Effects of *Saccharomyces cerevisiae* fermentation products (SCFP) and subacute ruminal acidosis (SARA) on feed intake, milk production and component, and fermentation in lactating dairy cows

By

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ABSTRACT

The effects of two *Saccharomyces cerevisiae* fermentation products (SCFP) and subacute ruminal acidosis (SARA) challenges on the dry matter intake (DMI), milk production, feed efficiency (FE), calculated as the ratio of the FCM and the DMI, rumen fermentation, and feces composition were determined in 32 lactating Holstein cows. Cows were assigned to four treatments including three SCFP supplementations as follows: i) control; ii), ii)14 g/d Diamond V Original XPC™ (XPC, Diamond V, Cedar Rapids, IA), iii) 19 g/d NutriTek® (NTL, Diamond V, Cedar Rapids, IA), or iv) 38 g/d NutriTek® (NTH, Diamond V, Cedar Rapids, IA) that started 28 d before the expected calving dates. Cows were monitored from wk 4 to wk 9 of lactation. The volume and kinetics of liquid rumen digesta were determined during wk 11. SARA challenges were conducted during wk 5 and wk 8 by switching from a moderate starch (18.6 % DM) to a higher starch (27.9 % DM) diet. These challenges increased the DMI, and the yield of milk and milk protein, but reduced the milk fat yield, the 3.5% fat corrected milk yield (FCM), and feces pH. The challenges also increased the rumen concentrations of propionate, butyrate and total VFA, and reduced those of acetate and ammonia-nitrogen. The SARA challenges only increased the duration of the rumen pH below 5.6 to more than 180 min/d, which was considered the threshold for SARA, in cows receiving the Control, XPC, and NTL treatments. However, even under these treatments, the rumen pH depression was mild. The first and second SARA challenge did not differ in the severity of the depression of rumen and feces pH, but the FE was lower during the second SARA challenge than during the first. The volume and kinetics of liquid rumen digesta were not affected by SCFP treatment.

Fecal pH was the lowest during the NTH treatment, suggesting this treatment shifted the fermentation of starch out of the rumen. The NTL and NTH treatments

reduced milk yield, the XPC treatment had a lower milk fat content than the NTH treatment, and the NTL treatment tended to reduce the FCM. Despite this, the SCFP treatments did not affect the FE, calculated as the ratio of the FCM and the DMI. Results show that the NTH treatment attenuated adverse impacts of grain-based SARA on the conditions in the rumen, without reducing milk production, and that repeated SARA challenges may increase the effect of such a challenge on the FE.

DEDICATION

This thesis is dedicated to my parents, Eslam Khalouei and Fatemeh Khayrali, who are the most valuable people in my life.

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FOREWORD

This thesis is formed based on the manuscript format and consists of one research manuscript.

This manuscript has been written in the formal of the Canadian Journal of Animal Science. The manuscript is: Khalouei, H., V. Senaratne, K. Fehr, J. Guo, I. Yoon, E. Khafipour and J.C. Plaizier. 2020. Effects of Saccharomyces cerevisiae fermentation products (SCFP) and subacute ruminal acidosis (SARA) on feed intake, fermentation and nutrient digestibilities in lactating dairy cows. The manuscript has been accepted for publication by the Canadian Journal of Animal Science.

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LIST OF ABBREVIATIONS

VFA	Volatile Fatty Acids
SARA	Subacute Ruminal Acidosis
LPS	Lipopolysaccharide
SAA	Serum Amyloid A
Hp	Haptoglobin
DM	Dry Matter
DMI	Dry Matter Intake
LBP	Lipopolysaccharide Binding Protein
SCFP	*Saccharomyces cerevisiae* Fermentation Products
NFC	Non-fiber Carbohydrates
MFD	Milk Fat Depression
NDF	Neutral Detergent Fiber
peNDF	Physically Effective Neutral Detergent Fiber
TMR	Total Mixed Ration

PSPS	Penn State Particle Separator
NRC	National Research Council
MgO	Magnesium Oxide
MON	Monensin Supplemented Cows
DIM	Days in Milk
TNF-α	Tumor Necrosis Factor Alpha
OTU	Operational Taxonomic Units
GPx	Glutathione Peroxidase
SOD	Super Oxide Dismutase
MDA	Malondialdehyde
SEM	Standard Error of Mean
FE	Feed Efficiency

Chapter 1: General introduction

High yielding dairy cows require energy-rich diets to meet their high energy requirements. These diets contain large amounts of starch, which is fermented in the rumen and leads to a large production of volatile fatty acids (VFA) and lactate. If the production of these organic acids exceeds the ability of the cow to clear them from the rumen and neutralize them, then they accumulate in the rumen and cause a pH depression that may lead to subacute ruminal acidosis (SARA) (Allen, 1997; Plaizier et al., 2008; Plaizier et al., 2018).

SARA is a common nutritional disorder in dairy farms that has a large economic impact on the dairy industry. Stone (1999) showed that each case of SARA in dairy cows in the United States costs about $1.12 USD/cow/d due to reduced milk fat and milk production, and increased culling. Plaizier et al. (2008) estimated that each case of SARA costs $400 CAD to Canadian dairy farmers as a result of educed milk production, reduced milk fat. and increased veterinary costs.

SARA is not only a costly disorder, but it is also very common on dairy farms. There have been various reports regarding the prevalence of SARA. Estimates of this prevalence are above 33% of dairy cows in Italy (Morgante et al., 2007), 13.8% in Netherland (Kleen et al., 2009), 27.6 % of Iranian dairy cows (Tajik et al., 2009) and 20% of lactating dairy cows in northern Germany (Kleen and Cannizzo, 2012).

There have been several definitions for SARA. Cooper et al. (1997) defined SARA as a rumen pH between 5.2 to 5.6. Another definition of SARA is a drop in the rumen pH to values below 5.8 (Beauchemin et al., 2003), as such a drop would compromise fermentation of fibre in the rumen. Gozho et al. (2005) defined SARA as a condition during which the rumen pH stayed between 5.2 to 5.6 for more than

180 min/d. This definition is based on the activation of the immune system that occurs during such a rumen pH depression. These researchers reported that if the rumen pH stays between 5.2 to 5.6 for such a duration, the concentration of lipopolysaccharide (LPS), an endotoxin in the outer membrane of the gram-negative bacteria, in rumen digesta increases, and that this results in inflammation. The SARA challenge in the study of Gozho et al. (2005) indeed increased the duration of the rumen pH below 5.6 to 180 min/d, increased the concentration of LPS in the rumen, and increased the concentrations of acute phase proteins, including serum amyloid A (SAA) and haptoglobin (Hp), in peripheral blood. These results are consistent with the findings of other reports (Zebeli et al., 2010; Li et al., 2016; Guo et al., 2019).

Other adverse effects of SARA on cow health and productivity include reduced and erratic dry matter intake (DMI), reduced fiber digestion, reduced milk and milk fat production, diarrhea, laminitis, inflammation and increased involuntary culling (Kleen et al., 2003; Krause and Oetzel, 2005; Plaizier et al., 2012).

Bauman and Griinari (2003) reported that a decline of the rumen pH alters the biohydrogenation pathways of the fatty acids in the rumen and increases the concentration of trans-octadecenoic acids in the rumen, such as *trans*-10 *cis*-12 C18:2. These fatty acids inhibit *de novo* milk fat synthesis in mammary glands. The reasons for decreased or erratic DMI during SARA include a decreased rumen pH, increased osmolality and VFA concentrations in the rumen (Kleen et al., 2003). However, despite observing a low rumen pH and increased rumen osmolality and VFA concentrations, Khafipour et al. (2009a) reported that SARA had no effect on DMI.

In order to reduce negative effects of SARA, various nutritional and management strategies have been used. These include feeding total mixed rations that prevent cows from sorting and ingesting excessive amounts of concentrate (Oetzel, 2007). Not only the mixing of the ration is important, but also meal size and meal frequency play a crucial role in preventing SARA. A reduction in the rumen pH after each meal is common, as fermentable carbohydrates enter the rumen and are subsequently fermented by the microbiota. Hence, increasing the meal frequency and decreasing the meal size spreads out the amount of organic matter that enters the rumen during the day, and reduces the diurnal variation of the rumen pH (Allen, 1997).

Buffers are another tool that used on dairy farms to prevent SARA in dairy cows. Sodium bicarbonate and magnesium oxide are common buffers that are supplemented to dairy cows (Erdman, 1988). The latter researcher reported that bicarbonate increases the DMI, milk production and milk fat yield in dairy cows, when the main forage source of the diet was corn silage. However, the results were inconclusive when the grass/legume silages were used as the main source of forage in the diet. The reason for this might be that diets with corn silage contain less effective fiber and protein that stimulate rumen buffering than grass/legume forage sources, and thus, supplementing the dairy cows with the buffers would have greater impacts in corn silage-based diets.

Other feed additives that have been used in dairy cow diets are yeast products. These products are available in two types, i.e. live yeasts and yeast culture fermentation products. One of the common yeasts that is used in the dairy industry is *Saccharomyces cerevisiae* (McAllister et al., 2011; Allen and Ying, 2012).

Studies show that adding yeast products, both live yeast and yeast culture fermentation products, increase the growth and activity of fiber digesting bacteria, stabilize rumen pH, and prevent SARA in dairy cows (Callaway and Martin, 1997; Chaucheyras-Durand et al., 2008; Poppy et al., 2012). It is believed that these beneficial effects are caused by components that these products contain, including organic acids, amino acids, polyphenols, and B vitamins that benefit the fiber-digesting bacteria, increase the fiber digestion, reduce the starch digestion rate and prevent SARA (Callaway and Martin, 1997). De Nardi et al. (2016) reported that adding polyphenols to the diet of cows that receive high grain diets increased the uptake of lactate, diversity and richness of the microbiota in the rumen, and reduced the time < 5.6 in the reticulum from 199 min/d to 18 min/d. They also reported that cows that were supplemented with polyphenols, compared to the control group, showed a reduced inflammatory response during a high grain feeding challenge (De Nardi et al., 2014). In the latter study, the concentration of the acute phase proteins SAA and lipopolysaccharide-binding protein (LBP) was lower than that of the control group. However, the concentration of haptoglobin (HP) did not differ between treatments. These researchers did not explain the reason for the lower inflammatory response in polyphenol supplemented cows, but a modified rumen fermentation pattern towards a reduced lactate concentration and a higher rumen pH may have caused this effect. In a study by Longuski et al. (2009), supplementation with 56 g/d of *Saccharomyces cerevisiae* fermentation product (SCFP) (Diamond V XP, Diamond V Mills, Cedar Rapids, IA) reduced the milk fat content in the cows that were challenged with a high starch diet. The mechanism behind this effect was not understood by these authors. However, the reduction in the rumen LPS concentration in SCFP-supplemented cows observed in a subsequent study by Li et al. (2016) may explain why these cows had higher milk fat yields than non-supplemented cows. Zebeli and Ametaj. (2009) reported a negative correlation between the rumen LPS concentration and milk fat content. These authors suggested that the body requirement for lipids in order to neutralize the LPS by binding to lipoproteins inhibits the availability for the precursors for *de novo* milk fat synthesis in the mammary gland.

In meta-analyses by Poppy et al. (2012) and Desnoyers et al. (2009), supplementation with live yeast and SCFP increased the DMI, milk yield, and milk fat yield in dairy cows. However, other researchers did not find such effects (Ali-Haimoud-Lekhal et al., 1999; Lescoat et al., 2000; Sauvant et al., 2004). These inconsistent results could be due to various factors including the number of animals per treatment, variation in diet composition, stage of the lactation, and dose of supplementation.

In a recent study by our group (Li et al., 2016), supplementation with 14 g/d of SCFP (XPC, Diamond V, Cedar Rapids, IA) reduced the variation in the rumen pH during control feeding, tended to reduce the LPS concentration in the rumen, increased the milk fat percentage, and reduced the inflammatory response in dairy cows by reducing pro-inflammatory cytokines in the peripheral blood circulation. The authors did not explain the mechanism behind the mode of action of these products. Nevertheless, the role of this SCFP supplementation in reducing the rate of starch digestion and increasing the functionality and abundance of fibrolytic bacteria will benefit the dairy cows (Allen and Ying, 2012).

We, therefore, aimed to conduct an experiment to investigate the effects of a new SCFP product (NutriTek®, Diamond V, Cedar Rapids, IA), which is claimed to contain more polyphenols and other beneficial components that other SCFP, on lactating dairy cows during normal/control feeding and grain-induced SARA challenge. In most of previous experiments, SARA challenges were conducted for a short period of time, which may not reflect the situation on dairy farms on which cows undergo SARA for prolonged periods. Supplementations with SCFP is expected to have greater impacts when the SARA challenge is more severe (when the pH stays below 5). Therefore, we decided to challenge dairy cows with two successive bouts of high-grain SARA challenge, in order to determine if a second

SARA challenge is more severe than the first. We also intended to determine if supplementation with SCFP has a greater effect during the second challenge compared to the first.

Chapter 2: Literature review

2.1 What is Sub-acute Ruminal Acidosis (SARA)? (definition)

Subacute ruminal acidosis is a common nutritional disorder in dairy cows, especially during the transition period and early lactation, in which the rumen pH drops reversibly to undesirable physiological levels for prolonged periods. However, there have been debates regarding the pH value that is considered as the threshold for SARA. This is partially due to the effects of the method of rumen sampling on the observed pH. For instance, a study by (Duffield et al., 2004) demonstrated that the rumen pH values differ among rumen sampling methods. They reported that the pH value was lowest when samples were collected using rumenocentesis. Also, they observed that the pH of rumen samples collected using a stomach tube and through rumen cannula were 0.35 and 0.33 pH units higher, respectively, than those of samples obtained by rumenocentesis. Thus, based on these findings, they suggested rumen pH thresholds of 5.5 when the rumen samples are collected by rumenocentesis, 5.8 when using rumen cannula, and 5.9 when using a stomach tube for diagnosis of SARA.

Cooper et al. (1997) defined SARA as the daily duration that rumen pH drops between 5.2 and 5.6. In contrast, studies have used a rumen pH below 5.8 as indicative of SARA (Beauchemin et al., 2003). More important than the pH threshold, is the time that rumen pH stays below these thresholds, as the duration that rumen pH stays below that threshold determines the negative effects of the rumen pH drop on the concentrations of free lipopolysaccharide (that comes from dead bacteria or released in the rumen during reproduction) in the rumen and blood, as well as the inflammation and the concentrations of acute phase proteins and cytokines in peripheral blood (Plaizier et al., 2012). It should be noted that diurnal 0.5 to 1 unit shift in rumen pH is expected during a day (Bergman, 1990).

Gozho et al. (2005) defined SARA by a drop in rumen pH below 5.6 for more than 180 min/d. These authors observed that the cows that met this criterion, showed reduced feed intake, and had inflammatory responses (e.g. elevated blood concentrations of interleukins and acute phase proteins).

2.2 Mechanism behind SARA and changes in rumen fermentation (How SARA occurs)

The rumen pH decreases when the amount of organic acids in the rumen, mostly VFAs and lactate, exceeds the capacity of the animal to clear and buffer these products (Krause and Oetzel, 2006; Plaizier et al., 2008).

It has been shown that the rumen pH of cows fluctuates throughout the day, and that a diurnal 0.5 to 1 unit shift in rumen pH is normal (Bergman, 1990). A part of this variation of the rumen pH is due to ingestion of fermentable organic matter during meals (Nocek et al., 2002). The severity of rumen pH depression after a meal is related to the amount of fermentation, as well as balance between acid production and clearance from the rumen. These factors depend on the DMI, and specifically on the amount of non-fiber carbohydrates (NFC) that an animal ingests, the rumen flow rate, and the dietary fiber composition (Plaizier et al., 2008). Increases in DMI, and, therefore, in the intake of NFC, lead to an increase in fermentation, and, subsequently, a reduction of the rumen pH (Oetzel et al., 1999).

High yielding dairy cows have been selected for a high milk production over last decades (Oltenacu et al., 2010). This has led to a shift from high forage diets to high grain diets to support their high energy and nutrient demands (Grummer et al., 2004). High concentrate diets contain large amounts of readily fermentable carbohydrates that are fermented by rumen microbiota that produce large amounts of VFAs and lactic acid in the rumen (Mertens, 1997). Lactic acid is 10 times stronger than VFAs. The pK_a = 4.8 for SCFPs while it is 3.86 for lactate [(where pK_a is $-\log(K_a)$ and K_a is the acid dissociation constant] . Also, the absorption rate of lactate is lower than other VFAs (Williams and Mackenzie, 1965). This means that accumulation of lactic acid leads to a greater rumen pH depression compared to accumulation of VFAs (Krause and Oetzel, 2006). The absorption rate of these organic acids by rumen papillae is another important factor that affects the rumen pH. A higher rate of absorption, when the VFA production rate is steady, means less accumulation of organic acids in the rumen, less acidity of rumen content and, therefore, a lower risk of SARA (Stone, 2004). Hence, cows with a high capacity of VFA absorption are at a low risk of SARA. The absorption process occurs through the rumen wall, which is covered by rumen papilla. These papillae increase the rumen surface and, therefore, the longer and denser the rumen papillae are, the higher their absorptive capacity is (Dirksen et al., 1985). These researchers also observed that the absorptive capacity of rumen papillae is higher when high grain diets are fed, comparted to when high forage diets are fed. This shows that the rumen papillae are adaptive and responsive to the composition of the dairy cow's diet. Hence, it is recommended that cows are gradually shifted from a high forage diet during the far-off dry period to a high grain diet after calving. This would help the rumen papillae to adapt to high grain diets and reduce the risk of SARA (Steele et al., 2009; Penner and Aschenbach, 2011).

The mechanism of VFAs absorption and stabilization of the rumen is shown in Fig 1.

As it is shown in this picture, part of the VFAs pass through the rumen epithelia to the cells via passive diffusion which is based on the lipophilicity of the epithelial membrane (Number 1). This pathway also facilitates the removal of protons from the rumen. The undissociated VFAs will then be dissociated in the cell. The released proton can react with the HCO3− and produce CO2 and H2O (3), be expelled by the Na+/H+ exchange back to the rumen (6) or to the blood (7) or coupled with VFAs metabolites and transferred to the blood circulation. The dissociated SCFA− in the epithelial cell will then go through one of four pathways; entering to the blood circulation through a basolateral ion channel (8), exchange with the HCO3− via ion exchange protein (9), combine with the free protons in the cytoplasm and make the undissociated HSCFA which will enter to the blood circulation via lipophilic diffusion (10), or metabolize into ketone bodies and co-transferred to the blood (11). The Na+/K+-ATPase is a key transport element required for the establishment of electrochemical gradients across epithelia (12).

The dissociate $SCFA^-$ in the rumen can also be exchanged with HCO_3^- by an anion exchange protein to stabilize the rumen pH (2). Lactate anions and protons in the rumen can enter to the cell via co-transportation (5).

During SARA, the amounts of the VFAs that are produced in the rumen, exceed the capacity of the rumen to clear them, thus the VFAs will be accumulated in the rumen and reducing its pH.

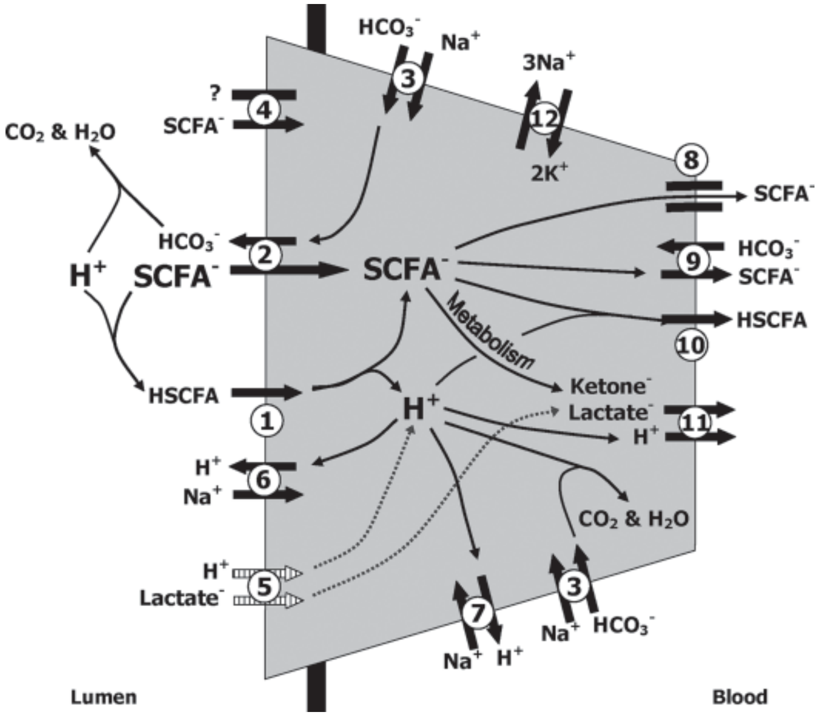

Figure 1. Model of organic acid transport in ruminal epithelial cells.

On the lumen-directed apical membrane, lipophilic diffusion of undissociated short-chain fatty acids (HSCFA; 1) is an efficient way to import acids with high lipophilicity (especially butyric acid) into the cell. These acids rapidly release their protons once inside the cell. All 3 short-chain fatty acids (SCFA; i.e., acetic, propionic, and butyric acids) additionally use transport proteins that exchange their anions (SCFA–) with HCO3 – for apical uptake (2). The SCFA–/HCO3 – exchange is especially important for acids with less lipophilicity, such as acetate, and is largely driven by HCO3 – imported from the blood via Na+/HCO3 – cotransport (3). The apically exported HCO3 – neutralizes 1 proton in the rumen; therefore, lipophilic diffusion and SCFA–/ HCO3– exchange have a largely comparable contribution to ruminal proton removal. The acetate anion may additionally use a poorly characterized apical uptake protein with undefined role for ruminal pH homeostasis (4). Lactate anions can enter the cell in cotransport with their protons (5). However, the responsible monocarboxylate cotransporter has an extremely low functional activity in roughage-adapted sheep and its quantitative importance remains to be determined. Protons taken up with HSCFA or lactic acid can either be neutralized by HCO3 – from basolateral Na+/HCO3 – cotransport (3) or expelled by Na+/H+ exchange across the apical (6) or basolateral membrane (7). The pathways of basolateral exit of SCFA have not been evaluated in detail but are likely to include an anion channel permeable to large anions (8), SCFA–/HCO3 – exchange (9), and lipophilic diffusion (10). Of these, SCFA–/HCO3 – exchange and lipophilic diffusion would contribute to net removal of protons from the epithelial cell into the blood. Butyrate is metabolized extensively to ketone bodies (i.e., β-hydroxybutyrate, acetoacetate) and propionate is metabolized partly to lactate inside the cells. These metabolic products and possibly apically imported lactate are expelled together with their protons across the basolateral membrane via monocarboxylate transporter 1 (MCT1) (11). All Na+-driven transport mechanisms are energized by the Na+/ K+ ATPase (12) at the basolateral membrane. The electrogenic efflux of Na+ via this pump requires a neutralizing efflux of anions through the basolateral anion channel (8). The HCO3 – and H+ pool inside ruminal epithelium is partly replenished from CO2 by carbonic anhydrase reaction (G.bel et al., 2002), which is not shown in the current model for clarity reasons. Adapted from (Stumpff et al., 2011). Published with the writer's permission.

2.3 Clinical signs

The clinical signs of the SARA are not readily apparent. Hence, the diagnosis and detection of SARA is difficult, since there is a gap between the time that SARA occurs in the rumen and the time that its clinical signs appear (Plaizier et al., 2008, 2012). Therefore, since diagnosis of SARA in farm condition is impossible, the SARA condition could not be treated. There are indicators in dairy cows that are considered as tools for the diagnoses of SARA, such as erratic DMI, laminitis, and reduced milk production and milk fat yield.

DMI has been used as a tool for SARA diagnosis in dairy cows It is reported that SARA can decrease the DMI (Kleen et al., 2003). It is assumed that an increased propionate concentration in rumen digesta results in increased blood glucose and a reduced appetite, and that a high osmolality of rumen fluid due to higher concentrations of VFAs also leads to a lower DMI (Allen, 2000; Gozho et al., 2006).

Activation of the immune system and inflammation may also reduce feed intake during SARA (Andersen, 2000). It has been reported that SARA leads to inflammation and elevated concentrations of acute phase proteins such as LBP, Hp and SAA, whose plasma concentrations increase during inflammation and are part of innate immune system, in blood (Gozho et al., 2006, 2007; Guo et al., 2019), and that inflammation suppresses feed intake (Andersen, 2000). This was also shown by Khafipour et al. (2009b) who induced SARA by feeding alfalfa-pellets in dairy cows. Whereas, the concentrations of rumen VFAs, rumen pH and the osmolarity of the rumen were similar to those during grain-based SARA challenges, the alfalfa pellet-based SARA did not cause inflammation and did not decrease the feed intake.

The redox potential (E_h) is another factor which is related to diet composition and DMI. This reducing condition in the rumen is essential for the function of fibrolytic bacteria (Marden et al., 2008; Pinloche et al., 2013). Reports have shown that feeding high concentrate diets increases the E_h from -210 to -171 in heifers (Monteils et al., 2009), and in dairy cows (Michelland et al., 2011; Friedman et al., 2017; Julien. 2011). The latter researcher observed the lowest E_h value in the rumen when the pH was the highest. Therefore, not only the rumen pH and DMI are related, but also the redox potential of the rumen is related to the DMI and diet composition in dairy cows.

A relationship between SARA and laminitis has also been reported (Stone, 2004). However, the exact mechanism behind this is not well understood. It has been proposed that feeding high grain diets results increases the concentrations of histamine and biogenic amines that increase the risk of laminitis (Nocek, 1997). SARA also increases the concentrations of methylamine which may be converted to formaldehyde and hydrogen peroxides and can further damage the capillaries in the hoof and cause laminitis (Saleem et al., 2012, 2013). High starch diets increase the production of histidine, which is converted to histamine in the rumen by *Allisonella histaminiforman* (Garner et al., 2002). Histamine also degrades hoof capillaries, which causes bleeding that leads to laminitis (Garner et al., 2002).

Milk fat depression (MFD) is also one of the signs that farmers monitor as indicator of SARA (Mertens, 1997). High starch diets that result in a low ruminal pH have been shown to induce MFD. A low ruminal pH causes incomplete biohydrogenation of fatty acids in the rumen, and increases the production of trans isomers of fatty acids, such as the *trans-10*, *cis*-12 C18:2 isoform, which inhibits *de novo* milk fat synthesis (Peterson et al., 2003).

Diarrhea is another symptom that SARA can cause in dairy cows (Oetzel, 2003). Large amounts of starch in high-grain/low-fiber diets may bypass digestion in the rumen and small intestine and reach the hindgut. Excessive starch fermentation in the hindgut leads to increased osmolality and acidity of hindgut digesta (Hall, 2002). This may damage hindgut epithelia and disturb its barrier function. Due to a high viscosity of the hindgut digesta, water flow from the blood to the hindgut will occur, which results in watery feces. Also, to protect itself, the hindgut will release more mucus to limit the damage to the epithelia (Argenzio et al., 1988), and, therefore, the feces contains mucus during periods of SARA.

Reduced fiber digestion and increased forage particles in the feces are also among the consequences of SARA. In a study by Plaizier et al. (2001), the NDF degradability of forages in the rumen was reduced due to high grain feeding and reduced rumen pH by between 19.6 and 21.8%. The reason for this was assumed to be the reduced abundance and functionality of fibrolytic bacteria in the rumen that are not resistant to rumen pH values below 6.0 (Shi and Weimer, 1992).

2.4 Prevalence and cost

Several studies have reported the prevalence of SARA in dairy herds. In a study of more than 10 dairy herds in Italy, over 33% of the cows in three of the herds had rumen pH values lower than 5.5, and were considered as SARA cases (Morgante et al., 2007). In another study by Kleen et al. (2009) using the rumenocentesis technique, 13.8% of the monitored dairy cows in 18 dairy herds in the Netherlands were diagnosed with SARA. Also, in a study in Iran on 10 dairy farms, the prevalence of SARA was reported as 27.6% (Tajik et al., 2009). Kleen et al. (2013) monitored 26 dairy farms and 315 dairy cows and reported that 20% of the cows were experiencing SARA. In these studies, the rumenocentesis technique and a threshold of 5.5 was used to diagnose SARA. The difference in prevalence of SARA in different countries could be due to many factors such as the farming system (Grazing or intensive), feeding management and strategies and many other factors.

Due to the invasive and expensive nature of the current diagnostic methods, diagnosing SARA is difficult. Also, common clinical signs of SARA, such as laminitis, milk fat depression, and diarrhea, are non-specific and their effects on animal health, production and the economic impact are difficult to ascertain. As a result, few studies have estimated the costs of SARA in dairy cows. In a study in United States by Donovan. (1997), the economic loss due to SARA was reported to be between US$ 500 million to US$ 1 billion per yr. Stone. (1999) studied 500 dairy cows in New York state, and estimated that SARA resulted in a loss of US$ 400 to US$ 475 per cow per yr. The latter study was based on the losses of reduced milk production, milk fat and true protein, and did not consider the economic losses due to lameness and its negative effects on reproduction, which may even be higher than those of reduced milk production.

2.5 Factors that affect the acidity of rumen contents

Dairy cows try to regulate their rumen pH in different ways, such as by increasing or decreasing DMI, buffering the rumen by increasing saliva secretion, and absorbing the acidic products of fermentation in the rumen. It is notable that the ability of the animal to stabilize rumen pH is limited, and when the amount of fermentable carbohydrates exceeds the capacity of the animal to absorb, clear, and neutralize the VFAs, the rumen pH declines (Krause and Oetzel, 2006). Beside this, other factors also affect the rumen pH, and can affect the severity of rumen pH depression. These are discussed below.

2.5.1 Amount and type of the cereals

High concentrate diets that are common in the dairy industry include large amounts of grain. These grains, mostly corn and barley, have high contents of non-structural carbohydrates, such as starch and sugars, which are highly fermentable. High intakes of NFC therefore increase the risk of SARA by production of large amounts of VFAs in the rumen.

The potential of a feed ingredient in causing SARA mostly depends on its starch and sugar contents and their digestibility in the rumen. As Sauvant et al. (1999) reported, there is a strong negative correlation (r = -0.97) between the rumen pH and the content of rumen digestible starch of the feed ingredients. In descending order based on the starch contents of common grains are: wheat (78%), corn and sorghum (72%), and barley and oat (58%) (Beauchemin et al., 2009). Not only the starch content of grain is important, but also the degradability of its starch in the rumen determines its potential for causing SARA (Callison et al., 2001).

In the feed industry, grains are processed in different ways, including grinding, roasting, steam-flaking and extrusion, in order to increase their starch availability. These methods not only increase the digestibility of feed ingredients, but can also increase the risk of SARA.

Based on their degradability rate, from the highest to the lowest, the grains are classified as oats, wheat, barley, high moisture corn, ground corn, and ground sorghum (Huntington, 1997; Offner et al., 2003). Many studies have reported that ruminal starch digestibly vary from 30% to 100%, due to the type of the grain and the type of processing. In agreement, Yang et al. (2001) observed a reduction in ruminal pH due to increased processing of barley (steam-rolled coarse, medium-flat, or flat-thickness) in lactating dairy cows. Therefore, assessing the amount of

NFC in a diet as a single factor to predict the risk of SARA can be misleading, since different processing methods may lead to different fermentation rates in the rumen and potentially increase the risk of SARA.

2.5.2 Amount of the fiber and particle size

One of the big challenges of the feeding high yielding dairy cows is how to maintain the balance between feeding high energy/grain diets, which is important to achieve the highest production potential of dairy cows, and feeding coarse fiber that is crucial for the rumen health (Zebeli et al., 2012).

The fiber content of a ration, which mostly comes from the forages, can reduce feed intake due to rumen fill. However, a minimum amount of coarse fiber in the diet is required to stimulate rumination, saliva secretion, rumen buffering, and maintain rumen health. It has been shown that an inadequate amount of coarse fiber in the diet reduces digestion and energy supply of nutrients to the animal (Yang and Beauchemin, 2006).

The National Research Council (NRC, 2001) suggests a minimum of 25% of NDF in dairy cow rations is required when corn silage and alfalfa silage are included in the diet as forage sources and corn as the primary concentrate. At least 75% of this NDF should come from the forage. It is notable that not only the percentage of the NDF is important, but also the physical characteristics of this fiber are crucial for rumen health and milk fat production. In order to standardize the coarse fiber concept, (Mertens, 1997) introduced a new concept for dietary fiber, named physically effective NDF (peNDF). This measure not only accounts for amount of NDF of diet, but also considers the particle size of the feed. The peNDF is defined as the portion of feed that stimulates chewing activity (Mertens, 1997). This index is calculated from the NDF content of a feed ingredient and its physical effective factor. This value varies from 0 (when NDF does not have any physical effect, e.g. ground corn, to 1 when NDF completely contributes to chewing activity and rumen buffering, such as fiber in long chopped hay (Allen, 1997).

Corn silage is a common forage source for dairy cows, however, using excessive amounts of this forage increases the risk of SARA, compared to diets that contain hay as the major forage source. Corn silage is chopped into short particles to maximize the fermentation process during silage preparation, whereas hay is not chopped (Kononoff and Heinrichs, 2003). Hence, corn silage may not provide enough physically effective fiber to cows. To prevent deficiencies in peNDF, hay and straw are, therefore, commonly added to dairy cow rations.

Very long feed particles may reduce the fiber digestibility and feed intake (Tafaj et al., 2007), and increase sorting of concentrates and small forage particles against coarse feed particles. This is not desirable due to an increased risk of SARA. Furthermore, short particles (4 mm to 6 mm) do not provide enough peNDF to maintain chewing activity, saliva secretion and buffering capacity of dairy cows on common dairy diets that contain between 50% and 60% concentrate (DM basis) (Kononoff and Heinrichs, 2003; Zebeli et al., 2007).

In order to measure the peNDF content of a total mixed ration (TMR), Lammers et al. (1996) developed the Penn State Forage Particle Separator (PSPS). This separator is made of three different sieves, with 19 mm, 8 mm and 1.18 mm mesh sizes, and a bottom pan. Heinrichs and Kononoff. (2002) suggested that to achieve the required chewing activity, 2% to 8% of the feed should remain on the first screen (19 mm), 30% to 50% on the second screen (8 mm), and 30% to 50% on the lowest screen and the bottom pan. Schadt et al. (2012) found that feed particles longer than 8 mm, i.e. the particles that remain on the first and second screens of

PSPS, are long enough to contribute to chewing activity and development of the rumen mat, but not the particles retained by the 1.18 mm sieve. These results are in agreement with the findings of Zebeli et al. (2008), who found that feed particles with a length of between 10 and 15 mm increase the digestibility of the feed without negative effects on fiber digestibility and rumen function. In a meta-analysis by Tafaj et al. (2007), increasing the peNDF from 50 g/kg DM to 310 g/kg DM of the diet, increased the rumen pH from 5.7 to 6.27, but no additional pH improvement was observed when more peNDF was provided. This meta-analysis also showed that when the amount of rumen degradable starch in the ration increased, the need for more peNDF to stabilize rumen pH increased quadratically. This indicates that the ration's peNDF content and feed particle size distribution are crucial in terms of chewing activity and rumen buffering, but that other factors, such as type and amount of the cereal, need to be considered to reduce the risk of SARA.

2.5.3 Feeding strategies

Not only the composition and structure of the diet are important for the prevention of SARA, but feeding management can also impact the risk of this disorder. Several of these feeding management factors are discussed below.

2.5.3.1 Adaptation

Cows rely on the microbiota in their digestive tract to digest and harvest the energy from their diet. The composition and functionality of these microbiota are affected by the composition of the diet (Plaizier et al., 2017). For instance, it is shown that feeding high concentrate diets changes the rumen microbiota composition towards lower abundances of fiber digesting bacteria and protozoa, since these organisms are intolerant to low ruminal pH. Adapting the gut microbiota to high concentrate diets, by gradually increasing the contents of highly fermentable substances, can attenuate this negative effect. Schwaiger et al. (2013) conducted a study on sixteen Angus heifers that were assigned to two different treatments, including long adaptations (LA) and short adaptations (SA) to high concentrate diets. Animals on the LA and SA treatments received a diet with a forage to concentrate (F:C) ratio of 60:40 for 33 and 7 days, respectively. Subsequently, the heifers in LA and SA were fed high concentrate diets with a F:C ratio of 9:91% for 34 and 8 days, respectively. Following this, SARA was induced. This induction involved feeding heifers with 50% of DMI:BW (Body weight) for 24 hr followed by a rumen infusion of ground barley at 10% of DMI:BW. After 1 hr, the heifers were fed their regular diet. The result of this study showed that LA heifers had greater SCFA and lactate absorption rates after the SARA induction, compared to the SA heifers. This shows that adaptation plays an important role in the risk of SARA.

Another aspect of adaptation to high grain diets involves the rumen epithelium. As noted earlier, the rumen epithelia respond to an increase in grain feeding by tissue proliferation and molecular changes of the cells (Gäbel and Aschenbach, 2007). Shifting from a forage-based diet to grain-based diet increases the proliferation and growth of rumen papillae during the transition period around calving, thereby increasing the capacity for the absorption of VFA (Penner et al., 2006). Also, a rapid increase in concentrate inclusion of the diet after parturition increases the rate of epithelial proliferation, compared to a more gradually increase in concentrate (Bannink et al., 2012).

Rumen VFA also promote growth and proliferation of the epithelial cells in the digestive tract (Sakata and Tamate, 1978). Among the VFA, butyrate has the largest effect on this growth and proliferation, and it increases the expression of genes that are related to growth and apoptosis of these cells (Penner and Aschenbach, 2011).

Hence, both the level and the rate of increase of fermentable carbohydrates in diets determine the growth of rumen papillae, and an increased production of VFA promotes the growth of rumen papillae, which increases the surface area for the absorption of fermentation acids.

In studies by Dohme et al. (2008) and Pourazad et al. (2016), repeated SARA challenges resulted in more severe pH depressions, increased duration below pH 5.8 and higher concentrations of VFA in the rumen than the first SARA challenge. In the study by Pourazad et al. (2016), two SARA challenge models were applied to dairy cows. The cows in the first treatment group received a continuous SARA challenge for 28 days (Persistent treatment), while cows in the second treatment group (Transient treatment) had a 7-day break between SARA challenges during which they were fed a forage-based diet. These researchers reported that despite the cows in both groups experienced the SARA challenge, the cows in transient group were more severely impacted during the second challenge based on the lower daily average ruminal pH and duration $\leq$ 5.8 (5.93 vs. 6.15 and 497 vs. 278 min/day for transient and persistent group, respectively). Since the DMI in both treatment groups were equal, provision of more fermentable carbohydrate did not cause the more severe SARA in the permanent group. The authors concluded that the VFA absorption rate in the persistent group was higher than that of the transient group. They hypothesized that the persistent SARA challenge may cause the rumen epithelium to adapt in order to increase the rumen absorption capacity. An increased capacity of VFA absorption has been shown to increase the flow of endogenous bicarbonate to the rumen, which is important for rumen buffering (Dijkstra et al., 2012). Pourazad et al. (2016) also concluded that prolonged rumen challenges are more favorable to ruminal microbiota, as they can adapt to the altered rumen conditions, and, thereby, decrease the severity of SARA compared to transient SARA challenges.

2.5.3.2 Feeding frequency and management

Increasing the feeding frequency can also stabilize the rumen by reducing the daily variation of the rumen pH, thereby lowering the risk of SARA. When cows are fed more frequently, meals will be more spread out, which will prevent ingestion of excessive amounts of concentrates during meals. This also increases the DMI (DeVries et al., 2003).

The method of feed preparation is also an important practice in dairy farms. Several studies have shown that cows select in favor of fine and small feed particles, which include concentrates, and select against long particles that mainly consists of forages (Kononoff and Heinrichs, 2003). This behavior will lead to an increase in ingesting the highly fermentable part of the diet and reduce the intake of peNDF, which will result in insufficient chewing activity, saliva production, and rumen buffering to prevent SARA. Thereby, feeding a TMR ration that prevents cows from sorting is beneficial to rumen health.

2.5.4 Feed additives

Feed additives have been receiving attention during the last years as a tool to prevent SARA. There are different types of such feed additives in dairy industry that have been used in this regard. However, there has been debate about the effectiveness of these products.

2.5.4.1 Buffers

Buffers are solutions that prevent pH changes in their environment. The most common buffer used in ruminant nutrition is sodium bicarbonate. This buffer increases DMI, milk production and milk fat content (Erdman, 1988). These positive effects where observed when the diets were based on corn silage as the forage, but such effects were not observed when sodium bicarbonate was added to grass or legume silage-based diets (Erdman, 1988; Staples and Lough, 1989). The positive effect of sodium bicarbonate when corn silage-based diets are fed, might be due to the higher capacity of corn silage in lowering rumen pH compared to grass or legume silage. It means that, using buffers in diets that have marginal amounts of dietary fiber and peNDF, may be more effective than using them in diets that have a higher content of these fibers.

Another buffer that is commonly used in dairy nutrition is magnesium oxide (MgO). Erdman. (1988) reported that using MgO increased the milk fat percentage, and that the effect of this buffer increased as the fiber content of the diet decreased. In some studies, adding MgO to the diet increased the milk fat percentage, without showing an effect on rumen pH (Emery et al., 1965; Huber et al., 1969). These authors suggested that roles of MgO are also outside of the rumen, including the uptake of acetate and triglyceride by mammary cells and, thereby, attenuating the suppressive effect of a low rumen pH on milk fat (Emery et al., 1965; Huber et al., 1969).

2.5.4.2 Ionophores

The term ionophore was first used in 1969 and refers to all carboxylic polyethers that have antibiotic properties (Pressman, 1968). These compounds are fermentation products of several actinomycetes (Bagg, 1997). Ionophores have been shown to improve feed efficiency and weight gain in ruminants (Russell and Strobel, 1989).

The most common ionophore in use in the dairy industry is monensin, which has been shown to increase the milk production in dairy cows by 1.3 kg/d (McGuffey et al., 2001).

The fermentation process by gram-positive bacteria in the rumen results in the production of methane, whereas fermentation pathways of gram-negative bacteria are more associated with the production of propionate and succinate (Russell and Strobel, 1989). As a result, fermentation by gram-negative bacteria produce less methane, and results in a more energy efficient utilization of feed compared to fermentation by gram-negative bacteria. Since the bacterial membrane of gram-positive bacteria is more permeable than that of gram-negative bacteria, the former are more sensitive and susceptible to ionophores (Russell and Strobel, 1989). Therefore, using antibiotics and ionophores reduces the population of gram-positive bacteria in the rumen, thereby increasing the production of propionate, inhibiting the methanogens activity, and increasing the fermentation efficiency (Russell and Strobel, 1989). Bergen and Bates (1984) concluded that many lactate-producing bacteria are not resistant against ionophores. However, this is not the case for lactatefermenting bacteria, such as *Selemonas ruminantium* and *Megasphaera elsdenii*. Hence, ionophores stabilize the rumen pH, increase propionate production, and decrease the concentration of lactate in the rumen.

Ionophores also increase the glucose availability to, and, by sending the satiety signals to the brain, prevent the animal from ingesting excessive amounts of feed in a meal. As a result, ionophores aid cows on high grain diets to stabilize their rumen (McGuffey et al., 2001). Increases in feed efficiency and propionate production also provide more energy to the animal, and decrease the energy requirement from body storages, such as adipose tissues. Reductions in body fat mobilization and, therefore, lower fatty acid metabolism, decrease the accumulation of triglycerides in the liver and reduce ketone body production (Ospina et al., 2010). Thereby, monensin prevents ketosis. Monensin can also increase gluconeogenesis in the liver either by increasing the propionate flux to the liver or by increasing the glucogenic enzyme activity in the liver (Karcher et al., 2007).

The beneficial effects of ionophores are especially important during the transition period, as they improve feed efficiency, reduce ketosis, and enhance rumen health. This was demonstrated by Markantonatos and Varga (2017) who studied the effects of monensin on cows 4 weeks before to 8 weeks after parturition. They supplemented 300 mg/cow per day of monensin during the whole experiment. They found that the contribution of blood glucose from sources other than ruminal propionate decreased in monensin supplemented cows (MON) comparing with the control group after parturition (2.26 vs. 1.09 g/min for MON and control group, respectively). They also observed that while the portion of the blood glucose pool coming from rumen propionate was increased from 22% in control cows to 31% in MON group, glucose oxidation significantly decreased in MON supplemented cows (1.67 vs. 0.92 gr/min in control and MON cows, respectively). These authors, therefore, concluded that monensin benefits cows by increased glucose production from propionate, and by decreased glucose oxidation in body tissues. Therefore, monensin can be used as a tool to reduce the risk of SARA and improve feed utilization efficiency in dairy cows, by reducing the need for dietary grain.

2.5.4.3 Live yeast and yeast cultures

The use of live yeast and yeast products in dairy cows as a tool to stabilize rumen environment, improve rumen function and production has gained considerable interest during the last several decades (Bach et al., 2007; Li et al., 2016; Shi et al., 2019). The most commonly used yeast products are *Saccharomyses cerevisiae* and its fermentation products. It should be noted that in order to be considered as a live yeast product, these products should contain > 1×10^{10} CFU/g DM live cells.

Williams et al. (1991) reported that *S. Cerevisiae* prevents the accumulation of lactate in the rumen, thereby helping cows to stabilize the rumen pH. Chaucheyras-Durand et al. (2008) showed that *S. Cerevisiae* increases lactate utilization by *Megasphaera elsdenii* and *Selenomonas ruminantium spp*. in the rumen, and therefore, reduces the accumulation of lactate, prevents the pH depression and stimulates the activity of cellulolytic bacteria in the rumen. This finding was in agreement with other reports, which claimed that *S. cerevisiae* increases the number and activity of cellulolytic bacteria, increases the rumen pH, and improves rumen function (Chaucheyras-Durand et al., 2008, 2016; Al Ibrahim et al., 2012).

Reports of the effects of live yeasts on cow health and performance are not conclusive. Some researchers have not seen any effect of *S. cerevisiae* supplementation on rumen function and animal performance (Kalmus et al., 2009; Chung et al., 2011; Malekkhahi et al., 2016; Ambriz-Vilchis et al., 2017), while others observed an improvement in rumen health and production (Fonty and Chaucheyras-Durand, 2006; Bach et al., 2007). Ambriz-Vilchis et al. (2017) conducted a survey on the effect of live yeast supplementation on 14 dairy cows. They carried out three trials including adding yeast to: 1) a standard diet, 2) to an acidogenic diet, and 3) to grazing dairy cow diet. They did not observe any effects on milk production, milk composition, body weight and body condition score, rumination time, and rumen pH in any of the 3 trials. Adding live yeast to the diet of transition dairy cows from three weeks before calving up to 12 weeks after calving also did affect the rumen pH and the cow's performance in the study of Hasunuma et al. (2016).

In a meta-analysis by Desnoyers et al. (2009), it was concluded that adding live *S. Cerevisiae* to the diet increased rumen pH, DMI, milk yield and organic matter digestibility, and tended to reduce the lactate concentration in the rumen.

These inconsistent findings among studies could be due to multiple factors, including the stage of the lactation, sample size, dosage of yeast supplementation, and diet composition. For instance, supplementing *S. Cerevisiae* was more effective in diets containing high amount of grains (Erasmus et al., 1992; Jouany, 1999), or when a lot of samples were taken into account as in meta-analyses. Hence, positive effects of yeast product are more obvious when the statistical power of the analysis increases.

Yeast culture products are another product that are used as feed additives. The most common yeast in this regard are *S. Cerevisiae* fermentation products (SCFP). The exact mechanism behind the effects of SCFP on animal performance is not yet well understood. However, it is hypothesized that the soluble compounds in SCFP, e.g. B vitamins, organic acids, polyphenols and amino acids, benefit the cows by stimulating proliferation and activity of lactic acid utilizing bacteria (preventing the accumulation of lactate in the rumen), fibrolytic bacteria (increasing digestibility of fiber and thus increased DMI), as well as by increasing microbial protein synthesis and outflow to the intestine (Nisbet and Martin, 1991; Callaway and Martin, 1997; Hristov et al., 2010).

Similar to the results of using live yeast, results of the effect of SCFP on animal health and performance are inconclusive. Whereas some studies observed improvements in DMI, milk production and milk components (Williams et al., 1991; Piva et al., 1993), other studies did not observe effects when SCFP was supplemented to dairy cows (Robinson, 1997; Robinson and Garrett, 1999). Periods of heat stress during which DMI is reduced or transition periods during which cows are shifting from a high-forage diet to a high-grain diet, are challenging to the gastrointestinal tract and the cow's metabolism. Hence, it is expected that SCFP is especially beneficial to dairy cows during these periods.

Schingoethe et al. (2004), carried out a study on 38 dairy cows at 105 days in milk (DIM) and onwards. They added 60 g/cow/day of a SCFP (XP, Diamond V Mills Inc., Cedar Rapids, IA) for 12 weeks during heat stress (average temperature of 38°C). Although, no effects of SCFP was observed on milk production, DMI, 4% fat corrected milk, and energy corrected milk, SCFP supplemented cows had a 7 % higher feed efficiency expressed as kg energy corrected milk per kg of DMI. These researchers did not measure the diet's digestibility but suggested that an improvement in this digestibility was the reason for this higher feed efficiency. Similar to the latter study, Hristov et al. (2010) did not find any effects of SCFP supplementation (56 g/head per day; XP, Diamond V Mills Inc., Cedar Rapids, IA) on ruminal fermentation, nutrient digestibility, N utilization, milk yield, and milk composition in mid-lactation dairy cows.

Longuski et al. (2009) proposed that the level of the fermentable carbohydrates in the diet affects the cow's responses to SCFP. They found that SCFP supplemented cows only had significantly higher milk fat yield and 3.5% fat corrected milk yield when high amounts of fermentable carbohydrate were fed. Such effects were not observed when the dietary content of fermentable carbohydrate was low. Erasmus et al. (2005) also reported that the effect of SCFP on DMI depends on the level of DMI of the cows. In their experiment, adding 2550 ppm of SCFP (Diamond V Mills Inc., XP Yeast Culture, Cedar Rapids, IA, USA) to the diet of cows with a DMI lower than 23.8 kg/d decreased the DMI, but it increased the DMI of the cows with a DMI higher than 23.8 kg/d. A survey by Allen and Ying. (2012) showed an interaction between SCFP supplementation and the level of starch in the diet. In SCFP supplemented cows, the rate of rumen starch digestibility was reduced when the DMI of the cows increased. In contrast, in cows without SCFP supplementation. the rate of the starch digestion increased when DMI increased. These findings, suggest that SCFP can attenuate the negative effects of high starch diets in high yielding dairy cows, and also lowers the cost of the ration in cows with a low DMI? (Allen and Ying, 2012).

Castagnino et al. (2017) conducted an experiment to determine if supplementation with SCFP (Diamond V XP Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA) increases the synthesis of B vitamins in the rumen./ However, supplementing 56 g/d of SCFP, did not increase the apparent synthesis of B vitamins and the amount of these vitamins reaching the duodenum.

In a recent study by Li et al. (2016), SCFP supplementation (14 g/d of Original XPC, Diamond V, Cedar Rapids, IA) did not affect DMI, milk yield, milk fat and protein yield of lactating dairy cows, but it reduced the rumen pH variation during non-SARA conditions, and increased milk fat percentage during SARA. The more stable rumen pH could be due to less variable rumen microbiota, and a better balance between the production and clearance of organic acids in the rumen in SCFP supplemented cows (Li et al., 2016). The increase in milk fat production due to SCFP supplementation in this study agrees with an earlier experiment (Longuski et al., 2009) that showed that supplementation with 56 gr/d of SCFP attenuated the milk fat depression during a SARA challenge. The mechanism behind this could be a limiting effect of SCFP on the production of the unsaturated fatty acids in the rumen that inhibit milk fat synthesis (Bauman et al., 2008). Another reason may be the attenuating effect of SCFP on the rumen LPS concentration, as rumen LPS reduces the milk fat content (Zebeli and Ametaj, 2009).

Li et al. (2016) also reported SCFP- supplemented cows tended to have lower rumen LPS, and less immune system activation and inflammation. This was shown by the lower blood concentrations of cytokines in SCFP- supplemented cows. These authors hypothesized that the stabilization of rumen conditions could be the reason for the lower rumen LPS concentration in the SCFP- supplemented cows. In a recent study (Guo et al., 2018), induction of SARA reduced the richness and evenness of rumen microbiota in both the fluid and solid fractions, but these reductions were attenuated by SCFP (Diamond V NutriTek, Cedar Rapids, IA) supplementation.

A big issue with the studies on the effects of SCFP supplementation on the health and production of cows is the limited number of animals per treatment and the resulting low statistical power. Hence, when the data of multiple publications are combined and analyzed in a meta-analysis, the effects of SCFP supplementation on production variables become more obvious. For example, a meta-analysis by Poppy et al. (2012), found that supplementation with SCFP resulted in higher DMI, milk yields, milk fat and milk protein yields, and higher energy corrected milk yields (increases of 0.62 kg/d, 1.18 kg/d, 0.06 kg/d, 0.03 kg/d and 1.65 kg/d, respectively). Hence, not only the diet composition, stage of the lactation, and dosage of SCFP supplementation affect a study's outcome, but also the statistical power of an experiment also.

2.6 Effects of high grain diets on rumen microbiota and metabolism

As discussed earlier, feeding energy-dense diets alter conditions in the rumen and hindgut by increasing VFA concentrations, reducing the rumen pH and increasing the osmolality in digesta (Krause and Oetzel, 2005; Plaizier et al., 2012). These changes affect the composition and functionality of microbiota in the rumen and hindgut (Plaizier et al., 2017).

Hungate et al. (1952) reported that high grain feeding results in the accumulation of VFAs, reductions in the relative abundance of cellulolytic bacteria, increases in the relative abundance of gram-positive bacteria, and the death of protozoa in the rumen. These authors also proposed that *Streptococcus bovis* is responsible for the rumen pH depression during high grain feeding.

Delivery of fermentable carbohydrates to the rumen increases the proliferation of rumen microbiota that use these carbohydrates as substrates, and, thereby, increases the production of fermentation products such as VFAs. Key microorganisms involved in carbohydrate fermentation are bacteria and protozoa. Fungi also produce amylases (McAllister et al., 1993), but their abundance decreases during high grain feeding (Obispo and Dehority, 1992).

Feeding a high grain diet initially increases the number of ciliated protozoa in the rumen. However, when the amount of grain increases to a point that leads to a depressed ruminal pH, their number start to decrease, as they are sensitive to a low pH (Dennis et al., 1983). Ciliated protozoa have a predatory activity on bacteria, and, thus, have a controlling role in the populations of bacteria. As a result, they have an inhibitory effect on the rate of fermentation, organic acid accumulation, and stabilize the rumen environment (Mendoza et al., 1993).

It has been demonstrated that feeding high grain diets reduces the diversity and richness of rumen microbiota (Petri et al., 2013; Plaizier et al., 2017).

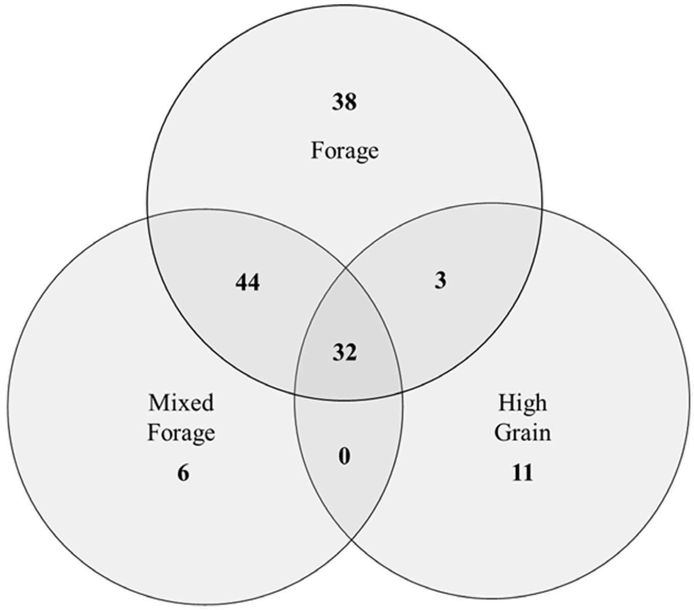

Figure 2. Number of OTUs in the rumen of the cows on different diets. Each circle shows the number of unique OTUs in the rumen, specific to the animals that consumed the specific diet. The numbers in overlapping area shows the common OTUs among the diets. Updated from Petri et al. (2013). Published with the writer's permission.

In the figure 2, it is shown that the number of operational taxonomic units (OTUs), defined as a groups of 16S RNA sequences with a 97% similarity (Konstantinidis et al., 2005)) is reduced in cows that are on high grain diets comparing to the cows that are consuming forage-based diets (Fig. 2).

A rich and diverse gut microbiota is indicative of a robust and functional microbiota and of a healthier host animal (Levin et al., 1999). When a microbial community is rich and diverse, elimination of some species from the environment due to a challenge like SARA, may not have a severe effect on the functionality of the whole microbiota, and the performance and health of the animals, since other species may functionally cover for the removed species. However, this may not occur in a less diverse and rich community. Also, it has been suggested that a high microbial richness increases the animal ability to use feed ingredients more efficiently (Plaizier et al., 2017).

Due to a challenge like SARA, may not have a severe effect on the functionality of the whole microbiota and the performance and health of the animals, since other species may functionally cover for the removed species. However, this may not occur in a less divers and rich community. Also, it has been suggested that a high microbial richness increases the animal ability to use feed ingredients more efficiently (Plaizier et al., 2017).

Studies have shown that grain-induced SARA increases the relative abundance of Firmicutes and decreases that of Bacteroidetes (Khafipour et al., 2009b; Fernando et al., 2010) in the rumen. Firmicutes are more efficient in degrading non-fiber carbohydrates and Bacteroidetes are more effective structural carbohydrate degraders. Therefore, a decreased relative abundance of Bacteroidetes may lead to a less efficient fiber digestion, which is not desirable (El Kaoutari et al., 2013).

High grain diets also reduce relative abundances of fibrolytic bacteria and increase the abundancs of amylolytic bacteria (Fernando et al., 2010). Amylotytic bacteria include *Streptococcus bovis*, *Ruminobacter amylophilus*, and *Selenomunas ruminantium*, each of which has high growth rates in the presence of starch (McAllister et al., 1990; Nocek, 1997). These bacteria have a major role in fermenting the starch and producing the VFA and lactic acid in the rumen when high grain diets are ingested. *Streptococcus bovis* is a potent starch fermenter and lactic acid producer, and a major cause of rumen pH depressions during high grain feeding, as lactic acid is more acidic than VFAs. As a result of such rumen pH depressions, the growth rate of most other rumen bacteria is inhibited, as they are sensitive to a such a low pH. Subsequently, Lactobacilli, which are resistant to low ruminal pH, become dominant and produce more lactate in the rumen, which drives the rumen pH down even more, and may ultimately result in acute acidosis (Gill et al., 2000).

During the normal rumen conditions, most lactate produced in the rumen is metabolized by lactate utilizing bacteria, such as *Megasphaera elsdenii, Selenomonas ruminantium,* and *Propionibacterium shermanii*. Of these bacteria, *Selenomonas ruminantium* is not resistant to low rumen pH and its activity is, therefore, inhibited during SARA. Hence, it does not play an important role during SARA and acute rumen acidosis. However, *Megasphaera elsdenii* is tolerant to a low ruminal pH, and can remove up to 74% of the lactate in the rumen (Counotte et al., 1981). It, therefore, has a crucial role in lactate metabolism and the prevention of lactate accumulation in the rumen during high grain feeding (Counotte et al., 1981). Robinson et al. (1992) induced acute ruminal acidosis in fattening steers and reported that the rumen pH in *Megasphaera elsdenii* supplemented animals exceeded 5.0, and that these animals had a 24% higher DMI comparing to the non-supplemented group. This shows that these bacteria are able to prevent acute ruminal acidosis. However, a problem with *Megasphaera elsdenii* is that their growth rate is much lower than that of *Streptococcus bovis*, so a lack of a balance between the growth rate of these bacteria can result in lactate accumulation in the rumen during the feeding of high grain diets (Oetzel, 2003).

Altogether, feeding high grain/energy dense diets can affect the rumen microbiota and metabolism greatly. The changes in the composition and functionality of rumen microbiota due to feeding these diets depend on the severity of pH depression and the amounts of fermentable substrates that are delivered to the rumen, as well as on the clearance of these acids from the rumen.

2.7 Acidosis and oxidative stress

Free radicals are end products of metabolism (Valko et al., 2007).These products are normally neutralized by antioxidants (e.g. glutathione peroxidase, Se, Vitamin E) in the body, and therefore, do not cause major problems. However, when the production of free radicals exceeds the ability of the body to neutralize these radicals, oxidative stress occurs (Mandelker and Vajdovich, 2011).

Oxidative stress can be the result of infections, the presence of toxins, drug metabolism, hyperlipidemias, and inflammation (Mandelker and Vajdovich, 2011). For example, it has been shown that neutrophil activation and phagocytosis extensively increases production of these free radical products, such as superoxide and hydrogen peroxide (Riley, 1994).

In dairy cows, the peripartum and early lactation periods are challenging to their health and production, as the cows are dealing with physiological and metabolic stresses. As a result, they are at a high risk of developing diseases, such as metritis, mastitis, retained placenta, and SARA (Goff and Horst, 1997; Plaizier et al., 2012). During this period, cows also commonly experience oxidative stress (Kankofer, 2002; Castillo et al., 2005). Antioxidant supplementation, i.e. with Se or Vitamin E, reduces the risks of mastitis (Allison and Laven, 2000), retained placenta, and ovary cysts (Harrison et al., 1984; Campbell and Miller, 1998). Free bacterial LPS, whose concentration in the gut is elevated during SARA, can be translocated from the digestive tract into the blood circulation (Khafipour et al., 2009b). This can activate an immune response, including the production of proinflammatory cytokines and that of oxygen radicals by Kupffer cells and neutrophils (Rietschel et al., 1994; Khafipour et al., 2009b; Mier-Cabrera et al., 2011). If the body fails to neutralize these radicals, this results in oxidative stress and tissue damage.

Abaker et al. (2017) induced SARA by excessive grain feeding in mid-lactation dairy cows. This increased the rumen and blood LPS concentrations in these cows. Cows with SARA had lower catalase and glutathione peroxidase (GPx) activities, and also had a lower total antioxidant capacity compared to control cows. The super oxide dismutase (SOD) activity and malondialdehyde (MDA) concentration was also higher in SARA cows, showing that high grain feeding caused oxidative stress in these animals. An increased SOD activity is due to an increased production of superoxide radicals (Hassan et al., 2005). These authors also concluded that translocation of LPS into the blood circulation, decreases the antioxidant activity of the cells in the liver, which also leads to oxidative stress.

The above findings suggest that dairy cows are at high risk of oxidative stress during SARA and other challenging periods, e.g. around parturition and during high grain feeding. Hence, adding antioxidants to the diet benefit these cows during the challenging periods.

Therefore, high grain feeding can provide more energy to dairy cows in order to meet their energy requirements, but may also have negative impacts on health and performance, and, thereby, reduce the energy supply for milk production. Many tools, such as increasing peNDF content of the diet, providing TMR to the cows, increasing the feeding frequency, and using feed additives such as SCFPs, have been applied to attenuate the negative impacts of SARA. However, the differences among available SCFP in the market with various dosage recommendations, and different experimental designs, has resulted inconclusive results regarding the effects of these feed additives. Also, in order to reduce the cost of the experiments, cows have normally been supplemented and challenged for short periods of time, which may not be long enough to show their effect. It is believed that these products are more beneficial when cows are at high risk of SARA and/or are challenged for a longer period. To our knowledge, SARA challenges on dairy cows have been mostly been conducted for a week, which might not represent an on-farm farm situation where animals are at high risk of SARA for a longer period of time. Hence, SCFP supplementation in cows that are subjected to consecutive bouts SARA, or are experiencing SARA for long periods, may be more beneficial. However, this has not yet been determined. Thus, in the current experiment, we decided to supplement the cows with SCFP from 4 weeks pre- up to 10 weeks after calving and to challenge them with 2 SARA challenges. We also tested a newly developed product (NutriTek®, Diamond V, Cedar Rapids, IA) on SARA-challenged cows and determined if the dose of this product affects its impacts.

Chapter 3: Hypothesis and objectives

3.1 Hypothesis

We hypothesized that grain-induced SARA reduces the pH, acetate to propionate ratio, and increases the concentrations of propionate and butyrate in the rumen and feces, as well as reduces feed intake, and that a second SARA challenge results in a more severe rumen pH depression than the first. We also hypothesized that SCFP Original XPC™ (XPC, Diamond V, Cedar Rapids, IA) and NutriTek® (Diamond V, Cedar Rapids, IA) reduce the impacts of SARA, but that NutriTek® has the largest effects, since this new product contains more beneficial components such as polyphenols which may benefit the animal since they act as antioxidants. We also hypothesized that these reductions depended on the dose of NutriTek® administered.

3.2 Objectives

The main objectives of our study were to compare the effects of supplementation with 14 g/d XPC, 19 g/d NutriTek® (NTL), and 38 g/d NutriTek® (NTH) on the pH and fermentation acids in the rumen and feces, and feed intake during normal feeding and grain-based SARA challenges. We also aimed to determine if these effects of SCFP are greater during a repeated SARA challenge compared to a first challenge, since as discussed earlier, a second challenge is expected to be more severe. The supplementation doses were determined based on the company's recommendations. For the NutriTek® product, a higher dose was included to assess if such a dose had a greater impact on animal performance.

Chapter 4: Manuscript

4.1 Abstract

Effects of *Saccharomyces cerevisiae* fermentation products (SCFP) on dry matter intake (DMI), pH, volatile fatty acid content, lactate, and ammonia-nitrogen in the rumen and feces were determined in 32 lactating Holstein cows. Cows were assigned to four SCFP treatments, including a Control and three SCFP supplementations: i). 14 g/d Diamond V Original XPC™ (XPC, Diamond V, Cedar Rapids, IA), ii) 19 g/d NutriTek® (NTL, Diamond V), or iii) 38 g/d NutriTek® (NTH, Diamond V). Cows were monitored from wk 4 to wk 9 of lactation. SARA challenges were conducted during wk 5 and wk 8 by switching from a moderate grain to a high grain diet that contained 18.6 and 27.9 % starch (DM basis), respectively. These challenges increased the DMI (17.2 vs. 21.6 kg/d, $P < 0.05$) and the rumen concentration of propionate, butyrate and total VFA, and reduced the feces pH (6.71 vs. 6.62, $P < 0.05$) and the rumen concentrations of acetate and ammonia-nitrogen. The duration of the rumen pH below 5.6 during the SARA challenges averaged 175.0, 233.8, 246.9, and 79.3 min/d, respectively, for the Control, XPC, NTL, and NTH treatments. This shows that these challenges did not induce SARA under the NTH treatment, and that the SARA under the other SCFP treatments was mild. The feces pH during the SARA challenges was lower during the NTH treatment than during the other SCFP treatments (6.55 vs. 6.74, $P < 0.05$), suggesting the NTH treatment shifted fermentation from the rumen to the hindgut. Results show that the NTH treatment attenuated adverse impacts of SARA.

Key words: Dairy cows, SARA, Saccharomyces cerevisiae fermentation products, digestibility.

4.2 Introduction

In order to support their high potential for milk production, dairy cows need to receive energy-rich diets with high starch and low fiber contents (NRC, 2001; Plaizier et al., 2008). Feeding these diets alters conditions in the rumen by decreasing the rumen pH and changing the VFA profile towards increased concentrations of total VFA, propionate, butyrate and lactate, and a reduced ratio of acetate to propionate (Li et al., 2012; Plaizier et al., 2018). Such a decrease of the rumen pH may lead to SARA, which has been defined as a moderate and reversible depression of this pH (Krause and Oetzel, 2006; Plaizier et al., 2008, 2018). This disorder affects the production and health of dairy cows by decreasing milk fat production, nutrient utilization, the functionality of the rumen epithelium, and feed intake, as well as by causing inflammation, laminitis, and diarrhea (Callaway and Martin, 1997; Li et al., 2016; Plaizier et al., 2018). In addition, SARA can reduce microbial digestion, especially that of fiber, in the rumen and hindgut, as many rumen and hindgut microorganisms, and their enzymes are sensitive to a low rumen pH (Russell and Dombrowski, 1980; Shi and Weimer, 1992; Russell and Wilson, 1996). In agreement, Plaizier et al. (2001) and Krajcarski-Hunt et al. (2002) observed that experimental induction of SARA by high grain feeding reduced the in situ 24 h NDF degradability of forages by between 19.6 and 20.5%. However, this does not imply that total tract NDF digestibility is also reduced, as hindgut fermentation may compensate the reduced fiber digestibility in the rumen (Demeyer, 1991). Nevertheless, as SARA may increase fermentation and acidity in the hindgut (Gressley et al., 2011; Li et al., 2012), this type of SARA may also reduce fiber digestion in the hindgut.

Saccharomyces cerevisiae fermentation products (SCFP) are produced by anaerobic fermentation with *S. cerevisiae*. These products differ from live yeast, as the latter have not gone through a fermentation process. These products include Original XPC (Diamond V, Cedar Rapids, IA) and NutriTek® (Diamond V, Cedar Rapids, IA), that contain beneficial compounds for cows and their gut microorganisms, such as amino acids (AA), polyphenols, antioxidants, and B vitamins (Schingoethe et al., 2004). Supplementation with SCFP has been shown to improve feed efficiency and stabilize rumen conditions of cows (Williams et al., 1991; Allen and Ying, 2012; Dias et al., 2018). This stabilization may, in part, result from lowering the digestion rate of starch in the rumen, and, thereby, reduce the accumulation of fermentation acids and limit the increase in acidity in this part of the digestive tract (Allen and Ying, 2012). In addition, SCFP may also stabilize the rumen of grain-fed cows by improving the functionality of lactate utilizing bacteria (Nisbet and Martin, 1991). Hence, administration of SCFP can result in reduced lactate concentrations in the rumen, increased functionality of fiber digesting bacteria (Callaway and Martin, 1997), increased fiber digestibility (Dias et al., 2018), and improved feed intake (Poppy et al., 2012). Li et al., (2016) observed that supplementation with XPC (Diamond V, Cedar Rapids, IA) reduced the variation in rumen pH and the inflammation resulting from grain-induced SARA. NutriTek® contains more antioxidants and polyphenols than XPC (Diamond V, 2019). Hence, it can be assumed that the SARA-mitigating effects of NutriTek® are greater than those of XPC. Reports on the effect of supplementation of yeast products on the DMI of dairy cows vary among studies (Desnoyers et al., 2009; Poppy et al., 2012). A meta-analysis conducted by Desnoyers et al. (2009) on the results of 157 experiments showed that, on average, the supplementation with yeast products increased DM digestibility and DMI. Poppy et al. (2012) conducted a meta-analysis using 61 research publications on SCFP supplementation of lactating dairy cows, and reported that during early lactation, supplementation increased DMI by 0.62 kg/d, whereas it decreased DMI by 0.78

kg/d thereafter. The variation of the impact of SCFP supplementation could be due to various factors, including differences in diet composition, experimental design, dose of supplementation, duration of supplementation, and stage of lactation (Robinson and Erasmus, 2009; Allen and Ying, 2012; Poppy et al., 2012). Aspects of the experimental design that could affect the outcome of such studies include insufficient statistical power and carry-over effects (Poppy et al., 2012; Li et al., 2016). It is expected that SCFP have more benefits when the cows experience nutritional challenges, such as SARA (Li et al., 2016; Plaizier et al., 2018).

We hypothesized that grain-induced SARA reduces the pH and acetate to propionate ratio and increases the concentrations of propionate and butyrate in the rumen and feces, as well as reduces feed intake, and that a second SARA challenge results in a more severe rumen pH depression than the first. We also hypothesized that SCFP Original XPC™ (XPC, Diamond V, Cedar Rapids, IA) and NutriTek® (Diamond V, Cedar Rapids, IA) reduce these impacts of SARA, but that NutriTek® has the largest effect. We also hypothesized that these reductions depended on the dose of NutriTek® administration. The main objectives of our study were to compare the effects of supplementation with 14 g/d XPC, 19 g/d NutriTek® (NTL), and 38 g/d NutriTek® (NTH) on the pH and fermentation acids in the rumen and feces, and feed intake during normal feeding and grain-based SARA challenges. We also aimed to determine if these effects of SCFP differed between a first and a repeated SARA challenge.

4.3 Materials and methods

4.3.1 Animals, diets and experimental design

The study was conducted at the Glenlea Research Station, University of Manitoba. It was pre-approved by the University of Manitoba Animal Care Committee and followed the guidelines of the Canadian Council for Animal Care (CCAC, 1993).

Thirty-two rumen-cannulated multiparous Holstein dairy cows were assigned to a randomized complete block design with 8 blocks to avoid carry-over effects of SCFP treatments. Cows were blocked based on parity, expected calving date and milk production during the previous lactation. The average parity and milk production in the previous lactation of the experimental cows were 2.67±0.91 and 10,49 ±1619 L (mean ± SD), respectively. Within each block, cows were randomly assigned to 4 treatments, i.e. Control, (No SCFP), and three different SCFP supplementations and were monitored between wk 4 and wk 9 after calving. Cows in the SCFP groups received 14 g/d Diamond V Original XPC™ (XPC), 19 g/d NutriTek® (NTL), or 38 g/d NutriTek® (NTH) mixed with 126, 121, and 102 g/d ground corn, respectively. The cows under the control treatment received only 140 g/d ground corn. These supplements were top dressed once daily immediately after the morning delivery of the diet at 0900. Cows were housed in individual stalls and had unlimited access to fresh water. The experiment was run between June 2016 and October 2017. Cows were fed a close-up total mixed ration (TMR) from 4 weeks pre-calving up to the calving date (Table 1, Table 2). After calving, cows were switched to a lactation TMR except for the 5th and 8th weeks after calving, during which SARA challenges were conducted. These challenges consisted of substituting TMR with pellets containing 50% ground wheat and 50% ground barley gradually over 3 days to reach a 20% grain inclusion of the total ration dry matter (Table 1, Table 2) (Khafipour et al., 2009b). The objective of the SARA challenge was to reduce the rumen pH to below pH 5.6 for more than 180 min/d. During the 2 wk recovery period between the SARA challenges, the lactation TMR was fed. Diets were provided ad libitum, allowing for between 5 and 10% orts, once daily at 0900. Weights of the feed and orts were recorded each morning before the first feed delivery.

4.3.2 Milk production and milk component

Milk production was recorded using Tru Test regulation meters (Westfalia Surge, Mississauga, ON) during the whole experiment. Milk samples were collected at four consecutive milkings during each week of the trial. Samples were preserved with 2-bromo-2-nitropropane-1,3 diol, stored at 4◦C until further analysis.

Table 1. Ingredient composition of experimental diets

	Lactating TMR	SARA TMR	Close-up dry cow TMR
	Ingredient compositions (% of DM)		
Round bale mixed alfalfa /grass silage	35	28	20
Alfalfa 1st cut haylage	-	-	8
Corn silage	-	-	37
Barley silage	20	16	-
Straw	-		13
Ground corn	20	16	-
Dairy Aide[1]	25	20	
Close-up dry cow supplement[2]	-		22
Wheat-Barley pellets	-	20	-

[1]Dairy Aide contained: Corn – Flaked (65.2%), Corn Distillers grain (7.1%), APF Fat Plus (3.6%), Feather Meal (3.6%), Porcine Meat Meal (2.9%, Soybean Meal (2.9 %), Canola Meal (2.9%), AV Fat Rothsay Feeders Choice (2.2%), sodium sesquicarbonate (SQ 810, 1.9%), Dicalcium Phosphate (1.5%), potassium chloride (DYNA K Red, 1.5%), Ground Limestone (1.5), Salt – Potash (1.5%), Dairy LMK Ultra micro (0.6 %), Magnesium Oxide (0.3%), Methionine Analogue – Novus (0.3%)[2] Dry cow supplement contained Landmark close-up dry cow pellets (50.0%)[3], flaked corn (20.0%), beet pulp pellets (15.0 %), rolled barley (12.5%), liquid molasses (2%), soy oil (0.5%), liquid caramel (0.01%) [3]Landmark close-up day cow pellets contained: barley (4.0%), limestone(7.0%), corn distillers grain (34.9%), dicalcium phosphate (1.8%), canola meal (23.0%), soybean meal (8.5%), wheat (15.0%), niacin (0.3%), biopowder SXC (0,05%), magnesium oxide (1.6%), transition VB 25K (2.9%) (Vitamin A 100,000 IU/Kg, vitamin D_3 30,000 IU/Kg, vitamin E 1,500 IU/Kg), dry cow microPX premix (1%) (Rice hulls, calcium carbonate medium, vitamin E 450000 IU, manganese sulfte 32%, optimin sey 3000, optimin manganese 15 %, mineral oil, intellibond Z 55%, Optimi pink 15%, zink sulphate 36%, intellibond c2 54%, copper sulphate 25%, optimin cooper 15%, vitamin A 1000 IU, selenium 4.5%, vitamin D_3 500 IU, eddi 80, cobalt carbonate 45%).

Table 2. Chemical compositions of experimental diets

	Lactating TMR	SARA TMR	Close-up dry cow TMR
DM, %	51	60	48
CP, % DM	17.9	17.2	15.5
Fat, % DM	4.3	3.6	2.8
NDF, % DM	34.9	28.2	38.7
ADF, % DM	26.0	19.9	26.7
Starch, % DM	18.6	27.9	17.6
Ca, % DM	1.32	1.06	1.26
P, % DM	0.45	0.47	0.37
Mg, % DM	0.37	0.34	0.41
Na, % DM	0.33	0.27	0.06
K, % DM	2.57	2.02	2.37

4.3.3 Rumen, feces, and urine pH

Rumen pH was monitored at 1-min intervals using indwelling pH data loggers (T7-1 LRCpH, DASCOR, Escondido, CA), which were placed in the ventral sac of the rumen of all cows, as described by Li et al. (2016). The data loggers were removed from the rumen every two weeks for cleaning and calibration, as described by (Penner et al., 2006). Cleaning was conducted by using a Terg-A-Zyme cleaning solution (Alconox, White Plains, NY). The standardization was performed using pH 4 and 7 buffers at 39°C. If a drift in pH recording was detected over time, then the recorded pH data were adjusted accordingly by calculating the slope between two calibrations and then adjusting the recorded pH values based on the slope. A rumen pH depression below 5.6 for more than 3 h/d was used as a threshold for defining SARA for dairy cows (Gozho et al., 2005). However, daily durations of rumen pH below 6.0, 5.8, and 5.6 were also determined and assessed in our study. Rumen liquid digesta was sampled twice weekly at 6 h after the 0900-feed delivery. Approximately 500 mL of whole rumen contents were collected through the canula from five sites (cranial, caudal, dorsal, caudal ventral), mixed thoroughly, and the solid and liquid digesta were separated using a Bodum French Press Coffee plunger. Subsequently, 12ml of the liquid digesta was centrifuged at 3000 RPM for 15 min, and 6 ml of the supernatant was collected. The subsample was mixed with 1.2 ml of 25% meta-phosphoric acid and stored at -20◦C until further analysis. After thawing at room temperature, these samples were analyzed for VFA and lactate using gas chromatography (model 3900 Star, Varian, Walnut Creek, CA) (Bhandari et al., 2007), and for ammonia-nitrogen using a colorimetric assay as described by Novamsky et al. (1974). Approximately 250 g of feces were collected daily at 6 h after the 0900-feed delivery from the rectum after cleaning the perianal area and mixed thoroughly. The pH of these samples was determined immediately after sampling using an Accumet Basic 15 pH meter (Fisher Scientific, Fairlawn, NJ) equipped with a Sensorex 450C Flat Surface Combination pH/Reference Electrode (Sensorex, Stanton, CA). Subsequently, samples were stored at -20◦C until further analyses. After thawing at room temperature, 20 g of subsample were mixed with an equal amount of 0.9% physiological saline, and analyzed for VFA, lactate and ammonia-nitrogen as previously described for rumen fluid samples.

Urine was collected twice weekly at 6 h after the 0900-feed delivery. Approximately 50 mL of mid-stream urine samples were collected by stimulating perineal area to initiate urination as described by Li et al. (2012). The urine samples were mixed thoroughly, and pH was measured immediately using the same equipment as used for the fecal pH measurement.

4.3.4 Chemical analysis and calculations

Feed, orts, and fecal samples were analyzed for DM by drying at 60°C for 48 h in a forced air oven. The DMI was calculated daily by subtracting amount of feed left from the amount of feed delivered per day on a DM basis. Dried samples were ground with a Wiley mill using a 1-mm screen (Thomas-Wiley, Philadelphia, PA) and kept in sealed bags for further analyses. Ground feed and fecal samples were pooled by stage relative to SARA induction for each cow. Analytical DM for pooled samples for each stage was determined (method 934.01; AOAC, 1990). All feed and feces samples were analyzed for NDF according to Van Soest et al. (1991) using α-amylase (Sigma No. A3306, Sigma Chemical Co., St. Louis, MO), and sodium sulfite, and corrected for ash concentration using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY), P (AOAC methods 968.08 and

935.13A; AOAC, 2005), and starch using the UV method (method 996.11; AOAC, 2005). Feed samples were also analyzed for crude protein using the CuSO4/TiO2 mixed catalyst Kjeldahl procedure (method 988.05; AOAC, 1990). Analyses of ADF, ether extract, and ash in feed samples were conducted using AOAC method 973.18 (AOAC, 1990), AOAC method 920.39 (AOAC, 1990), and AOAC method 923.03 (AOAC, 2005), respectively. Calcium, P, K, Mg, and Na in feed samples were measured by inductive coupled plasma emission spectroscopy (method 968.08; AOAC, 1990) using an Atom Scan 25 Plasma Spectrometer (Thermo Jarrell Ash Corp., Grand Junction, CO) after acid digestion.

4.3.5 Statistical analysis

Data were analyzed with the MIXED procedure of SAS (Ver. 9.3, SAS Inst., Inc., Cary, NC) using the following model:

Yikl = μ + Ti + Sj + TSij + Bk + eijk.

Where Yijk = observations for dependent variables, μ = overall mean, Ti = fixed effect of SCFP supplementation (CON, XPC, NTL, or NTH), Sj = fixed effect of stage of SARA (pre-SARA1, SARA1, post-SARA1 SARA2, and post-SARA2), TSij = effect of SCFP supplementation and stage of SARA interaction, Bk = random effect of Block (1 to 8) and eijk = residuals. Stage of SARA was considered a repeated measure for the subject of cow within block with the AR (1) covariance structure. Normality of distributions of residuals was tested by the Shapiro–Wilk's statistics using Proc UNIVARIATE of SAS ver.9.3. If needed, the data was transformed by the natural logarithm or raising the variable to the power of lambda to alleviate heterogeneity of residual variances. The required lambda value was the lambda calculated using a Box-Cox transformation analysis using

TRANSREG procedure of SAS ver.9.3. Homogeneity of variance tested by combining stage of SARA and SCFP treatment into a single factor and analyzed as a one-way ANOVA with Levene's test in PROC GLM of SAS ver. 9.3. If the assumption of equal variances was not met, then in the following analysis with PROC MIXED of SAS ver.9.3. the GROUP = group in the REPEATED statement was included to allow the variances to be estimated separately, and the DDFM = SATTERTHWAITE option was added in the MODEL statement to adjust the degrees of freedom for the unequal variances. The PDIFF option was applied to evaluate pairwise comparisons between treatments and stages. The significant effects of SCFP treatment, Stage of SARA, and their interactions were discussed at $P < 0.05$, and tendencies were reported at $0.05 < P < 0.10$.

4.4 Results

4.4.1 DMI, milk production and milk component

The DMI did not differ among SCFP treatments (Table 3). However, across SCFP treatments, the DMI was higher during the SARA1, pre-SARA2, SARA2, and post-SARA2 stages than during the pre-SARA1 stage (Table 3).

The milk yields of the Control (43.4 kg/d) and XPC (44.7 kg/d) treatments were higher ($P = 0.01$) than those of the NTL (40.7 kg/d) and NTH (41.3 kg/d) treatments. The FCM of the NTL treatment (41.4 kg/d) tended ($P = 0.10$) to be lower than those of the Control (44.5 kg/d), XPC (43.0 kg/d), and NTH (44.1 kg/d) treatments. The SCFP treatments did not differ in FE. The milk fat content tended to be lower for the XPC treatment than for the NTH treatment (3.56 vs. 3.91%, $P = 0.10$), but the milk fat yield was not affected by SCFP treatment. The milk protein content of the XPC treatment (2.96 %) was higher ($P = 0.05$) than those of the Control (2.84 %) and NTH (2.90 %) treatments. The effect of the interaction between the SCFP and stage of SARA was not significant for any milk production variable.

The milk yields were higher ($P < 0.01$) during the SARA1 and SARA2 stages than during the pre-SARA stages (Table 3). However, the FCM yields were lower ($P < 0.05$) during the SARA1 and SARA2 stages, than during the other SARA stages. Both fat yields and fat contents were lower ($P < 0.001$) during the SARA1 (3.43% and 1.34 kg/d) and SARA2 (3.43% and 1.33 kg/d) stages than during the pre-SARA1 (4.31 and 1.66 kg/d), pre-SARA2 (3.86% and 1.55 kg/d, and post-SARA2 (3.71% and 1.54 kg/d) stages. The milk protein content was higher during the pre-SARA1 (2.98%) stage than during the SARA1 (2.88%), pre-SARA2 (2.86%), and SARA2 (2.89%) stages. The milk protein yield was lower ($P = 0.03$) during the pre-SARA1 (1.14 kg/d) stage than during the SARA1 (1.24 kg/d), SARA2 (1.23 kg/d), and post-SARA2 (1.23 kg/d) stages. The FE was lower during the SARA1 and SARA2 stages, than during the pre-SARA1 stage, and the FE was also lower during the SARA2 stage than during the SARA1 stage.

Table 3. Effects of SCFP treatment (control, XPC, NTL, and NTH) and stage of SARA (Pre-SARA1, SARA1, Pre-SARA2, SARA2, and Post-SARA2) on milk production variables, dry matter intake (DMI), and feed efficiency (FE).

	SCFP Treatment				Stage						Effects, *P- values*		
	Con	XPC	NTL	NTH	Pre-SARA1	SARA1	Pre-SARA2	SARA2	Post-SARA2	SEM[1]	Treat	Stage	Treat× Stage
Milk yield, kg/d	43.4^{a}	44.7^{a}	40.7^{b}	41.3^{b}	39.4^{b}	43.6^{a}	42.1^{a}	43.1^{a}	42.5^{a}	1.25	0.01	<0.01	0.92
3.5%FCM	44.5^{x}	43.0^{x}	41.4^{y}	44.1^{x}	43.9^{a}	40.6^{b}	43.4^{a}	40.2^{b}	43.5^{a}	1.57	0.10	0.05	0.87
Fat content, %	3.69^{xy}	3.56^{y}	3.63^{xy}	3.91^{x}	4.31^{a}	3.43^{c}	3.86^{b}	3.43^{c}	3.71^{b}	0.12	0.10	<0.001	0.98
Fat yield, kg/d	1.59	1.46	1.47	1.62	1.66^{a}	1.34^{b}	1.55^{a}	1.33^{b}	1.54^{a}	0.08	0.16	<0.001	0.97
Protein content, %	2.84^{b}	2.96^{a}	2.94^{ab}	2.90^{b}	2.98^{a}	2.88^{b}	2.86^{b}	2.89^{b}	2.92^{ab}	0.04	0.05	0.01	0.34
Protein yield, kg/d	1.23	1.20	1.19	1.20	1.14^{b}	1.24^{a}	1.18^{ab}	1.23^{a}	1.23^{a}	0.04	0.48	0.03	0.87
DMI, kg/d	21.9	20.4	20.7	20.3	17.2^{c}	19.3^{c}	21.7^{b}	23.9^{a}	22.0^{ab}	1.03	0.20	<0.01	0.85
FE[2]	2.03	2.11	2.00	2.17	2.55^{a}	2.10^{b}	2.00^{b}	1.68^{c}	1.98^{b}	0.11	0.45	<0.01	0.93

[1]SEM =standard error of mean.
[2]FE = 3.5%FCM/DMI.
[a,b,c]means with different superscripts within SCFP treatments and SARA stages differ (P < 0.05).
[x,y]means with different superscripts within SCFP treatments and SARA stages ten to differ (P < 0.10).

4.4.2 Rumen digesta, urine and feces

The average daily rumen pH, and the durations of the rumen pH below pH 6, 5.8, and 5.6 were affected by the interaction of SCFP treatment and stage of SARA (Table 4). This interaction tended to be significant for the urine pH, but was not significant for the fecal pH. Due to the interaction of the effects of SCFP and SARA stage on rumen pH variables, the effects of these factors were evaluated within classes of the other factor. The effects on the duration of the rumen pH below 5.6 were compared within stage of SARA and within SCFP treatment, as this duration was used as the threshold of SARA (Gozho et al., 2007). The effect of SCFP treatment on this duration was significant during the SARA1 and SARA2 stages, but not during the pre- and post-SARA stages (Table 4). During SARA1 and SARA2, the NTH treatment reduced ($P < 0.05$) this duration, but the XPC and NTL treatments did not have such an effect. During the SARA1 and SARA2 stages, the durations of the rumen depression below 5.6 were higher than the threshold of 180 min/d for the XPC and NTL treatments. However, for the NTH treatment, this duration (79.3 min/d) did not exceed the threshold. Across all SCFP treatments, the durations of the rumen pH below 5.6 were equal during the pre-SARA1, pre-SARA2, and post- SARA2 stages (Table 5). In cows on the Control and NTH treatments, this duration was longer during the SARA1 stage than during the SARA2 stage (228.4 vs. 121.6 min/d, and 104.6 vs. 53.9 min/d, respectively, $P < 0.05$). However, during the other SCFP treatments, this duration did not differ between the SARA1 and SARA2 stages.

The urine pH only differed significantly, but not substantially, among SCFP treatments and stages of SARA from 7.99 to 8.09. The fecal pH during the SARA1 and SARA2 stage were 6.61, and 6.63, respectively, and were lower ($P < 0.05$) than those during the pre-SARA1, post-SARA1, and post-SARA2 stages, which were 6.71, 6.70, and 6.84, respectively (Table 4).

Across stages of SARA, the fecal pH of the NTH treatment (6.55) was lower ($P = 0.02$) than those of the Control, XPC, and NTL treatments, which were 6.74, 6.75, and 6.74, respectively (Table 4). The SARA challenges increased the rumen concentrations of total VFA, propionate, butyrate, total VFA, and lactate, whereas they reduced those of acetate, other VFA, and ammonia-nitrogen, and reduced the acetate to propionate ratio (Table 6). These concentrations and this ratio did not differ between the first and the second SARA challenge. The NTL treatment had a higher ($P < 0.05$) rumen propionate concentration than the NTH treatment, and the NTH treatment tended ($P < 0.10$) to have a lower rumen total VFA concentration than the Control and XPC treatments. The XPC treatment had a lower rumen ammonia-nitrogen concentration than the Control and NTH treatments. The effect of the interaction between the SCFP treatment and the stage of SARA induction was not significant for any of these rumen variables.

Table 4. Effects of SCFP treatment (control, XPC, NTL, and NTH) and stage of SARA (PreSARA1, SARA1, Pre-SARA2, SARA2, and Post-SARA2) on rumen, fecal, and urine pH variables.

	Treatment				Stage						Effects, *P-values*		
	Con	XPC	NTL	NTH	Pre-SARA1	SARA1	Pre-SARA2	SARA2	Post-SARA2	SEM	Treat	Stage	Treat*Stage
Avg. Rumen pH	6.28	6.25	6.26	6.36	6.35	6.04	6.46	6.09	6.5	0.02	<0.001	<0.001	0.004
Time < pH 6.0, min/d	331.4	356.0	330.5	223.3	139.1	627.3	105.2	590.9	89.1	38.9	<0.001	<0.001	0.003
Time < pH 5.8, min/d	182.1	196.3	199.0	98.5	40.8	355.5	52.1	363.0	33.3	32.0	<0.001	<0.001	<0.001
Time< pH 5.6, min/d	82.3	99.1	106.1	33.2	13.6	179.3	19.0	178.0	10.8	22.1	<0.001	<0.001	<0.001
Fecal pH	6.74[a]	6.75[a]	6.74[a]	6.55[b]	6.71[a]	6.61[b]	6.70[a]	6.63[b]	6.84[a]	0.05	0.01	0.02	0.87
Urine pH	8.08	7.99	8.06	8.06	8.00	8.06	8.09	8.06	8.02	0.03	0.01	0.07	0.08

[a,b]means with different superscripts within SCFP treatments and SARA stages differ (P < 0.05)

Table 5. Effect of SCFP treatments (control, XPC, NTL, and NTH) and by stages of SARA (pre-SARA1, SARA1, pre-SARA2, SARA2, post-SARA2) on rumen Time < pH 5.6.

Stage	Treatment					
	Control	XPC	NTL	NTH	SEM[1]	Effects, *P-values*
Pre-SARA1	7.1	3.2	2.9	6.3	3.6	0.60
SARA1	228.4[a]	183.1[ab]	241.0[a]	104.6[b]	64.1	0.01
Pre-SARA2	26.58	6.28	24.08	4.26	10.1	0.10
SARA2	121.6[b]	284.4[a]	252.8[a]	53.9[b]	45.4	<0.001
Post-SARA2	14.6	27.6	3.1	21.3	9.7	0.32

[a,b]means with different superscripts within SARA stage differ ($P < 0.05$).
[1]SEM = standard error of mean.

Table 6. Effects of SCFP treatment (control, XPC, NTL, and NTH) and stage of SARA (Pre-SARA1, SARA1, Pre-SARA2, SARA2, and Post-SARA2) on rumen VFA, lactate, and ammonia-N.

	Treatment				Stage						Effects, *P- values*		
	Con	XPC	NTL	NTH	Pre SARA 1	SARA 1	Pre-SARA 2	SARA 2	Post-SARA 2	SEM[1]	Treat	Stage	Treat× Stage
Acetate, mmol/L	81.9	81.0	77.6	78.6	83.6[a]	73.2[b]	83.4[a]	76.9[b]	82.9[a]	2.01	0.23	<0.001	0.97
Propionate, mmol/L	30.5[ab]	30.2[ab]	31.1[a]	26.8[b]	20.9[b]	44.3[a]	21.2[b]	44.3[a]	20.9[b]	1.78	<0.01	<0.001	0.76
Butyrate, mmol/L	12.6	13.9	12.9	12.5	11.2[c]	14.5[a]	12.2[b]	14.2[a]	12.5[b]	0.96	0.24	<0.001	0.96
Other VFA, mmol/L	5.3	5.2	5.4	5.6	6.0[a]	4.0[c]	6.7[a]	4.5[c]	5.7[b]	0.38	0.38	<0.001	0.87
Total VFA, mmol/L	130.2[x]	130.5[x]	126.9[xy]	122.1[y]	121.4[c]	131.7[a]	122.4[a]	140.4[a]	122.1[c]	4.4	0.09	<0.001	0.94
Ac/Pr[2]	3.15[ab]	3.13[ab]	3.07[b]	3.48[a]	4.13a	1.97b	3.98[a]	1.91[b]	4.06[a]	0.22	<0.01	<0.01	0.70
Lactate, mmol/L	6.65	6.61	6.12	6.72	5.22[b]	7.44[a]	5.09[b]	8.26[a]	5.16[b]	1.05	0.12	<0.01	0.21
Ammonia-N, mg/dL	11.9[a]	9.8[b]	11.7[ab]	12.9[a]	14.3[a]	8.4[b]	12.5[b]	8.9[b]	13.7[b]	1.31	0.04	<0.01	0.99

[a,b,c]means with different superscripts within SCFP treatment or SARA stage differ ($P < 0.05$)
[x,y]means with different superscripts within SCFP treatment or SARA stage tend to differ ($0.05 < P \leq 0.10$)
[1]SEM = standard error of mean
[2]Ac/Pr = acetate to propionate ratio

The concentration of propionate in feces was lower during the pre-SARA2 stage, than during the SARA1 and SARA2 stages (Table 7). Also, the fecal acetate to propionate ratio was lower during the SARA1 and SARA2 stages, than during the pre-SARA1 and pre-SARA2 stages. Other VFA, lactate and ammonia-N variables in feces were not affected by the stage of SARA. The fecal concentrations of acetate, and propionate were higher ($P < 0.05$) for the NTH treatment, than for the other SCFP treatments. The concentration of butyrate in feces was higher in the NTH treatment than in the NTL treatment, whereas the fecal concentration of total VFA was higher for the NTH than for the XPC and NTL treatments. The fecal concentrations of ammonia-nitrogen were lower in the XPC and NTL treatments than in the Control treatment.

Table 7, Effects of SCFP treatment (control, XPC, NTL, and NTH) and stage of SARA (PreSARA1, SARA1, Pre-SARA2, SARA2, and Post-SARA2) on fecal VFA, lactate, and ammonia-N.

	Treatment				Stage						Effects, *P- values*		
	Con	XPC	NTL	NTH	Pre-SARA1	SARA1	Pre-SARA2	SARA2	Post-SARA2	SEM[1]	Treatment	Stage	Treat× Stage
Acetate, mmol/L	24.0^{b}	22.1^{b}	21.8^{b}	26.7^{a}	23.8	24.0	22.8	24.4	23.0	1.23	<0.01	0.64	0.11
Propionate, mmol/L	4.5^{b}	4.1^{b}	3.8^{b}	5.1^{a}	4.2^{ab}	4.8^{a}	3.9^{b}	4.8^{a}	4.2^{ab}	0.26	<0.01	<0.01	0.22
Butyrate, mmol/L	2.2^{ab}	2.1^{ab}	2.0^{b}	2.4^{a}	2.3^{a}	2.2^{ab}	2.1^{ab}	2.2^{ab}	2.0^{b}	0.14	0.03	0.41	0.28
Other VFA, mmol/L	1.0	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.65	0.41	0.36	0.10
Total VFA, mmol/L	33.5^{ab}	30.5^{b}	29.8^{b}	36.5^{a}	32.4^{ab}	33.7	31.4	33.8	31.5	1.68	<0.001	0.41	0.11
Ac/Pr[2]	5.5^{b}	5.5^{b}	5.9^{a}	5.4^{b}	5.9^{a}	5.2^{b}	5.9^{a}	5.2^{b}	5.7^{ab}	0.19	0.01	<0.001	0.94
Lactate, mmol/L	1.5	1.1	1.2	1.5	1.3	1.4	1.2	1.4	1.5	0.18	0.15	0.70	0.26
Ammonia-N, mg/dL	3.4^{a}	2.7^{b}	2.7^{b}	3.0^{ab}	3.0	2.9	2.8	3.1	3.0	0.25	<0.01	0.70	0.48

[a,b]means with different superscripts within SCFP treatment or SARA stage differ (P < 0.05).
[1]SEM = standard error of mean.
[2]Ac/Pr = acetate to propionate ratio.

4.5 Discussion

4.5.1 Effects of SARA challenges

In our study, both SARA challenges increased milk yield, but they reduced the FCM and milk fat content. The milk yield and milk fat content were higher, respectively, lower during the pre-SARA2 and post-SARA2 stages than during the pre-SARA1 stage, but the FCM yield did not differ among these stages. Reported effects of grain-induced SARA on milk yield vary from a reduction (Khafipour et al., 2009b), to no impact (Gozho et al., 2007; Danscher et al., 2015; Li et al., 2016), and an increase (Steele et al., 2016). When the SARA is mild and it does not substantially reduce the DMI, like in our study, then the increase in the energy density of the diet may increase milk yield, but due to a milk fat depression, likely not increase the FCM yield and the FE (Plaizier et al., 2008; Steele et al., 2016). If the induction of SARA increases the energy availability in the rumen, then it may also increase microbial protein production and milk protein production (Plaizier et al., 2008; Khafipour et al., 2009b). The latter was indeed observed in our study. As the milk protein yield was higher during the SARA1, SARA2, and post-SARA2 stages, this may have occurred in our study.

A milk fat depression resulting from the SARA challenges has been reported previously (Bauman and Griinari, 2003; Plaizier et al., 2008, 2018). It is assumed that this depression is due to changes in the biohydrogenation of unsaturated fatty acids in the rumen that result from a low rumen pH, and that this depression increases of the production of fatty acid isomers, such as trans- 10, cis- 12 C18:2 (Bauman and Griinari, 2003). These isomers reduce *de novo* milk fat synthesis in the mammary gland, and, thereby, reduce the supply of fatty acids for milk fat synthesis (Bauman and Griinari, 2003). Also, high levels of bacterial toxins in the blood, such as lipopolysaccharide, that occur during SARA, may inhibit body fat mobilization (Waldron et al., 2006), which also reduces the availability of fatty acids for milk fat synthesis (Bauman and Griinari, 2003).

Despite a quick recovery of the rumen conditions from the SARA challenges, the FE did not recover quickly, resulting in the lowest FE during the SARA2 stage. This low FE was the result of a combination of low milk fat yield and FCM yields, and a high DMI. The increase in DMI from the SARA challenges contributed more to the decrease in FE, than the decrease in FCM. Hence, the cost of this reduction in FE may, therefore, depend on the feed cost, which are commonly higher for a high grain diet then for a more moderate grain diet (Ho et al., 2018).

Reasons why SARA can reduce DMI include reduced rumen motility, increased rumen concentrations of VFA and endotoxins, inflammation, and increased rumen osmolality (Kleen et al., 2003; Plaizier et al., 2008, 2012). Rumen motility and osmolality were not measured in our study, but the effects of the SARA challenges on the pH, VFA concentrations, and markers of inflammation (Guo et al., 2019) were limited. Whereas a severe grain-based SARA reduces feed intake, a mild SARA can increase DMI (Plaizier et al., 2008; Khafipour et al., 2009b; Pourazad et al., 2016). The latter effects may be explained by a reduction in physical rumen fill due to the higher inclusion of grain and the lower inclusion of forages in the diet (Allen, 2000; Plaizier et al., 2008, 2018). The SARA challenge diet in our study contained substantially more grain (36 vs. 20 % DM) and less forage (44 vs. 55% DM) than the lactating diet, and, therefore, contained less physical fill than the control diet. Hence, the lower physical fill of the SARA diet may be the reason why the DMI was higher during the SARA2 stage than during the pre-SARA1 stage. A reason for the absence of a difference in DMI between the pre-SARA1 and SARA1 stages may be that it took the conditions in the rumen that affected the

DMI more than 1 wk to adapt to the increase in grain feeding during the SARA1 stage. The lower DMI during the pre-SARA1 stage compared to the pre-SARA2 and post-SARA2 stages may indicate that it took these conditions also more than 1 wk to adapt from a reduction in grain feeding.

The rumen pH depression during SARA is not well defined, and studies have used different threshold values for this. Cooper et al. (1999) used a threshold of rumen pH between pH 5.2 and 5.6, while Beauchemin et al. (2003) used a rumen pH threshold of 5.8. In the present study, a rumen pH depression below 5.6 for more than 180 min/d was used as the threshold for SARA, as only equal or greater rumen depressions reduced feed intake and caused an inflammatory response in the study of Gozho et al. (2007). This threshold was also chosen, as it allowed comparisons with our previous studies on SARA. The threshold was barely exceeded during the SARA challenges in cows on the Control, XPC, and NTL treatments, and was not exceeded during the NTH treatment. Based on rumen pH values, the SARA induced in our study can, therefore, be considered as mild. The SARA challenges also increased the rumen concentrations of propionate, butyrate, total VFA and lactate, and reduced that of acetate and the acetate to propionate ratio. These changes are commonly observed during high grain feeding and grain-induced SARA (Gozho et al., 2007; Li et al., 2016; Pourazad et al., 2016), and reflect that fermentation of non-fiber carbohydrates produce more VFA and lactate than fermentation of fiber, and that amylolytic bacteria produce more propionate and less acetate than fibrolytic bacteria (Russell and Rychlik, 2001; Plaizier et al., 2018).

The SARA challenges also reduced the fecal pH. This suggests that the SARA challenges increased fermentation in the hindgut, most likely by increasing the starch content of hindgut digesta (Li et al., 2012, 2016). The latter increase can also explain why the SARA challenges increased the propionate content of the feces (Russell and Rychlik, 2001; Plaizier et al., 2018). The size of the depression of the fecal pH during the SARA challenges, however, does not indicate that hindgut acidosis was induced (Gressley et al., 2011; Plaizier et al., 2018).

In our study, two successive SARA challenges were conducted, to determine if repeated SARA challenges have a more severe impact than a single SARA challenge. This was done as Dohme et al. (2008) and Pourazad et al. (2016) reported that repeated SARA challenges resulted in more severe rumen pH depressions than the initial SARA challenge. These studies contrast with our findings, as for cows on the XPC and NTL treatments the rumen pH depression below pH 5.6 did not differ between the first and second SARA challenge, and for cows on the Control treatment, the rumen pH depression during the second challenge was lower than that during the first SARA challenge. Also, the duration below rumen pH 5.6 did not differ among the pre-SARA1, pre-SARA2, and post SARA2 stages, suggesting that, based on the rumen pH depression, cows recovered from the SARA challenges very quickly. Based on the rumen VFA concentrations, this recovery was also very quick. The recovery from a SARA challenge may be long when this challenge decreased the absorptive capacity of the ruminal epithelium and altered the composition and functionality of the rumen microbiota (Dohme et al., 2008). Based on rumen pH values, the SARA induced by Dohme et al. (2008) and Pourazad et al. (2016) were more severe than that induced in our study. Hence, the impact of the SARA challenges, including their effects of the microbiota and epithelia in the rumen, may have been lower, and, therefore, the recovery from SARA more rapid in our study. The fecal pH also did not differ between the first and second SARA challenges, suggesting that the recovery of the hindgut pH from SARA challenges is also rapid.

4.5.2 Effects of SCFP treatments

Results on the effects of SCFP supplementation on DMI vary among studies, but the meta-analysis by Poppy et al. (2012) on sixty-one research publications concluded that, across these studies, SCFP increased DMI by 0.62 kg/d. Due to the high variation among cows, many individual studies may not have had sufficient statistical power to find significant effects of SCFP supplementation on feed intake. Several individual studies did indeed not observe an effect of SCFP on DMI (Allen and Ying, 2012; Li et al., 2016; Olagaray et al., 2019). However, whereas Shi et al. (2019) reported that DMI during the close-up and fresh periods was not affected by supplementation with 19 g/d of NutriTek, but they found that SCFP supplementation during post-fresh period tended to reduce DMI. In contrast, Dann et al. (2000) observed that supplementation with 60 g/d of Diamond V XP increased DMI of dairy cows during the immediate pre- and post-partum periods, but not later in lactation. These authors attributed this increase in DMI to an improved fiber digestion and reduced fill in the rumen. The reason that this supplementation did not increase DMI later in lactation could be that at that stage of lactation, physical fill is no longer rate limiting for DMI (Allan et al., 2000). In agreement, a parallel study to our experiment (Senaratne, 2019) found that supplementation with NutriTek increased fiber digestibility.

Ramsing et al. (2009) also reported an improved DMI before calving as well as an increase in milk production of 10% in dairy cows supplemented with 57 and 227 gr/d of SCFP (XP, fully fermented yeast culture of *S. cerevisiae*, Diamond V Mills, Cedar Rapids, IA). Despite this, these authors did not find that supplementation with SCFP increased DMI after calving. An explanation for the effect of SCFP on the rumen pH of cattle on high grain diets may be that the fermentation of starch in the rumen is reduced, whereas that in the hindgut is increased (Allen and Ying, 2012; Shen et al., 2018). This is confirmed by our study, as NTH treatment increased the rumen pH during the SARA challenges and decreased the concentration of total VFA in the rumen and the fecal pH and increased the concentration of propionate in feces. The more favorable environment in the rumen during the NTH treatment could have attenuated the milk fat depression resulting from the SARA challenges (Bauman and Griinari, 2003), but that did not occur during this study. Nevertheless, across the stages of SARA, the FCM of NTH was higher than that of the NTL treatment, and the fat content of the NTH treatment tended to be higher than that of the XPC treatment. Hence, the effects of the NTH treatment on milk production was more favorable than those of the other SCFP treatments.

Across the stages of SARA, cows on the NTH treatment had lower rumen concentrations of propionate and a higher acetate to propionate ratio than cows on the NTL treatment. Also, the concentration of total VFA in the rumen was lower during the NTH treatment than during the Control and XPC treatments. In contrast, the concentrations of acetate and propionate was highest in NTH cows among all treatments, and the concentration of total VFA was higher than NTL and XPC, and the acetate to propionate ratio in the feces were lower during the NTL treatment than during the other treatments. Together with the effects of the NTH treatment on the rumen and feces pH, these changes in VFA show that the NTH treatment shifted fermentation from the rumen to the hindgut, thereby creating a more favorable rumen environment during SARA. Such a shift in fermentation may not be entirely beneficial when it induces hindgut acidosis (Gressley et al., 2011; Plaizier et al., 2018). However, the fecal pH values during the NTH treatment do not suggest that hindgut acidosis occurred. Our results also show that an increased digestion of starch in the small intestine must not have compensated for the reduced fermentation of starch in the rumen, as the increased fermentation in the hindgut during the NTH treatments was likely the result of an increased starch content of hindgut digesta during this treatment (Li et al., 2012, 2016).

It has also been proposed that the positive effects of yeast products are not solely through a direct action on the rumen pH, but also through a modulatory effect on the fermentation process and ruminal microbiome, such as by stimulation of lactate utilizers and an increase in certain cellulolytic bacteria and fungi (Calsamiglia et al., 2012). The soluble growth factors in SCFP have been shown to stimulate growth of pure cultures of ruminal bacteria that digest cellulose and utilize lactate in vitro (Callaway and Martin, 1997). Also, Calsamiglia et al. (2012) proposed that positive effects of yeast products are modulatory effects on the fermentation process and ruminal microbiome, such as by stimulation of lactate utilizers and fibrolytic microorganisms. This may explain why Mao et al. (2013) observed that XP (Diamond V, Cedar Rapids, IA) SCFP increased the populations of fibrolytic microorganisms including protozoa, fungi, *F. succinogenes*, *R. albus*, and *R. flavefaciens* during in vitro incubations. In agreement, Harrison et al. (1988) found that supplementation with 114 g/d of a SCFP tended to increase the abundance of anaerobic bacteria and increased that of cellulolytic bacteria in the rumen of dairy cows.

A main finding from our study is that the NTH treatment is able to attenuate the impact of SARA on the rumen pH depression, whereas the effect of other SCFP treatments on this impact was not significant. Despite this, our study did not find any attenuating effects of NTH on milk yield, milk fat yield, and milk protein yield during SARA challenges. Possibly more experimental units may have been needed to detect significances of these effects (Poppy et al., 2012).

The SCFP NutriTek is different from XPC, in that it provides enhanced bioactive compounds which include those found in XPC, new fermentation metabolites, and additional propriety antioxidants, including polyphenols (Diamond V Mills Inc. Cedar Rapids, IA). Several of the symptoms of grain-induced SARA, including inflammation, may be, at least partly, due to the accompanying oxidative stress (Gäbel et al., 2002; Karmin et al., 2011; Plaizier et al., 2018). Polyphenols have antioxidative and anti-inflammatory effects in mammals (Gessner et al., 2016). In agreement, De Nardi et al. (2016) observed that administration of 100 g/d of a polyphenol mixture increased the rumen pH and reduced the concentrations of neutrophils and acute phase proteins in peripheral blood in dairy heifers on a high-grain diet. In addition, polyphenols may increase the activity of lactate-consuming bacteria and the growth of propionate-producing bacteria (Balcells et al., 2012).

Hence, the higher content of polyphenols and other antioxidants in NutriTek compared to XPC may explain why NutriTek at a dose of 38 g/d had the larger effect on attenuating the drop in rumen pH and fiber digestion caused by the SARA challenges.

4.6 Conclusions

The rumen pH depressions, feed intakes, and concentrations of fermentation acids in the rumen and feces show that the SARA challenges induced a mild form of SARA in cows under the control, XPC, and NTL treatment. Based on the rumen pH data, the SARA challenges did not induce SARA in cows on the NTH treatment. The latter may have been due to a shift of the digestion of starch from the rumen to the intestines, creating a better rumen environment. This shift was not obviously associated with improvements in the yield of milk and milk fat, and in

FE. However, the FCM was higher for the NTH treatments than for the NTL treatment, and the milk fat content was tended to be higher for the NTH treatment than for the XPC treatment. The rumen pH of the cows recovered quickly from the SARA challenges, and the first and second SARA challenges did not differ in the severity of their rumen pH depression. In contrast, the milk fat content and FE did not make a fast recovery from these challenges. Our data suggest that the NTH treatment can limit the depressions of the rumen pH that are associated with high grain feeding without reducing milk production.

Chapter 5: General discussion

The SARA in our study was milder than that in earlier studies. Nevertheless, the rumen pH depressions during the SARA stages in our study were considerably lower than that during the pre-SARA stage. Despite this, the rumen pH depression was not so substantial that it greatly impacted the health and production of the cows. The rumen pH data from our study showed that the cows experienced a mild form of SARA, compared to the study by Li et al. (2016) who observed a rumen pH below 5.6 350 min/d. This mild form of SARA explains why the SARA challenges did not reduce feed intake and milk production. The DMI and milk production in our study were increased by the SARA challenges, while a more severe SARA reduced both feed intake and milk production in a previous study (Li et al., 2016). One reason that a grain-induced SARA is expected to reduce the DMI is that it can reduce the dry matter digestibility and, especially, the NDF digestibility (Plaizier et al., 2001). This reduces the DMI due to an increase in rumen fill. In agreement with this, the parallel study of Senaratne. et al. (2019) reported that SARA reduced the total tract apparent digestibility of the NDF from 61.1 to 49 %.

Another mechanism that controls DMI is the metabolic response to the ingestion of feed. It is believed that increased levels of propionate in the blood suppresses DMI. Infusion of propionate into the rumen of the dairy cows has been shown to reduce meal length, meal size and DMI in dairy cows (Choi and Allen, 1999). In other studies on dairy cows (Stocks and Allen, 2012; Gualdrón-Duarte and Allen, 2017), infusions of propionate decreased the feed intake compared to iso-energetic infusions of a VFA mixture and acetate. These findings show that the hypophagic effects of propionate go beyond regulating the energy supply to the cows, since the same amount of the energy that was provided by infusion of acetate or butyrate did not have the same effect. Therefore, there may be other mechanisms involved related to the role of propionate in satiety. It has been shown that the metabolism of acetate in the liver of dairy cows is limited while the metabolism of propionate is extensive due to the high activity of propionyl CoA and limited activity of acetyl CoA in hepatocytes (Ricks and Cook, 1981; Demigné et al., 1986).

An additional factor that could reduce DMI and milk yield during SARA is inflammation. It has been shown that SARA triggers an immune response, and this is likely due to translocation of LPS from digestive tract into blood circulation (Gozho et al., 2007; Li et al., 2016; Guo et al., 2019). This endotoxin is part of the outer membrane of gram-negative bacteria, and its release into the rumen and hindgut is increased during SARA. Earlier studies have shown that SARA also reduces the barrier function of epithelia of the digestive tract. The combination of this reduced barrier function with the increases in luminal LPS increase the translocation of LPS (Plaizier et al., 2012). The presence of LPS in the blood circulation results in an inflammation characterized by an acute phase response (Plaizier et al., 2012; Li et al., 2016; Guo et al., 2019). The parallel study by Guo et al. (2019) confirmed that the SARA challenges increased LPS in rumen digesta and in feces, and that this was accompanied by increased in the blood plasma concentrations of the acute phase proteins SAA and Hp. However, these increases were small compared to those observed in earlier studies with more severe SARA challenges. This provides additional evidence that the SARA induced in our study was moderate. In the study from Guo et al. (2019), the NTH treatment reduced the LPS concentration in the rumen as well as the blood concentrations of acute phase proteins. This indicated that the high dose of NutriTek was able to attenuate negative effects of the SARA challenge. These findings are consistent with our finding that NTH supplemented cows did not undergo SARA during the feeding of the high grain diet.

Another parallel study (Senaratne, 2019) observed that the SARA challenges reduced the apparent total tract digestibility of NDF. This confirmed the observations of Plaizier et al. (2001), that a grain-based SARA challenge reduced the *in situ* NDF digestibility in the rumen. Reasons for this reduction are likely reductions in the abundances and functionality of the rumen microbiota, and especially of fiber-digesting microbiota, as the latter bacteria are sensitive to a low rumen pH (Shi and Weimer, 1992; Russell and Wilson, 1996). In agreement with this, the parallel study of Guo et al. (2018) showed that high grain feeding reduced the alpha diversity of the ruminal microbiota during the control treatment. However, this was not the case in NTH supplemented cows. This may be due to the more stable rumen conditions in these cows.

Various tools have been applied in order to reduce negative impacts of SARA, such as feeding TMR, increasing the feed delivery frequency, and supplementing yeast products, such as SCFP. In a previous study, supplementation with 14 g/d/head of Diamond V Original XPC™ (XPC, Diamond V, Cedar Rapids, IA) to SARA challenged cows reduced the variation in rumen pH, tended to decrease the

LPS concentration in the rumen, and reduced blood levels of the pro-inflammatory cytokine TNF-α (Li et al., 2016). The SARA challenge in this earlier studies took place for only one week, while the cows in commercial dairy farms are likely at high risk of SARA for a longer periods of time, and may experience multiple bouts of SARA during their lactation (Garrett et al., 1997; Oetzel et al., 2000). This is expected to challenge cows more compared to a single SARA challenge (Dohme et al., 2008). In contrast with our hypothesis, we did not observe any differences between the first and second SARA challenges regarding the rumen pH depression, and the fermentation in the rumen and hindgut. This shows that cows recovered rapidly from the first SARA challenge. As discussed earlier, this may be due to the mild form of SARA.

The cows on the NTH treatment showed a shift in fermentation from the rumen to the hindgut, thereby reducing the rumen pH depression, without compromising hindgut fermentation. The lower fecal pH in NTH cows combined with the higher fecal concentrations of acetate and propionate, demonstrated that this treatment increased the amount of dietary starch that bypassed rumen fermentation and digestion in the small intestine.

Chapter 6: General conclusion

In conclusion, the SARA challenges in our study were mild and the rumen and fecal pH and VFA concentrations, rumen NDF digestibility, rumen LPS, and immunological markers in the blood shifted back to the pre-SARA stage values rapidly after the challenges. In addition, supplementing NutriTek at the higher dose showed the potential to attenuate negative impacts of SARA, as the cows on this treatment had a higher rumen and fecal pH values, lower concentrations of markers of inflammation and a higher fiber digestibility during the SARA challenges, compared to cows that were not supplemented with SCFP. This result may, in part, be explained by a stabilizing effect of this NutriTek supplementation on the rumen microbiota during SARA. This stabilizing effect may be due to increased by-pass of starch from the rumen to the lower gut that leads to a decrease in fermentation in the rumen. It is believed that polyphenols, which are assumed to be higher in NutriTek comparing to XPC, increase the uptake of the lactate in the rumen, increased the diversity and richness of the rumen microbiota, and reduce the time pH < 5.6 in the rumen when high grain diet are fed. However, the exact mechanism for this is yet to be uncovered. It is expected that the higher ruminal pH and lower fecal pH in NTH cows in our study is due to the bypass of the starch from the rumen. It should be noted that bypassing the starch to the lower gut may lead to increased fermentation in the hindgut and may, therefore, cause hindgut acidosis. The fecal pH in our study did not indicate that hindgut acidosis occurred in the cows. An important point that needs to be investigated is how much of the starch that escaped the rumen fermentation was digested in the small intestine and could be utilized by the cows.

Chapter 7: Future directions

Future directions include:

To supplement the cows with the other doses of NutriTek in order to investigate the cow response.

To apply genomics, transcriptomics and metabolomics to investigate the mechanisms through which these products affect the microbiota of the digestive tract, as well as their interactions with each other and with the host.

To induce a more severe form of SARA to study the effects of the SCFP during a more severe SARA challenge.

To uncover the mechanism of bypassing the starch from the rumen and to determine how much of the starch bypasses the rumen and excreted in the feces to determine if using the SCFP increase the amount of the starch that was not being used by the animal.

To use this product in a larger scale e.g. a large dairy farm with larger numbers of dairy cows involved in the experiment to increase the accuracy and the power of the statistical tests on variables that require a high number of replicates per treatment, such as DMI and milk production.

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