on d 1 and 5 after calving, indicating that SCFP supplementation resulted in a smoother increase in DMI after calving. The decrease in serum Hp concentration due to SCFP supplementation may also be an indicator of reduced sickness, which led to increased feed intake after calving by cows supplemented with SCFP.

Serum SAA concentrations had a minor positive relationship with plasma concentrations of glucose (Figure 3.3, $P = 0.03$). The immune system uses a substantial amount of glucose, but this relationship indicates that although SAA was increased, it was accompanied by an increase in glucose. This suggests that the animal was still able to maintain glucose concentrations and maintain an immune response. Although there was no difference in serum SAA concentration between treatments, serum concentration of Hp were positively related to serum concentrations of SAA ($P < 0.01$, Figure 3.4). This is a further indication of the association between these two acute phase proteins. Serum concentrations of Hp were positively related to plasma concentrations of free fatty acids (Figure 3.5, $P = 0.05$) and BHB (Figure 3.6, $P < 0.01$), but not to plasma glucose concentrations ($P > 0.05$). Greater fat mobilization is associated with inflammation (Bradford et al., 2015). Inflammation is a process that uses copious amounts of glucose, and during the transition period the cow is forced to increase fat mobilization to support glucose production in the liver. Lipolysis, the breakdown of fat, increases plasma concentrations of free fatty acids, which are used by tissues and the liver for energy when there is a lack of glucose (Grummer, 1993). The association between low serum concentrations of Hp and low plasma concentrations free fatty acids or BHB suggests that cows with lower serum Hp were not mobilizing body reserves at the rate of cows with higher serum Hp because of reduced immune system activation.

All cows experience inflammation during the calving transition, and it is an important physiological process (Bradford et al., 2015). However, continuous inflammation is indicative of continuous exposure to harmful stimuli, such as lipopolysaccharide (LPS) as a result of SARA. Subacute rumen acidosis challenges have resulted in the increase in serum concentrations of SAA (Gozho et al., 2007; Khafipour et al., 2009a; Rodríguez-Lecompte et al., 2014; and Li et al., 2016). Serum haptoglobin concentrations also increased as a result of the SARA challenge (Khafipour et al., 2009a; Li et al., 2016). Feeding a low starch diet may reduce incidence of SARA, and therefore reduce activation of an acute phase response. However, in the current study, there were few differences in rumen fermentation parameters due to a low starch diet, and on d 6 after calving a low starch diet increased duration of pH < 5.8 (Shi et al., 2018b), but this increase in duration of pH < 5.8 did not contribute to higher acidosis index (Shi et al., 2018b). In the current study the differences in serum Hp concentrations was not due to a reduced incidence of SARA. Differences in rumen fermentation due to dietary starch content were minimal, and sampling frequency may not have been sufficient enough to detect a difference in serum concentrations of acute phase proteins due to dietary starch content.

3.3.3 Oxidative Status

Plasma concentrations of ROM were not different between treatments (Table 6). Cows supplemented with SCFP had greater plasma concentrations of MDA than cows not fed SCFP on d 21 after calving (Table 6). Plasma concentrations of TOAC tended to decrease on d 1 and 7 due to SCPF supplementation (Table 6). Supplementation of SCFP and dietary starch content tended to interact to affect plasma concentrations of TAOC d 21 after calving. Animals fed SCFP+LS had lower plasma concentrations of TAOC than animals fed CON+LS.

While plasma concentrations of TAOC tended to be lower d 1 and 7 when feeding SCFP, this may not indicate oxidative stress at this time point. Total antioxidant capacity may decrease because antioxidants are being utilized to neutralize oxidants. If there is not an increase in oxidants there may be sufficient antioxidant capacity to counteract the oxidants. There were no differences between treatments in plasma concentrations of ROM or MDA on d 1 and 7, suggesting that the reduction in TAOC was not associated with oxidative stress. Reactive oxygen metabolites are released during many processes in the body, including oxidative burst by

immune cells and during respiratory processes such as β-oxidation of free fatty acids in the liver (Miller et al., 1993). If not neutralized, ROM can break down tissues. Malondialdehyde is a marker of lipid peroxidation, or the breakdown of lipids such as the lipid membrane of tissues by oxidants (Halliwell and Chirico, 1993). Plasma MDA concentrations were increased for SCFP supplemented cows and decreased for cows not supplemented with SCFP at d 21. Oxidative stress is the imbalance between oxidants and antioxidants. While this may suggest that cows experienced oxidative stress, this may not have been the case. Compared to concentrations of MDA in literature (Bernabucci et al., 2005; Castillo et al., 2006), the plasma concentrations of MDA in the current study did not increase to the same extent. While there was a significant difference, the values were not high enough to have an effect on the animal.

The increase in MDA at d 21 when supplementing MDA may have been a result of increased metabolism. The β-oxidation of free fatty acids results in the production of oxidants (Rosca et al., 2012). Supplementing with SCFP may have resulted in increased metabolism, increased oxidant output, and increased oxidative damage, shown by an increase in plasma concentrations of MDA at d 21 for cows supplemented with SCFP. However, it cannot be ruled out that the oxidative stress on d 21 for cows supplemented with SCFP was due to more than increased metabolism.

The effect of dietary starch content on indices of oxidative stress has not been explored, but may have an indirect impact on oxidative status. Multiple in vitro studies and reviews have implicated free fatty acids as the cause of oxidative stress because of β-oxidation of free fatty acids in the liver (Sordillo and Raphael, 2013). Diets fed post-calving may have an impact on the metabolic status of the animal, but results are inconclusive. A low starch diet has increased plasma concentrations of free fatty acids and BHB after calving (McCarthy et al., 2015a). But another study found that there was no difference between high or low starch diets on metabolic status (Dias et al., 2018). Dietary starch content may not have an effect on indices of oxidative stress because of the limited effect on plasma free fatty acid concentrations.

3.3.4 Adaptive Immune Response

There was no effect of treatment on serum concentrations of IgG (Figure 3.10). The ovalbumin challenge was successful; there was an effect of day on serum IgG concentrations. Serum IgG

concentrations were lowest at d 7, and increased from d 7 to 21 and from d 21 to 35. The increase in serum IgG concentration from d 7 to 21 suggests an initiation of an adaptive immune response. It is important to note that the analysis for IgG was general IgG content, not anti-ovalbumin IgG.

A greater response to an ovalbumin challenge is beneficial because it indicates the immune system is capable of mounting an adaptive response. There is also an association between response to an ovalbumin challenge and milk yield: multiparous animals that were categorized as higher responders to an ovalbumin challenge had greater projected 305d milk yield (Wagter et al., 2003). Animals that have a high response to an ovalbumin challenge may be able to quickly and efficiently clear a pathogen and return to immune homeostasis sooner, resulting in decreased use of glucose by the immune system.

An important component of *Saccharomyces cerevisiae* are β-glucans in the yeast cell wall, and they have been shown to increase humoral response to vaccination in broilers (Muthusamy, et al., 2011) and to an ovalbumin challenge in pigs (Li et al., 2005). Additionally, in vitro, SCFP has been shown to increase B-cell activation (Jensen et al., 2008). However, in vivo, results have not been conclusive. Zaworski et al (2014) did not conduct an ovalbumin challenge, but they measured serum IgG concentrations and found no difference between SCFP supplemented cows and cows not supplemented with SCFP. An ovalbumin challenge conducted in pre-weaning calves also resulted in no difference between SCFP supplemented and control calves (Magalhães et al., 2008). Contrary to this, enzymatically hydrolyzed yeast supplementation resulted in a greater response to an ovalbumin challenge than unsupplemented cows (Yuan et al., 2015). It may be possible that there are greater concentrations of β-glucans in hydrolyzed products than in yeast culture products, and may be why I did not see a greater response to the ovalbumin challenge in cows supplemented with SCFP.

As far as I am aware, no studies have been conducted to evaluate the effects of dietary starch content on the adaptive immune response. Multiple in vitro studies have determined that increasing free fatty acid concentrations may reduce lymphocyte function (Lacetera et al., 2004, 2005). A low starch fresh diet may increase free fatty acid concentrations (McCarthy et al.,

2015a). In the present study a low starch diet increased concentrations of free fatty acids (Shi et al., 2018a), but this had no effect on response to the ovalbumin challenge. The results from in vitro studies may not be applicable to in vivo situations.

A limitation of the ovalbumin challenge is that capability of lymphocytes is not measured. For example, cows diagnosed with endometritis had greater concentrations of leukocytes (a general class of immune cells that includes lymphocytes) than cows not diagnosed with endometritis. However, leukocytes from cows diagnosed with endometritis had decreased killing capability (Kim et al., 2005). Function of lymphocytes may be more important to the adaptive response than concentration, however in this experiment, capability of lymphocytes was not measured.

3.4 Conclusion

All cows experienced inflammation after calving, but cows supplemented with SCFP had lower systemic inflammation at d 7 after calving. However, SCFP supplementation resulted in oxidative stress d 21 after calving. There were no differences between diets for tight junction gene expression, but a low starch fresh diet reduced mRNA abundance of IRAK1 in rumen tissue d 21 after calving. Supplementing SCFP and reducing dietary starch content after calving may reduce inflammation and improve health status of the animal after calving.

Table 3.1 Animal information at enrollment (28 days before expected calving date).

Item		Treatments			
		CON+LS	SCFP+LS	CON+HS	SCFP+HS
No. of animals		9	10	10	9
	Primiparous	3	3	3	3
	Multiparous	6	7	7	6
Average parity		2.00	2.00	1.90	1.89
Body Condition Score		3.31	3.25	3.35	3.29

Table 3.2 Feed ingredients of experimental diets.

Ingredient, % DM	Prepartum		Postpartum			
			Low starch		High starch	
	CON	SCFP	CON	SCFP	CON	SCFP
Barley silage	46.9	46.9	46.5	46.5	46.5	46.5
Alfalfa hay	—	—	2.77	2.77	2.77	2.77
Barley straw	29.1	29.1	—	—	—	—
Barley rolled	7.46	7.46	8.92	8.92	18.1	18.1
Corn gain, ground, dry	—	—	12.5	12.5	12.5	12.5
Canola meal mech. Extract	8.19	8.22	10.2	10.2	7.96	7.96
Soybean meal, Sol 44% CP	—	—	3.00	3.00	3.00	3.00
Corn gluten meal, dried	0.45	0.45	0.85	0.85	2.00	2.00
Amino Plus	1.81	1.81	2.00	2.00	2.00	2.00
Beet pulp	—	—	8.34	8.34	—	—
F-100, Dairy fat	—	—	1.10	1.10	1.10	1.10
Magnesium Oxide	0.35	0.34	0.18	0.18	0.18	0.18
Limestone	0.72	0.7	1.38	1.38	1.60	1.60
Salt	0.14	0.14	0.48	0.48	0.48	0.48
Magnesium Sulfate (H2O)	0.59	0.59	—	—	—	—
Potassium carbonate	—	—	—	—	0.09	0.09
Calcium Chloride	0.43	0.43	—	—	—	—
Calcium Sulfate	0.48	0.48	—	—	—	—
Vitamin premix	0.05	0.05	0.02	0.02	0.02	0.02
Trace mineral premix	0.01	0.01	0.01	0.01	0.01	0.01
Selenium	0.006	0.006	0.004	0.004	0.006	0.006
Nutri A-Z C	0.1	0.1	0.05	0.05	0.05	0.05

Biotin 2%	—	—	0.005	0.005	0.005	0.005
Zinpro 4 plex C	—	—	0.03	0.03	0.03	0.03
NutriTek Premix CON[1]	1.92		1.06	—	1.06	—
NutriTek Premix TRT[2]	—	1.92	—	1.06	—	1.06
Rumensin premix[3]	0.8	0.8	0.58	0.58	0.58	0.58
Urea	0.5	0.5	—	—	—	—

[1]Contains 99.0% of dry, ground corn and 1.0% of canola oil, providing 0 g/d of NutriTek.

[2]Contains 7.9% of NutriTek, 91.1% of dry, ground corn, and 1% of canola oil, providing 19 g/d of NutriTek based on expected dry matter intake of the cow (11kg/day prepartum; 20 kg/d postpartum).

[3]Contains 1.21% of Rumensin, 97.54% of dry, ground barley, and 1.25% of canola oil, providing 242 mg of monensin sodium activity for close-up cows to the amount of 22 mg/kg monensin sodium total diet dry matter and 320 mg of monensin sodium activity for lactating cows to the amount of 16 mg/kg monensin sodium total diet dry matter.

Table 3.3 Nutrient composition of experimental diets.

Nutrient composition, DM basis	Prepartum		Postpartum			
	CON	SCFP	Low starch		High starch	
			CON	SCFP	CON	SCFP
NDF, %	49.5	49.4	33.3	32.7	31.7	31.5
Forage NDF, %	45.1	45.1	24.0	24.0	24.0	24.0
ADF, %	31.6	31.5	20.9	20.3	19.3	19.2
NFC, %	25.6	25.6	39.3	41.3	41.1	42.8
CP, %	15.3	15.3	17.3	17.0	17.5	16.9
Starch, %	13.8	13.9	21.6	22.5	27.0	29.5
Fat, %	2.5	2.5	3.6	3.6	3.7	3.7
TDN, %	61.0	61.0	71.0	72.0	72.0	73.0
NE_L, Mcal/kg DM	1.43	1.43	1.60	1.62	1.63	1.64
Calcium, %	0.94	0.92	1.09	0.95	1.10	0.73
Phosphorus, %	0.33	0.33	0.42	0.42	0.43	0.42
Magnesium, %	0.43	0.43	0.33	0.31	0.30	0.28
Potassium, %	1.69	1.69	1.48	1.46	1.47	1.44
Sodium, %	0.19	0.19	0.32	0.28	0.29	0.24
Chloride, %	0.86	0.86	0.6	0.62	0.62	0.62
Sulfur, %	0.40	0.40	0.25	0.25	0.26	0.26
Cobalt, ppm	0.55	0.59	1.89	1.89	1.92	1.92
Copper, ppm	12.1	12.0	21.2	19.7	28.1	16.7
Iodine, ppm	0.59	0.59	0.66	0.66	0.66	0.66
Manganese, ppm	39.6	40.1	48.5	44.0	44.3	40.5
Selenium, ppm	0.27	0.27	0.27	0.26	0.28	0.28
Zinc, ppm	44.7	53.3	80.1	72.1	91.6	69.6

Vitamin A, 1000 IU/kg	30	30	10.6	10.6	10.6	10.6
Vitamin D, 1000 IU/kg	3.0	3.0	1.1	1.1	1.1	1.1
Vitamin E, IU/kg	99.8	99.8	35.1	35.1	35.1	35.1
DCAD, mEQ/kg	26	25	192	164	170	138
DM, %	50.2	50.2	50.2	49.9	50.2	50.1

Table 3.4 Primer Sequences for genes measured for mRNA abundance.

Gene	Accession #[1]	Forward Primer (5'-3')	Reverse Primer (5'-3')	bp	Sources
TLR4	NM_174198.6	TGCGTACAGGTTGTTCCTAACATT	TAGTTAAAGCTCAGGTCCAGCATCT	110	Moyes et al., 2010
IRAK1	NM_001040555.1	CCTCAGCGACTGGACATCCT	GGACGTTGGAACTCTTGACATCT	103	Moyes et al., 2010
NFKB1	NM_001076409.1	TTCAACCGGAGATGCCACTAC	ACACACGTAACGGAAACGAAATC	95	Moyes et al., 2014
OCLN	NM_001082433.2	GCCATTTTCGCCTGTGTTG	CCAAAGGCACTTCCTGCATAA	101	Minuti et al., 2015
CLDN1	NM_001001854	GGCATCCTGCTGGGACTAATAG	CAGCCATCCGCATCTTCTGT	100	Minuti et al., 2015
CLDN4	NM_205801	CCCGCGCCCTCATCGTCATC	GTTGGCCGACCAGGACACCG	185	Malmuthuge et al., 2013
TJP1	XM_002696650.3	GCACATAGGATCCCTGAACCA	TGCTTCCGGTAGTACTCCTCATC	107	Minuti et al., 2015

[1]Gene accession number from NCBI (http://www.ncbi.nlm.nih.gov).

Table 3.5 The effect of supplementation of a *Saccharomyces cerevisiae* fermentation product on relative mRNA abundance on d -10, ± 3 relative to expected calving date.

	Treatments[1]			P-value[2]
Gene	CON	SCFP	SEM	SCFP
TLR4	1.53	1.12	0.221	0.22
IRAK1	1.35	1.15	0.205	0.52
NFKB1	1.04	1.12	0.144	0.70
OCLDN	1.29	1.33	0.175	0.88
CLDN1	0.84	0.86	0.105	0.91
CLDN4	1.70	2.01	0.485	0.67
TJP1	0.97	1.00	0.072	0.75

[1]Treatments: CON = TMR without SCFP; SCFP = TMR with SCFP

[2]SCFP = SCFP supplementation

Table 3.6 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and reducing dietary starch content on relative mRNA abundance on d 21, ±3 after calving.

	Treatments[1]							
	Low		High			P-value[2]		
Gene	CON	SCFP	CON	SCFP	SEM	S	SCFP	S*SCFP
TLR4	1.06	0.63	1.01	1.15	0.227	0.32	0.53	0.23
IRAK1	1.02	0.87	1.50	1.25	0.185	0.04	0.30	0.81
NFKB1	0.86	0.74	0.88	0.96	0.095	0.23	0.88	0.32
OCLDN	1.25	1.23	1.30	1.41	0.189	0.55	0.83	0.76
CLDN1	0.73	0.86	0.93	0.80	0.081	0.38	0.99	0.12
CLDN4	1.99	4.13	3.13	3.55	1.818	0.88	0.50	0.64
TJP1	0.99	1.06	0.97	0.92	0.116	0.49	0.92	0.60

[1]Treatments: CON = TMR without SCFP; SCFP = TMR with SCFP; Low = low starch fresh TMR; High = high starch fresh TMR

[2]S = Starch content; SCFP = SCFP supplementation; S*SCFP = Interaction between starch and SCFP

Table 3.7 The effect of sampling day on relative mRNA abundance in rumen epithelium.

	Day, ± 3			P-value
Gene	d -10	d 21	SEM	
TLR4	1.27	0.94	0.135	0.08
IRAK1	1.24	1.16	0.118	0.58
NFKB1	1.09	0.85	0.070	0.04
OCLDN	1.30	1.29	0.102	0.95
CLDN1	0.85	0.84	0.054	0.86
CLDN4	1.87	3.25	0.762	0.21
TJP1	0.99	0.99	0.056	0.99

Table 3.8 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and reducing dietary starch content on serum concentrations of haptoglobin (Hp) and serum amyloid-A (SAA).

	Treatments[1]							
	Low		High			P-value[2]		
Item	CON	SCFP	CON	SCFP	SEM	S	SCFP	S*SCFP
SAA, mg/L								
d -10[3]	26.6	37.9			5.92		0.17	
d 1[3]	101.5	132.4			16.32		0.17	
d 7	126.4	90.0	99.4	111.5	20.45	0.89	0.55	0.24
d 21	43.0	41.0	31.3	37.2	11.20	0.48	0.86	0.72
d 42[4]	62.1	56.4	60.6	58.2	16.40	0.99	0.80	0.92
Hp, mg/mL								
d -10	0.08	0.09			0.006		0.17	
d 1	0.19	0.15			0.056		0.59	
d 7	0.68	0.23	0.56	0.29	0.165	0.84	0.03	0.60
d 21	0.10	0.12	0.11	0.12	0.039	0.84	0.69	0.97
d 42	0.13	0.10	0.11	0.12	0.035	0.96	0.76	0.55

[1]Treatments: CON = TMR without SCFP; SCFP = TMR with SCFP; Low = low starch fresh TMR; High = high starch fresh TMR

[2]S = Starch content; SCFP = SCFP supplementation; S*SCFP = Interaction between starch and SCFP

[3]On d -10 and d 1 dietary starch content was not a treatment because animals were receiving close-up diets.

[4]All animals were fed high starch diets after d 21. Carryover effects of fresh diets are of interest.

Table 3.9 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and reducing dietary starch content on plasma concentrations of reactive oxygen metabolites (ROM), malondialdehyde (MDA), and total antioxidant capacity (TAOC).

	Treatments[1]							
	Low		High			P-value[2]		
Item	CON	SCFP	CON	SCFP	SEM	S	SCFP	S*SCFP
ROM, U CARR								
d -10[3]	132	138			14.1		0.70	
d 1[3]	112	122			9.0		0.41	
d 7	117	131	116	114	12.8	0.50	0.60	0.52
d 21	103	113	116	101	7.8	0.94	0.79	0.11
d 42[4]	131	153	135	126	10.7	0.28	0.49	0.15
MDA, µM								
d -10	17.5	17.8			1.11		0.84	
d 1	16.7	16.9			1.11		0.87	
d 7	17.3	16.2	15.2	19.3	1.63	0.79	0.35	0.12
d 21	18.9	19.6	16.0	20.2	1.19	0.33	0.04	0.13
d 42	21.1	24.4	22.7	22.7	1.48	0.99	0.26	0.26
TAOC, mM								
d -10	0.75	0.73			0.06		0.83	
d 1	0.99	0.80			0.07		0.06	
d 7	1.07	0.75	0.93	0.87	0.11	0.93	0.07	0.22
d 21[5]	1.04[a]	0.74[b]	0.93[ab]	0.95[ab]	0.09	0.58	0.13	0.09
d 42	0.94	0.81	0.83	0.92	0.08	0.99	0.77	0.17

[1]Treatments: CON = TMR without SCFP; SCFP = TMR with SCFP; Low = low starch fresh TMR; High = high starch fresh TMR

[2]S = Starch content; SCFP = SCFP supplementation; S*SCFP = Interaction between starch and SCFP

[3]Starch was not a treatment on d -10 and d 1 because cows were receiving the close-up diets.

[4]All animals were fed high starch diets after d 21. Carryover effects of fresh diets are of interest.

[5]Superscripts with differing letters in the same row represent a tendency for a difference.

Table 3.10 Health events during the experimental period.

	Treatments[1]			
	Low		High	
Health Event[2]	CON	SCFP	CON	SCFP
Retained Placenta	0	0	1	0
Metritis	1	1	1	0
Mastitis	0	1	1	0
Displaced Abomasum	3	0	1	1

[1]Treatments: CON = TMR without SCFP; SCFP = TMR with SCFP; Low = low starch fresh TMR; High = high starch fresh TMR

[2]Health events were recorded according to the following parameters: retained placenta (fetal membranes retained for greater than 24 h), metritis (purulent vaginal discharge), mastitis (abnormal milk when fore stripping before milking; swollen, hot, and/or hard quarters), and displaced abomasum (diagnosed and surgically treated by veterinarian). Values represent number of animals diagnosed.

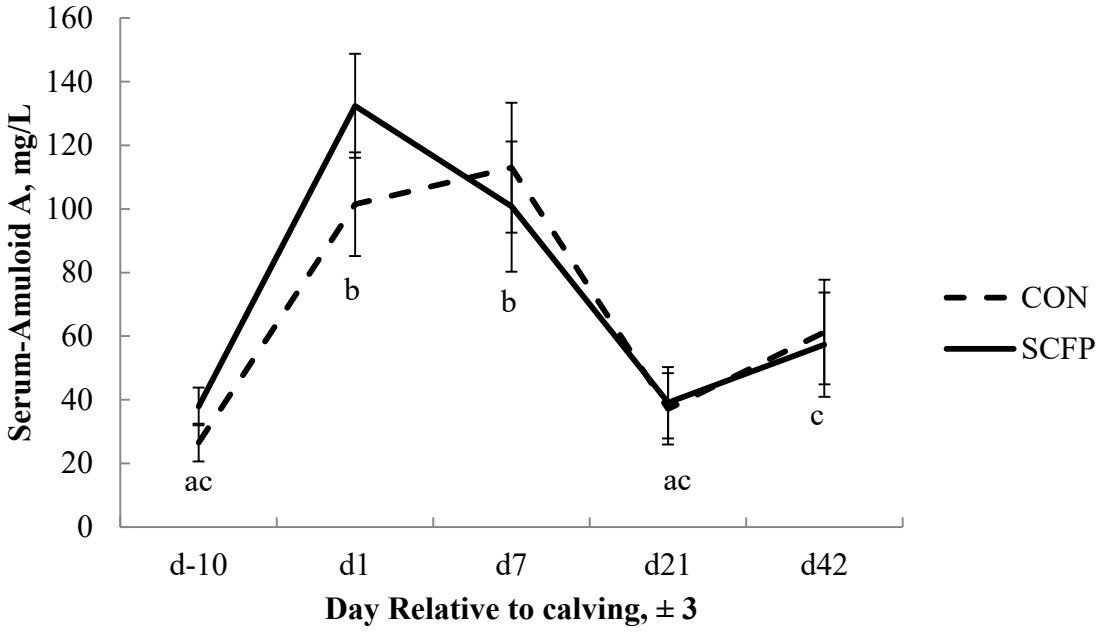

Figure 3.1 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product on serum concentrations of serum amyloid-A (SAA). Effect of day, $P < 0.01$; effect of SCFP supplementation, $P = 0.53$; interaction of day by SCFP supplementation, $P = 0.41$. Superscripts with different letters indicate week effect and $P < 0.05$.

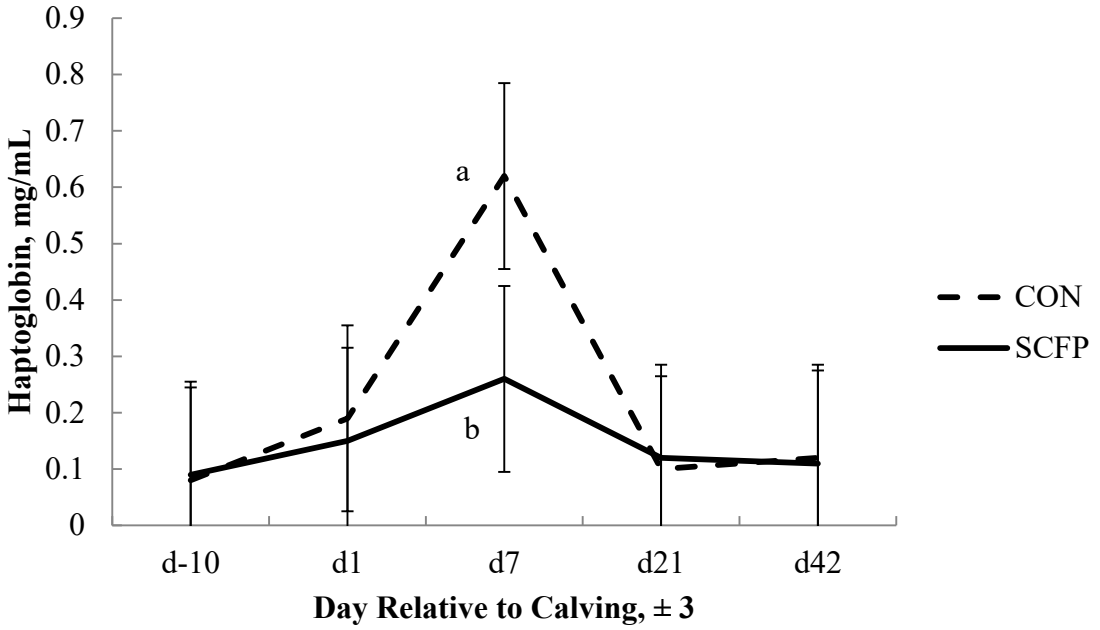

Figure 3.2 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product on serum concentrations of haptoglobin (Hp). Effect of day, $P < 0.01$; effect of SCFP supplementation, $P = 0.08$; interaction of day by SCFP supplementation, $P < 0.01$. Superscripts with different letters indicate day x SCFP interaction and $P < 0.05$.

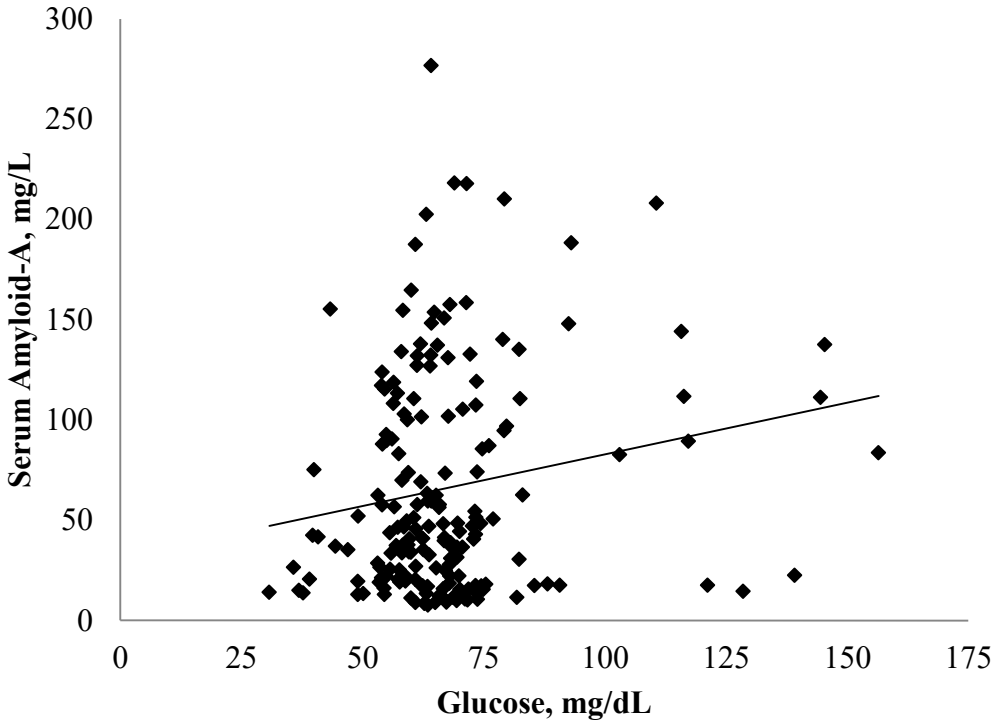

Figure 3.3 The relationship between serum concentrations of serum amyloid-A (SAA) and plasma glucose concentrations, $P = 0.03$, $r = 0.16$.

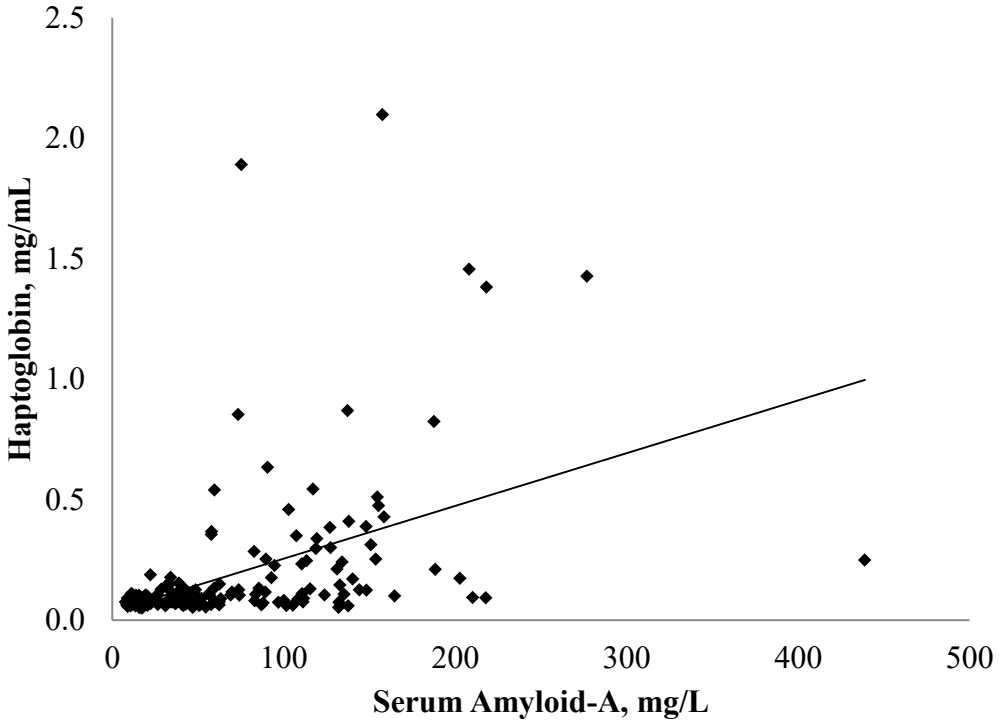

Figure 3.4 The relationship between serum concentrations of haptoglobin (Hp) and serum amyloid-A (SAA), $P < 0.01$, r = 0.46.

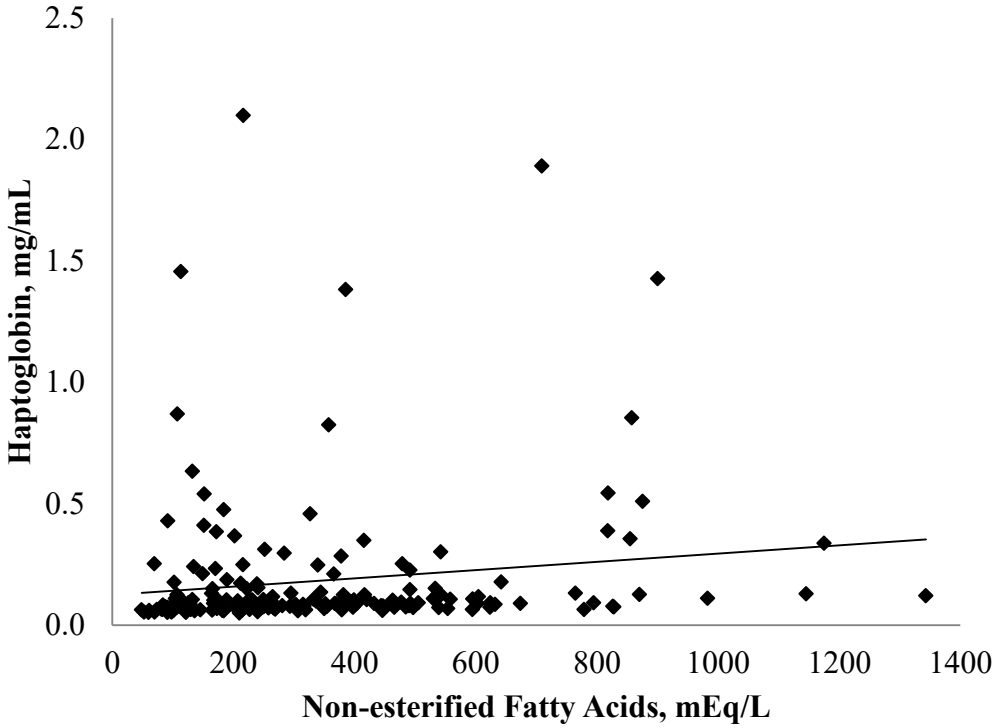

Figure 3.5 The relationship between serum concentrations of haptoglobin (Hp) and plasma concentrations of free fatty acids, $P = 0.05$, $r = 0.14$.

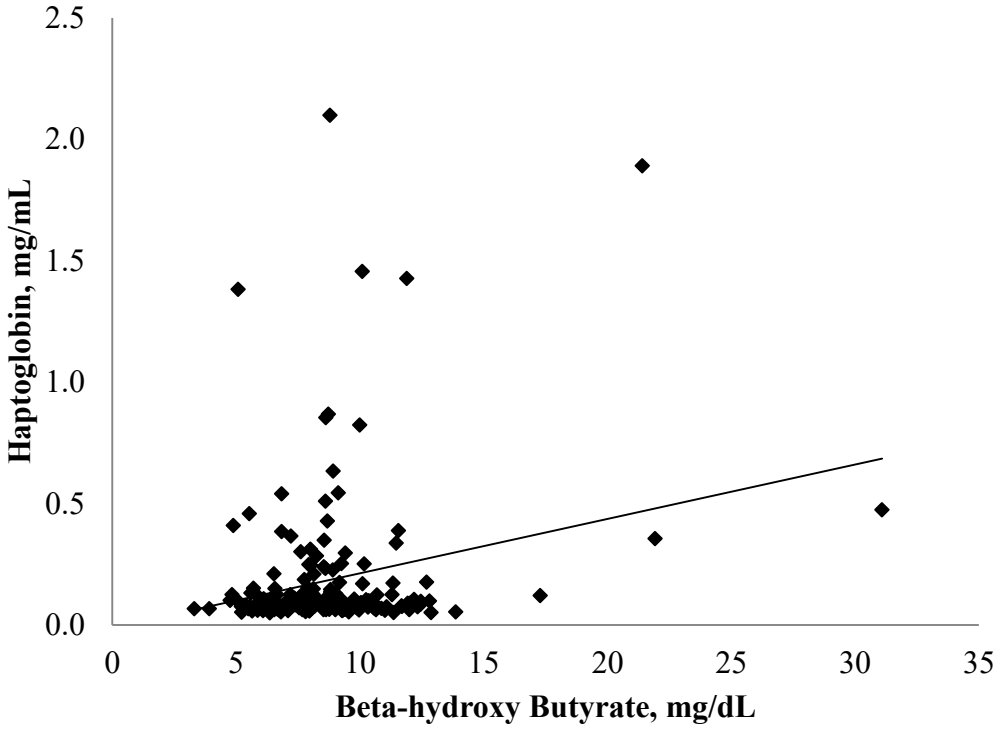

Figure 3.6 The relationship between serum concentrations of haptoglobin (Hp) and plasma BHB concentrations, $P < 0.01$, r = 0.23.

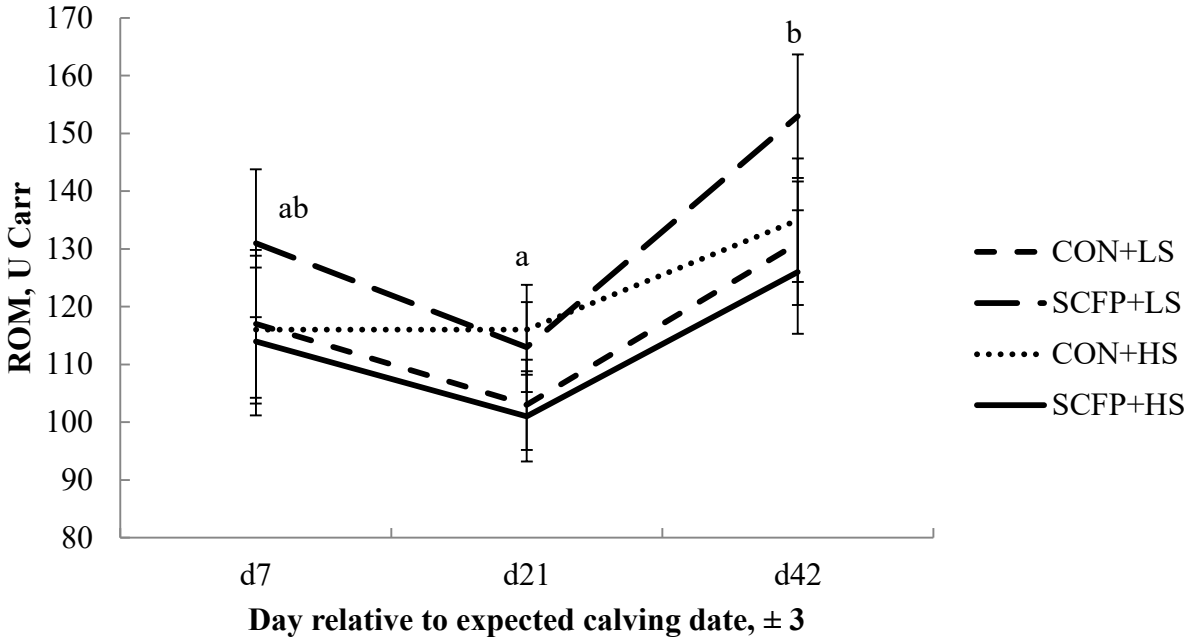

Figure 3.7 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and decreasing dietary starch content on plasma concentrations of reactive oxygen metabolites (ROM) after calving. Effect of day, $P = 0.01$; effect of SCFP supplementation, $P = 0.66$; effect of fresh diet starch content, $P = 0.47$; interaction of day by SCFP supplementation, $P = 0.69$; interaction of day by fresh diet starch content, $P = 0.58$; interaction of day by SCFP supplementation and fresh diet starch content, $P = 0.87$. Superscripts with different letters indicate day effect and $P < 0.05$.

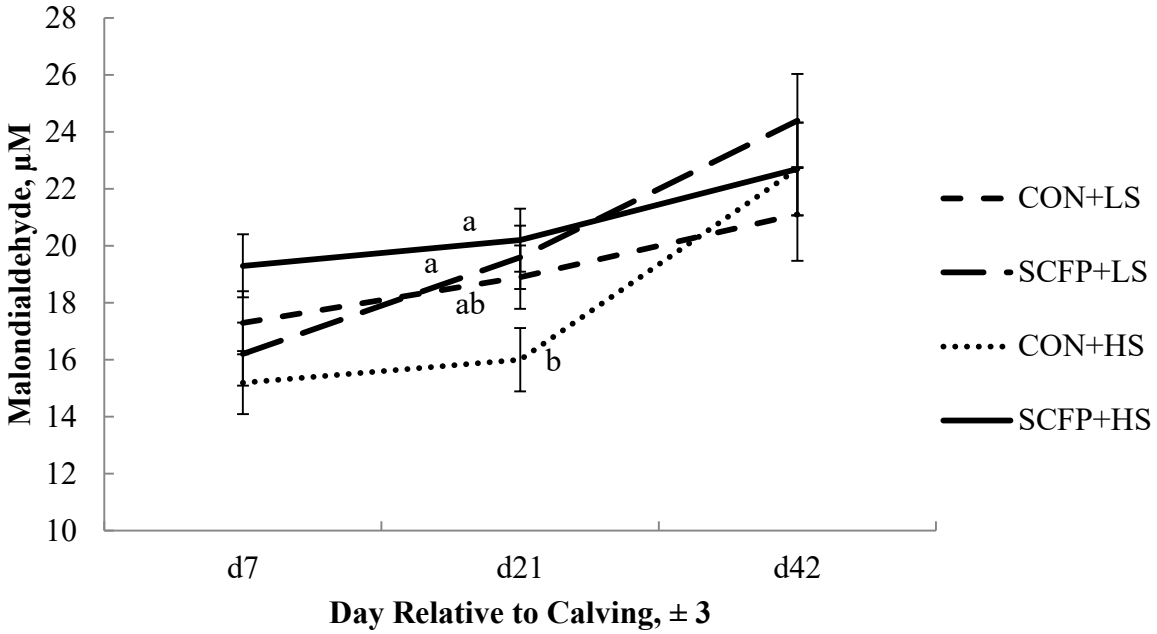

Figure 3.8 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and decreasing dietary starch content on plasma concentrations of malondialdehyde (MDA) after calving. On d 21, $P = 0.04$. Superscripts with different letters indicate day effect and $P < 0.05$.

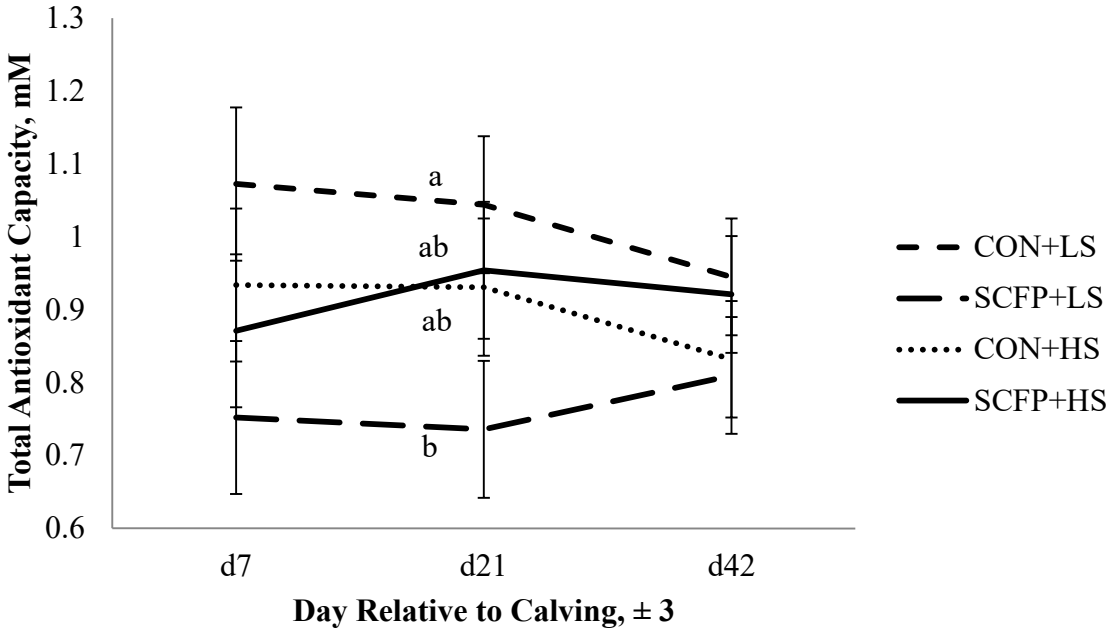

Figure 3.9 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and decreasing dietary starch content on plasma total antioxidant capacity (TAOC) concentrations after calving. On d 21, $P = 0.09$. Superscripts with different letters indicate day effect and $P > 0.05 < 0.10$.

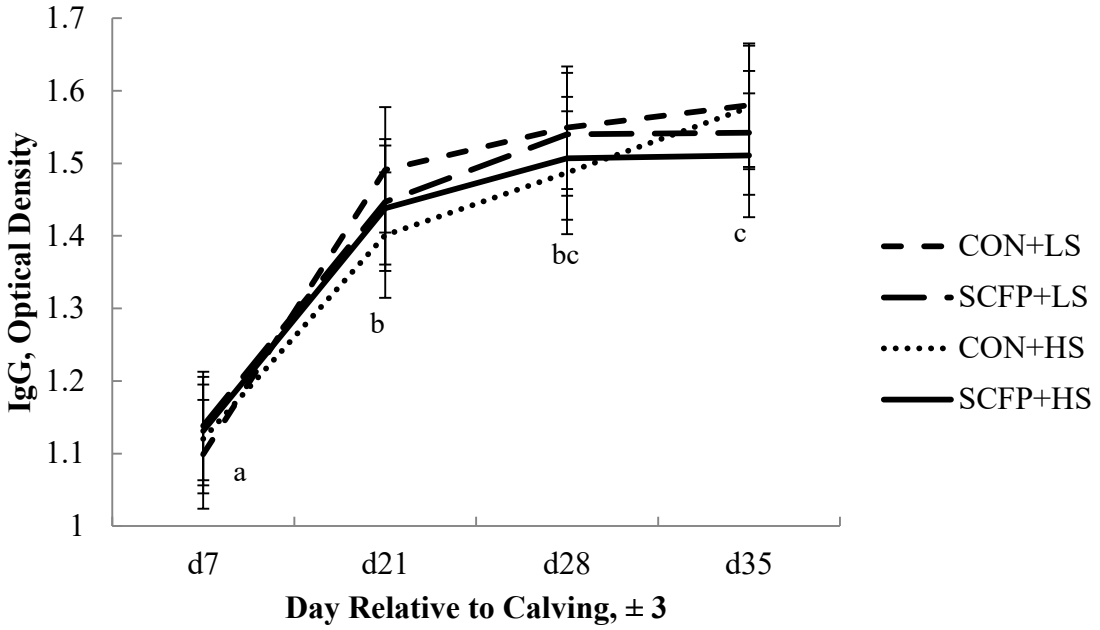

Figure 3.10 The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product and decreasing dietary starch content on serum concentrations of IgG in response to an ovalbumin challenge. On d 7 and 21, ± 3, after calving animals were injected with 1.0 mg ovalbumin and 0.25 mg Quil-A dissolved in 1 ml of phosphate buffered saline. Immediately before injection and on d 28 and 35 approximately 5 mL of blood was taken from the coccygeal vessels into a glass tube to collect serum. Effect of day, $P < 0.01$; effect of SCFP supplementation, $P = 0.81$; effect of fresh diet starch content, $P = 0.83$; interaction of day by SCFP supplementation, $P = 0.55$; interaction of day by fresh diet starch content, $P = 0.86$; interaction of day by SCFP supplementation and fresh diet starch content, $P = 0.85$. Superscripts with different letters indicate day effect and $P < 0.05$.

Chapter 4: General Discussion

4.1 Major Findings

There were three major findings in this thesis:

1) Supplementing SCFP decreased serum concentrations of Hp on d 7 after calving
2) Feeding a low starch diet after calving increased apparent total tract NDF digestibility on d 7 after calving
3) Feeding a low starch diet after calving decreased mRNA abundance of IRAK1 in rumen tissues at d 21 after calving

The supplementation of SCFP through the transition period reduced systemic inflammation at d 7 after calving. This suggests that cows fed SCFP returned to immune homeostasis faster than cows not supplemented with SCFP. Feeding a low starch diet after calving increased apparent total tract NDF digestibility d 7 after calving. Cows fed a low starch diet digested more of the fibre in the diet. Additionally, a low starch diet reduced mRNA abundance of IRAK1 d 21 after calving. This reduced mRNA abundance suggests reduced transcription of IRAK1, and therefore reduced immune system activation in rumen tissue d 21 after calving.

4.2 Limitations

As has been discussed previously, the initiation of inflammation is important for parturition and expelling the placenta (van Engelen et al., 2009; Boro et al., 2014). The resolution of inflammation is also key for a healthy and productive animal. The immune system primarily utilizes glucose, and this puts a strain on the animal. In this study, sampling was not frequent enough to determine if there was an effect of treatment on serum SAA concentrations. Additionally, sampling in this study was not frequent enough to detect an increase in serum concentrations of Hp in cows supplemented with SCFP. The answer to the question is important because cows must be able to return to baseline concentrations soon after calving, in the absence of health events. If I conduct my experiment again, I would take daily blood samples from calving to d 7 after calving. This would allow me to determine the day the SAA and Hp peaked, and determine which treatment may lead to faster resolution of inflammation. Serum concentrations of SAA were increased on both d 1 and 7. Additional blood samples would also allow me to determine if serum concentrations of Hp increased and then returned to baseline for

SCFP cows, or if concentrations did not increase at all. The additional data would also allow me to determine if there was an interaction between SCFP supplementation and dietary starch content.

Symptoms of sickness can be measured by rectal temperature and the animal's general appearance in the form of behavior scores (Smith and Risco, 2005). In order to measure sickness symptoms daily rectal temperatures should have been measured from calving to d 7 after calving. The normal rectal temperature of a healthy cow is around 38.5°C. Rectal temperature elevated to 39.5°C indicates fever and potential infection or inflammation (Smith and Risco, 2005). Additionally, I would take daily appearance scores of the cows from calving to d 7 after calving according to standards set by Smith and Risco (2005). Appearance scores include eye depth as an indicator of dehydration. Another score is ear height. Upright ears above the head indicate an animal is alert. A sick cow may have ears that droop below the poll and indicates depression, pain, and fever. Scores of eye depth and ear height taken by one consistent scorer for the length of the study this would give an accurate assessment of the animal's general appearance and attitude. This additional information would have allowed us to further speculate the effects of SCFP on the sickness response after calving.

There was no difference between treatments in response to the ovalbumin challenge. However, concentration of IgG may not be the only indicator of the function of the adaptive response. Regardless of concentration of IgG, B-cells may have reduced capability to react to stimulation and reduced antibody production (Kim et al., 2005). The ability of lymphocytes to react to stimulation should be measured in future studies to ascertain the function of the adaptive response after calving.

4. 3 Future Research

I cannot specify the mode of action of SCFP on oxidative status. Nutritek® is a proprietary product, and I can only speculate as to why SCFP supplementation led to the responses seen in this study. In order to determine the mode of action of the antioxidants in SCFP, future research should measure plasma concentrations of other indicators of oxidative status such as antioxidants

glutathione peroxidase, glutathione, and vitamin C, and oxidants such as the active form of vitamin E, α-tocolpherol and hemoglobin. This may shed more light on why the cows supplemented with SCFP may have experienced oxidative stress on d 21 after calving.

In this study I speculate that the low starch diet was especially beneficial because the change from the close-up diet to fresh diet may be less drastic than a high starch fresh diet. This may be why I saw the response in mRNA abundance of IRAK1 in rumen tissue d 21 after calving due to a low starch fresh diet. In this study we fed a basal controlled energy close-up diet to all cows. We could not address the effects of a high energy close-up diet in combination with a low starch fresh diet on mRNA abundance of genes in rumen tissue. Future research should address if the benefit seen when feeding a controlled energy close-up diet may be seen when feeding a high energy close-up diet.

Saccharomyces cerevisiae fermentation products have multiple components such as the components of yeast, β-glucans and mannitol, and vitamins and antioxidants. These components may interact to have the effect seen on Hp, or they may act separately. Future research should address the mode of action of SCFP on the acute phase response. In order to test this question all components of the diet should remain the same except for the experimental focus, which is the addition of β-glucans, mannitol, and the vitamins and antioxidants found in SCFP. Specifying which component had the positive effect of the fermentation product on inflammation would enable the development of specialized products to target specific immune processes. For instance, if one component were able to manipulate the acute phase response by reducing systemic inflammation after calving this would benefit the animal by improving health and productivity, such as the results seen in this study.

4.4 Industry Implications

I would suggest supplementing SCFP during the transition period because SCFP supplementation decreased systemic inflammation d 7 after calving. This may have coincided with increased feed intake at d 1 and 5 after calving and a smoother increase of DMI after calving (Shi et al., 2018a). This is beneficial to the cow because it can reduce the extent of NEB. Negative energy balance is associated with reduced fertility (Leroy et al., 2008). Another benefit

may be the reduction in incidence of SARA. Constant feed intake, rather than intermittent and fluctuating intake, may prevent a reduction in rumen pH (Krause and Oetzel, 2006). A reduction in incidence of SARA may reduce immune system activation and improve cow health.

Feeding a low starch diet for the first three weeks after calving was beneficial and increased apparent total tract NDF digestibility. In order to reduce dietary starch content, I replaced rolled barley grain with beet pulp. Reducing dietary starch content by replacing grain with a highly fermentable byproduct feed such as beet pulp may reduce costs associated with grains, and increase apparent total tract NDF digestibility. This increase in digestibility may have contributed to the increase in milk yield seen in cows fed a low starch diet after calving (Shi et al., 2018a).

When a dairy producer is feeding a controlled energy close-up diet, feeding a low starch fresh diet for the first three weeks after calving may decrease immune activation in rumen tissue after calving. Feeding a controlled energy close-up diet in combination with a low starch fresh diet may decrease the incidence of SARA by reducing the impact of the dietary change from the close-up period to the fresh period on the rumen. This combination of diets may have reduced concentrations of LPS in rumen fluid after calving, and reduced activation of immune system. Reducing immune activation in rumen tissue at week 3 after calving may have potential benefits including reduced glucose use by the immune system and reduced strain on the animal's ability to produce glucose.

4.5 Conclusion

In conclusion, reducing dietary starch content and supplementing with SCFP had positive effects on the animal. Inflammation was reduced when supplementing SCFP. Reducing dietary starch content increased apparent total tract nutrient digestibility on d 7 after calving and decreased immune system activation in rumen tissue on d 21 after calving. These effects will be beneficial on a commercial operation because animals may be healthier than cows not supplemented with SCFP or fed a high starch diet.

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