THE EFFECTS OF PARTIAL REPLACEMENT OF BARLEY STARCH WITH SUGARS ON RUMINAL FUNCTION, OMASAL NUTRIENT FLOW, UREA-N RECYCLING, AND PRODUCTION PERFORMANCE IN DAIRY COWS.

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Animal and Poultry Science
University of Saskatchewan
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ABSTRACT

A considerable number of studies have shown that the partial substitution of corn starch with sugars is beneficial in terms of improving dry matter (DM) intake, milk yield and efficiency of nitrogen utilization in dairy cows without putting them at risk of developing ruminal acidosis; however, research with dairy cows fed barley-based diets is limited. The objective of this study was to determine the effects of partial replacement of barley starch with dried whey permeate (DWP; contained 83% lactose) on ruminal acidosis, transport of short-chain fatty acids (SCFA) across the ruminal wall, urea-N recycling to the digestive tract, ruminal microbial protein production, omasal nutrient flow, and production performance in dairy cows. Eight Holstein cows (97 ± 10 days-in-milk; 733 ± 63 kg body weight) were used in a replicated 4 × 4 Latin square design with 28-d periods (18 d of adaptation and 10 d of measurements). Four cows in one Latin square were ruminally-cannulated to facilitate ruminal fluid and omasal digesta sampling. Cows were fed a barley-based diet (3.5% total sugar [TS]; control), or diets that contained 6.5, 9.5 or 12.5% TS (DM basis). Diets were isonitrogenous (17.3% crude protein) and contained 24.3, 22.2, 21.2, and 19.1% starch for the control, 6.5, 9.5 or 12.5% TS diets, respectively. Dietary inclusion of DWP did not affect DM intake (mean = 26.6 kg/d) and milk yield (mean = 34.9 kg/d); however, milk lactose content quadratically changed (4.40, 4.42, 4.46, and 4.40% for the control, 6.5, 9.5, and 12.5% TS diets, respectively) across diets. Ruminal concentration of acetate, propionate, and total SCFA were not affected by dietary treatment; however, ruminal concentration of butyrate was cubically increased (12.8, 13.0, 14.1, and 14.9 m*M* for the control, 6.5, 9.5, and 12.5% TS diets, respectively) as dietary content of DWP increased. When expressed as absolute (mmol/h) or fractional (%/h) rates of absorption, the rates of acetate, propionate, and total SCFA absorption across the ruminal wall were cubically decreased by the 9.5% TS diet compared to control and 6.5% TS diets, then increased by the 12.5% TS diet compared to the 9.5% TS diet. The rate of butyrate absorption across the ruminal epithelium tended to change cubically as level of dietary DWP increased. For individual and total SCFA, Cl^--insensitive and Cl^--competitive rates of absorption across the ruminal wall were largely unaffected by dietary treatment, except that the Cl^--competitive absorption of propionate tended to be cubically altered as dietary content of DWP increased. Mean ruminal pH tended to change quadratically (6.32, 6.31, 6.34, and 6.22 for the control, 6.5, 9.5, and 12.5% TS diets, respectively) as dietary content of DWP increased. Dietary addition of DWP cubically changed

the ruminal concentration of ammonia-N (NH_3-N; 12.1, 12.3, 10.9, and 9.40 mg/dL for the control, 6.5, 9.5, and 12.5% TS diets, respectively). The omasal flow of bacterial N quadratically changed (513, 517, 539, and 468 g/d for the control, 6.5, 9.5, and 12.5% TS diets, respectively) as dietary content of DWP increased. The ruminal digestibility of DM linearly increased as dietary content of DWP increased, but total-tract DM digestibility was unchanged. The ruminal and total-tract digestibilities of water-soluble carbohydrates (WSC) cubically increased as dietary content of DWP increased. There were no dietary effects on ruminal and total-tract digestibility of neutral detergent fiber and acid detergent fiber. Total urinary N excretion quadratically changed (223, 255, 238, and 223 g/d for the control, 6.5, 9.5, and 12.5% TS diets, respectively), and fecal N excretion linearly increased as dietary content of DWP increased; however, productive N was unaffected by dietary TS content. Quadratic changes were observed in absolute amounts of endogenous production of urea-N (i.e., UER; 467, 531, 522, and 472 g/d for the control, 6.5, 9.5, and 12.5% TS diets, respectively). Although the absolute amount of urea-N transferred to the gastrointestinal tract (i.e., GER; 307, 354, 340, and 309 g/d for the control, 6.5, 9.5, and 12.5% TS diets, respectively) tended to change quadratically, there was no difference across diets when GER was expressed as a proportion of UER. Increasing dietary TS by adding DWP tended to change microbial utilization of recycled N in a cubic manner (15.8, 13.7, 15.8, and 15.9 % for the control, 6.5, 9.5, and 12.5% TS diets, respectively); however, recycled N that was utilized for anabolism (UUA) was unaffected by diets. These results suggest that the partial replacement of barley starch with DWP does not affect the production performance of dairy cows; however, dietary TS concentration of 9.5% potentially improve ruminal N efficiency by decreasing NH_3-N concentration and increasing omasal microbial N flow.

ACKNOWLEDGEMENTS

I express my sincere gratitude to Dr. Timothy Mutsvangva, my thesis supervisor for always being an excellent guide and motivation to achieve all my goals in the master's program. I would like to extend my appreciation to the rest of the committee including my co-supervisor, Dr. Greg Penner, Dr. Andrew Van Kessel, and Dr. Fiona Buchanan for their valuable support and constructive criticism.

I should take my hat off to all the staff in the Rayner dairy research and teaching facility for their enormous support for my animal experiment.

I am grateful to Natalia Rudnitskaya, the lab manager in the animal and poultry science department, for her valuable and kind support in order to conduct my laboratory analysis. I also appreciate the effort of Stocki Myles who conducted the isotope analysis for me without any delay.

I owe my friends, Jolet, Sam, Elizabeth, Fostin, JK, Rodrigo, Janna, Basim, and Khalil a debt of gratitude for providing their invaluable help whenever I wanted.

I gratefully acknowledge the government of Saskatchewan, Ministry of agriculture for funding for research project through agriculture development fund (ADF).

My lovely wife Namala, thank you so much for being with me in every sorrow and joy in my life and for the inspiration.

Last but certainly not least, from the depth of my heart, I thank my mother, father and grandmother for your guidance, encouragement, and support that allow me to follow my ambitions.

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LIST OF ABBREVIATIONS

[$^{15}N^{15}N$]-urea, double-labeled urea

AA, amino acids

ADF, acid detergent fiber

AOAC, association of official analytical chemists

BCFA, branched-chain fatty acid

BHBA, beta-hydroxybutyrate

BW, body weight

CP, crude protein

Cr-EDTA, chromium-ethylenediaminetetraacetic acid

DIM, days in milk

DM, dry matter

DMI, dry matter intake

DWP, dried whey permeate

EE, ether extract

FAB, fluid-associated bacteria

FP, fluid phase

GC, gas chromatography

GER, GIT entry rate (urea-N transfer to the GIT)

GIT, gastro-intestinal tract

iNDF, indigestible NDF

LPP, large particle phase

MNE, milk N efficiency

MUN, milk urea nitrogen

N, nitrogen

NAN, non-NH_3-N

NANBA, non-NH_3 non-bacterial N

NDF, neutral detergent fiber

NFC, non-fiber carbohydrate

NH_3, ammonia

NPN, non-protein N

NRC, national research council

NSC, non-structural carbohydrate

OM, organic matter

OMTDR, OM truly digested in the rumen

OTD, omasal true digesta

PAB, particle-associated bacteria

PP, particle phase

PUN, plasma urea nitrogen

RDP, ruminally-degradable protein

RFC, readily-fermentable carbohydrate

ROC, urea-N return to the ornithine cycle

RUP, ruminally-undegradable protein

SARA, sub-acute ruminal acidosis

SCFA, short-chain fatty acid

SPP, small particle phase

TMR, total mixed ration

TS, total sugar

UER, urea-N entry rate (endogenous production of urea)

UFE, urea-N loss to feces

UUA, urea-N utilized for anabolism

UUE, urinary urea-N excretion

WRR, temporary isolated washed reticulo-rumen

WSC, water-soluble carbohydrate

$YbCl_3$, ytterbium chloride

1. GENERAL INTRODUCTION

In high producing ruminants (dairy and beef cattle), carbohydrates are the major source of energy. For most dairy diets, carbohydrates contribute 60 to 70% of the total feed on a dry matter (DM) basis (NRC, 2001). In the rumen, carbohydrates undergo microbial fermentation, providing energy for microbes, with the production of short-chain fatty acids (SCFA) as a byproduct. The SCFA that are produced from microbial fermentation provide the majority of the energy requirements for maintenance, growth and lactation in dairy cows (Bergman, 1990). As one of the major dietary carbohydrates, starch plays a vital role in high-producing ruminants in terms of energy supply. Barley that contains 57 to 58% starch on a DM basis (Huntington, 1997) is the major cereal grain that is included in dairy cow diets as a source of energy in western Canada.

One of the detrimental effects of barley-based dairy diets that contain high levels of highly-fermentable starch is that they can induce ruminal acidosis (Klevenhusen et al., 2013). In dairy cows, the most prevalent form of ruminal acidosis is subacute ruminal acidosis (SARA; Enemark, 2008). Because of the extensive fermentation of barley reaching the rumen, high-producing dairy cows fed barley-based diets have a high risk of SARA. In western Canada, SARA is a persistent problem in high-producing dairy herds that are fed barley-based diets. Sub-acute ruminal acidosis negatively affects the cow and the herd in many ways, including reduction of milk production efficiency, premature culling (Krause and Oetzel, 2005), and laminitis (Nocek, 1997). Economic losses due to SARA in North American dairy herds are estimated around 1.3 USD per day per affected animal (Stone, 2004). Therefore, it is important to develop strategies to reduce the prevalence of SARA in dairy herds so as to improve their overall performance, productivity, health, and economic outcomes.

Reducing diet fermentability in the rumen is a possible approach to minimize SARA. However, its implementation is limited due to the potential negative impacts on animal performance. Partial replacement of grain starch with sugars is an alternative approach to mitigate the risk of SARA. Even though sugars are known to be highly fermentable in the rumen compared to starch (e.g., sugars: 40-60 %/h; starch: 20-40 %/h; Amburgh et al., 2015), several studies have demonstrated the ability to use sugars and byproducts containing sugars without detrimental effects on dairy cattle performance, productivity, and ruminal health. A study

conducted by Broderick and Radloff (2004) showed an increase in dry matter intake (DMI) and quadratic changes in milk yield when corn starch was partially replaced with molasses. Penner and Oba (2009) observed increased DMI and a tendency for increased milk fat yield when corn starch was partially replaced with sucrose. Also, DeFrain et al. (2004) observed a tendency for increased DMI when corn starch was replaced with lactose. In addition, recent studies have reported increased (Khorvash et al., 2014), or a tendency for increased (Penner and Oba, 2009), or unaffected ruminal pH (DeFrain et al., 2004; Broderick et al., 2008) when dietary starch was partially replaced with sugars. These data suggest that cereal grain starch can be partially replaced with highly-fermentable sugars in order to improve production performance in high producing ruminants without putting them at risk of SARA.

Mechanisms for the regulation of ruminal pH when cereal grain starch is partially substituted with sugars are not well understood. Increased ruminal concentration of butyrate (DeFrain et al., 2004, 2006) after the replacement of starch with sugars as has been reported is known to stimulate ruminal epithelial proliferation (increased length, width, and surface area of ruminal epithelium; Sakata and Yajima, 1984; Penner et al., 2011), which may enhance the absorption of SCFA. Chibisa et al. (2015) demonstrated an increase in Cl^--competitive absorption of acetate and propionate when corn or barley starch was partially replaced with lactose. Based on that finding, they suggested that the reduced risk of ruminal acidosis when dietary starch is partially replaced with sugars is partly due to increased SCFA absorption across the rumen epithelium. However, there could be other mechanisms that can explain the reduced risk of SARA associated with diets that contain sugars.

Nitrogen (N) is an important constituent of milk protein. However, the efficiency of incorporating N from dietary crude protein (CP) into milk is relatively low (Hristov et al., 2004). Dairy cows capture between 25 and 35% of dietary CP as milk N (Sinclair et al., 2014), thus as much as 72 to 73% of dietary N is excreted in urine and feces (Tomlinson et al., 1996). Excessive excretion of N from the intensive cattle industry has become an environmental burden. Therefore, more attention should be focused on improving the efficiency of N utilization in dairy industries. The efficiency of N utilization can be improved in dairy cattle either by reducing N intake or maximizing N incorporation into milk protein (Higgs et al., 2013). In order to improve N utilization, it is important to enhance the efficiency of microbial protein production and urea

recycling into the rumen. Since sugars are more fermentable than starch in the rumen, substitution of starch with sugars may rapidly provide energy for microbial protein synthesis. As a result of this increased rate of carbohydrate fermentation and rapid supply of energy for microbes, urea recycling to the rumen becomes more efficient, thus improving the efficiency of N utilization in cattle. Urea is the major nitrogenous waste in the urine and a major contributory factor to environmental pollution. However, cattle have a physiological mechanism that allows them to recycle urea to the digestive tract where it can be used for microbial protein synthesis that, subsequently, supplies amino acids (AA) to the host animal (Marini et al., 2004). Urea is synthesized in the liver and, on average, 67% of hepatic urea-N output returns to the digestive tract (Lapierre and Lobley, 2001). Between 35 and 55% of urea-N that returns to the digestive tract (Lapierre and Lobley, 2001) is utilized as a N source (as NH_3-N) by rumen microbes to synthesize microbial protein. Increased intake of cereal grain-based diets containing readily-fermentable carbohydrates (RFC) increases urea recycling into the gut (Huntington, 1989), thereby reducing urinary N excretion (Higgs et al., 2013). Replacing dietary starch with sucrose decreased urinary urea-N excretion (Broderick et al., 2008), and partially replacing corn or barley starch with dried whey permeate (DWP) improved N utilization efficiency in dairy cows (Chibisa et al., 2015). However, in the literature, there are no published studies that have examined the effects of partially replacing starch with sugars on urea-N recycling in dairy cows fed barley-based diets that are typically fed in western Canada.

Dietary inclusion of sucrose up to 10% total sugar (TS) levels in corn-based diets has been demonstrated to be beneficial on DMI and milk production, and N utilization of high-producing cows (Broderick et al., 2008); however research work is limited on barley-based diets. Recently, Chibisa et al. (2015) explored the effects of partial replacement of corn or barley starch with DWP up to 8% TS level on animal performance. In that study, 8% TS level did not increase the risk of SARA, suggesting that DWP can be used to substitute barley starch. However, feed intake and milk production were not affected when DWP was fed, suggesting that the 8% TS level might not have been adequate to elicit positive responses. Therefore, the aim of my thesis research was to determine the effects of partial replacement of barley starch with DWP up to 12.5% TS level on ruminal function, omasal nutrient flow, urea-N recycling, and production performance in dairy cows.

2. LITERATURE REVIEW

2.1. Feeding cows towards a sustainable dairy industry

Improving the efficiency of milk production and profit maximization are major goals of a sustainable dairy industry (Bauman et al., 1985). Over the past few decades, the production potential of dairy cows has been substantially increased by the advancement of modern genetics and effective management strategies (VandeHaar and St-Pierre, 2006). One of the important management strategies is nutritional management, which can strongly influence milk production and cow health. Feeding diets that can provide adequate amounts of nutrients to meet the metabolic demands of lactating cows plays a significant role in terms of improving the quality and quantity of milk. Switching from forage-based diets to grain-based diets is a strategy to increase energy availability to dairy cows and, subsequently, to improve their milk production (VandeHaar and St-Pierre, 2006). Energy is the primary factor that limits milk production in dairy cows (Allen, 2000). In comparison to high-forage diets, feeding grain-based diets that are more energy-dense helps to overcome the limitation of energy availability to high-producing dairy cows. Although grain-based diets can provide adequate amounts of nutrients to meet the nutrient requirements of high-producing dairy cows, the drawback of feeding high-grain diets is that they may negatively influence the health and performance of lactating cows by increasing the risk of SARA (Krause and Oetzel, 2006).

One of the goals for sustainable dairy production is to have environmentally-friendly production systems (VandeHaar and St-Pierre, 2006). However, dairy as a part of the livestock industry, negatively affects the environment in several ways, including air and water pollution (Steinfeld et al., 2006). Excessive excretion of N from intensive dairy operations is one of the major factors that results in air and water pollution (Tamminga, 1992; Hutson et al., 1998). Inefficient capturing of dietary CP as milk protein (Hristov et al., 2004) not only leads to excess N excretion, but also has implications on the profitability of dairy operations as protein supplements are some of the most expensive feed ingredients in dairy diets. Therefore, it is extremely important that nutritionists explore feeding strategies that optimize animal health and productivity while ensuring that environmental sustainability is maintained.

2.2. Carbohydrates as the major energy source in dairy diets

As the major source of energy in dairy diets, carbohydrates play a vital role in improving the lactational performances of high-producing dairy cows. According to NRC (2001), lactating cow diets contain 60 to 70% carbohydrates on a DM basis. Dietary carbohydrates can be partitioned into structural carbohydrates (i.e., cellulose and hemicellulose) and non-fiber carbohydrates (NFC) which includes pectin, non-structural carbohydrates (NSC), and organic acids (NRC, 2001). Non-structural carbohydrates are composed of starch, soluble sugars, and fructans (Beauchemin et al., 1997; NRC, 2001). In comparison to structural carbohydrates, NSC are more dense in energy. Early lactating cows have a high energy demand for milk synthesis (NRC, 2001), in addition to their growth and maintenance energy requirements. Therefore, most of the lactating cow diets are formulated to have greater proportions of NSC than structural carbohydrates. In the rumen, microbes ferment dietary carbohydrates (Russell et al., 1992) in order to acquire energy for their protein synthesis and other cellular activities. During microbial fermentation of carbohydrates, SCFA are produced as a byproduct. Once absorbed into the bloodstream from the rumen, these SCFA supply the majority of the energy requirements for lactation, growth, and maintenance of dairy cows (Bergman, 1990). When the dietary availability or ruminal digestion (rate and/or extent) of NSC is increased, both the energy supply to support ruminal microbial growth and, as a consequence, SCFA absorption for animal use, also increase. Starch is the primary NSC component in lactating cow diets and dietary starch concentration is usually increased by adding cereal grains (Dyck et al., 2011). In North American dairy diets, either corn or barley is included as the major cereal grain due to their abundant supply. Barley, which contains 57 to 58% starch on a DM basis (Huntington, 1997) is the major cereal grain that is included in lactating cow diets in western Canada.

Sugars such as lactose are also a rich source of fermentable carbohydrates, but their use as an alternative energy source in dairy diets is limited due to lack of knowledge on sugar metabolism and fermentation characteristic in the rumen (Oba, 2011). Sugars are water-soluble in nature and can be categorized into disaccharides or monosaccharides. Sucrose, lactose and maltose are major disaccharides, whereas glucose, fructose, and galactose are major monosaccharides. The majority of feed ingredients that are commonly included in dairy cow diets contain trace amounts of sugars, such that dairy cow diets typically contain 2 to 4% TS

(Oba, 2011). Molasses (a source of sucrose) is used in total mixed rations (TMR) not only as an energy source (Broderick and Radloff, 2004; Oelker et al., 2009), but also to improve palatability and reduce feed sorting behavior of cows (DeVries and Gill, 2012). In addition, whey products that contain lactose have the potential to be included in lactating cow diets (Huber et al., 1967; Bragg et al., 1986).

2.3. Carbohydrate metabolism in dairy cows

2.3.1. Ruminal microbes that are responsible for carbohydrate degradation

Polysaccharide (e.g., cellulose, hemicellulose, pectin, starch, etc.) degradation is a result of complex symbiotic efforts of various microbes in the ruminal ecosystem (Flint et al., 2008). Plant cell wall polysaccharides are degraded by the combined effort of bacteria, protozoa and fungi (Wang and McAllister, 2002). *Ruminococcus flavefaciens*, *R. albus*, and *F. succinogenes* are major cellulolytic bacteria in the rumen (Koike and Kobayashi, 2009). *Prevotella ruminicola*, *P. bryantii*, *B. fibrisolvens*, and *S. bovis* are few of the amylolytic bacteria responsible for starch degradation (Cerrilla et al., 2003; Flint et al., 2008). Polysaccharide-degrading enzymes (e.g., cellulase, hemi-cellulase, pectinase, amylase, etc.) that are produced by ruminal microbes are capable of hydrolyzing polysaccharides to glucose. Dietary disaccharides (i.e., sucrose, maltose, lactose) are also hydrolyzed to monosaccharides such as glucose, galactose, and fructose. Sucrose that contains glucose and fructose is the most common and naturally occurring disaccharide in plant materials (Hall, 2010). Maltose is composed of two glucose units, whereas lactose is composed of glucose and galactose. Because disaccharides and monosaccharides are highly water-soluble in nature, sugars are mainly in the fluid fraction of rumen digesta. Before being fermented to yield ATP, ruminal bacteria should come in contact with the sugar molecule and absorb that sugar into the cytoplasm prior to fermentation; ion gradient-linked active transport, substrate-binding transport, and group translocation are some of the mechanisms of transporting sugars across bacterial cell membrane (Russell and Wallace, 1997). *Prevotella ruminicola*, *B. fibrisolvens*, *S. bovis*, and *S. ruminantium* are some of the microbes that can hydrolyze disaccharides in the rumen (Russell and Baldwin, 1978). Ruminal microbes utilize simple sugars to produce ATP, whereas SCFA are produced as byproducts (van Houtert, 1993).

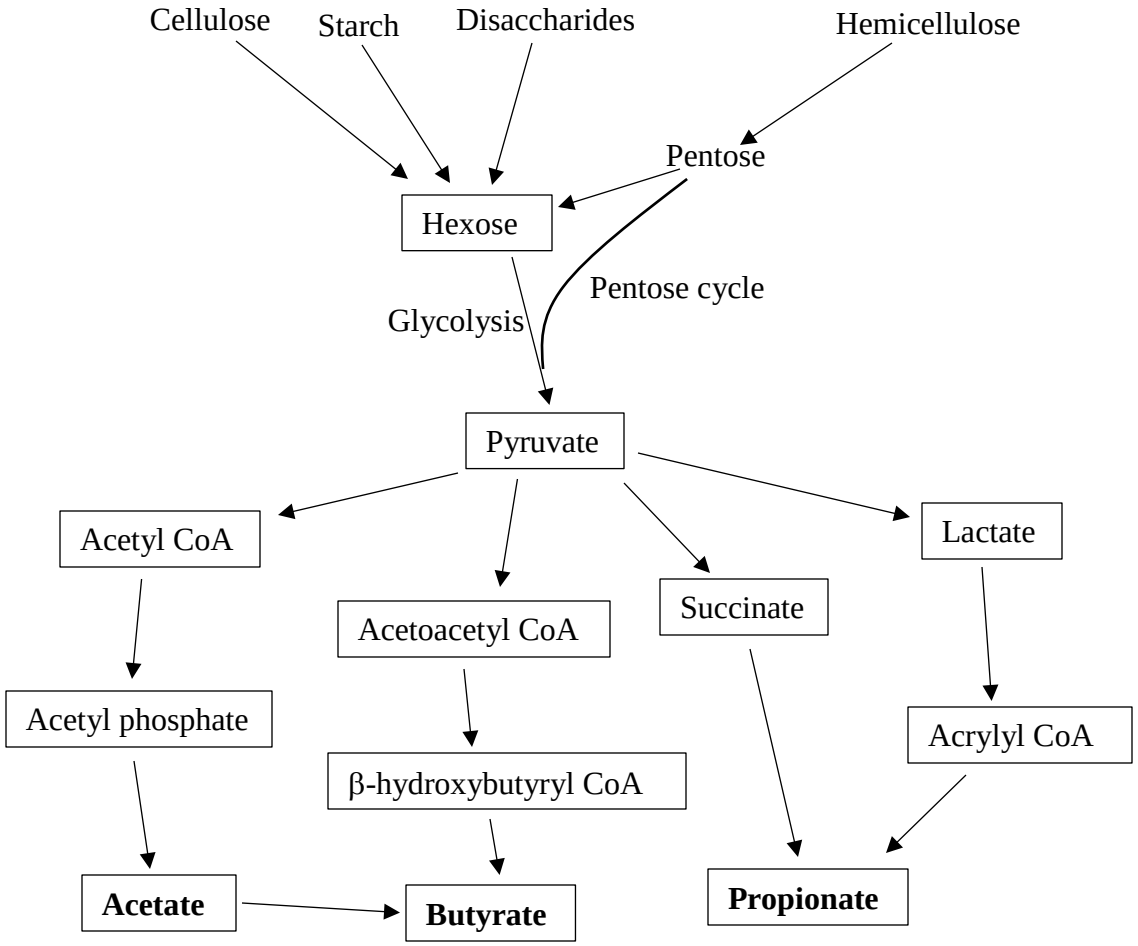

Figure 2.1 Production of short-chain fatty acids (SCFA) in the rumen (adapted from van Houtert 1993; Dijkstra and France 2005). Microbial degradation of polysaccharides and disaccharides in the rumen releases hexose and pentose sugars. These hexoses are further fermented by ruminal microbes in order to generate ATP for their maintenance and growth, whereas SCFA are produced as a byproduct. Acetate, propionate, and butyrate that provide the majority of the energy requirements for maintenance, growth, and lactation in dairy cows, are the major types of SCFA produced in the rumen. Each type of SCFA is produced through a different biochemical pathway. The acrylate pathway that produces propionate form pyruvate via lactate is more prominent in cows fed high concentrate diets.

2.3.2. Short-chain fatty acid production in the rumen

Acetate, propionate and butyrate are the major SCFA that are produced in the rumen (Figure 2.1); however, isobutyrate, valerate, and isovalerate are also produced in small amounts. Net production and molar proportions of each individual SCFA vary with dietary forage to concentrate ratio (Sutton et al., 2003). As an example, dairy cows fed high forage diets (forage:concentrate = 60:40), had molar proportions of 68, 19.4, and 12.6 mol/100 mol of acetate, propionate and butyrate, respectively, with a net production of 79.8 mol/d of SCFA. When the same cows were fed a high concentrate diet (forage:concentrate = 10:90), net production of SCFA was 90 mol/d and molar proportions of acetate, propionate and butyrate were 48.6, 36.6 and 8.8 mol/100 mol, respectively (Sutton et al., 2003).

Pyruvate that is synthesized as a result of the fermentation of hexoses and other monosaccharide (e.g., xylose catabolism) is converted to SCFA via several biochemical pathways with the aid of different microbial enzyme catalytic reactions in the rumen (van Houtert, 1993). Several pathways can be involved in acetate synthesis in the rumen. One of the major pathways for acetate production is the breakdown of pyruvate to acetyl phosphate and formate by pyruvate-formate lyase enzyme (van Houtert, 1993). Acetyl-phosphate is then converted to acetate under the influence of acetyl kinase. In another pathway, acetyl CoA is converted to acetyl phosphate with the influence of phosphotransacetylase and subsequently to acetate. *Ruminococcus flavefaciens* is one of the major acetate-producing bacteria in the rumen (Stewart et al., 1997). In addition, *M. elsdenii* has the ability to produce acetate from lactate (Prabhu et al., 2012).

Propionate is produced through two major pathways in the rumen (van Houtert, 1993; Figure 2.1). The succinate or dicarboxylate acid pathway is the most significant pathway of propionate production in ruminants that are adapted to high forage diets. Pyruvate is converted to succinate through several intermediates such as oxaloacetate, malate and fumarate. Succinate undergoes a series of decarboxylation reactions to produce propionate. In the acrylate or glyoxalate pathway, propionate is produced from pyruvate through lactate and acrylate. The acrylate pathway is more prominent in cows fed high concentrate diets. *Megasphaera elsdenii* is capable of producing propionate via the acrylate pathway in the rumen (Prabhu et al., 2012).

In order to synthesize butyrate, acetyl CoA is converted to β-hydroxybutyryl CoA via acetoacetyl CoA (van Houtert, 1993). Malonyl CoA, which can react with acetyl CoA to produce acetoacetyl CoA, also contributes to butyrate production in the rumen. β-hydroxybutyryl CoA is subsequently converted to butyrate through a series of chemical reactions. In addition, butyrate can be generated from acetate as a result of extensive carbon interchange among SCFA and this conversion is catalyzed by butyryl CoA/acetate CoA transferase enzyme (van Houtert, 1993). *Butyrivibrio fibrisolvens* is one of the major butyrate producers that exist in ruminal microbiota (Miller and Jenesel, 1979).

In addition, the carbon skeletons from the deamination of AA (e.g., valine, leucine, isoleucine, etc.) can be precursors for branched-chain fatty acids (BCFA) such as isobutyric, isovaleric and isobutyric acids in the rumen (Dijkstra and France, 2005).

2.3.3. Short-chain fatty acid absorption across the ruminal epithelium

Short-chain fatty acid absorption across the ruminal epithelium is accompanied with regulation of ruminal pH (Aschenbach et al., 2011). Each individual SCFA has the ability to release H^+ ions to the ruminal environment; however, SCFA themselves can act as buffers (i.e., HCO_3^- dependent absorption of $SCFA^-$ across the ruminal epithelium), thus facilitating the regulation of ruminal pH (Aschenbach et al., 2011). The absorption of SCFA contributes to 60% of ruminal buffering capacity in ruminants fed diets containing greater proportion of non-structural carbohydrates (Dijkstra et al., 2012). Therefore, SCFA absorption may play a significant role in pH homeostasis in high-producing dairy cows fed cereal grain-based diets.

2.3.3.1. Mechanisms for short-chain fatty acid absorption

Around 50 to 85% of SCFA produced in the rumen get absorbed across the ruminal epithelium, and several known mechanisms are involved in this absorption (Aschenbach et al., 2011). Passive diffusion is one of the mechanisms that facilitate the absorption of SCFA produced in the rumen. Short-chain fatty acids in the undissociated state are lipophilic and easily diffuse through the epithelium of the rumen (Gäbel et al., 2002). Acetate, propionate and butyrate have pKa values of 4.75, 4.87 and 4.81, respectively (Kristensen et al., 1998). As calculated based on the Henderson-Hasselbalch equation, almost 90% of the SCFA are dissociated at pH 5.8 (Aschenbach et al., 2011), suggesting that the proportion of SCFA in the

undissociated state is greater at low ruminal pH. This may suggest that passive diffusion could be more important at low ruminal pH; however, this notion has been shown not to be true as other gradients (e.g., gradient of HCO_3^- between the rumen and blood) also changed as ruminal pH decreased (Aschenbach et al., 2009). Butyrate has the greatest lipophilic permeability whereas that of acetate is the lowest (Walter and Gutknecht, 1986). Once diffused into the ruminal epithelial cells, non-ionized SCFA rapidly dissociate to their ionized forms due to the high cytosolic pH (Penner, 2014). Bicarbonate-dependent absorption is the most prominent mechanism for the absorption of ionized or dissociated SCFA across the ruminal epithelium and this mechanism is important for the absorption of less lipid-soluble SCFA such as acetate (Gäbel et al., 1991; Aschenbach et al., 2009). The exchange of ionized SCFA with HCO_3^- is facilitated by transport proteins in the ruminal epithelial cells (Aschenbach et al., 2011). Apical exportation of HCO_3^- is significantly important as HCO_3^- has the capability of neutralizing protons, thus contributing to the regulation of ruminal pH. Nitrate-sensitive SCFA absorption, proton-coupled $SCFA^-$ transport, and electrogenic SCFA transport are the other possible mechanisms for SCFA absorption across the ruminal epithelium (Penner, 2014).

2.3.3.2. Factors that regulate the short-chain fatty acid absorption

Rumen volume has a considerable effect on SCFA absorption across the ruminal epithelium. Fractional absorption rates of SCFA are inversely related to the volume of the rumen. (Dijkstra et al., 1993; Júnior et al., 2006). An increased rumen volume may increase the distance of SCFA in the ruminal fluid to the ruminal mucosa, thus reducing the rate of absorption (Dijkstra et al., 1993). The rate of SCFA absorption across the ruminal epithelium also relies on the rate of SCFA release from the ruminal mat to the ventral pool. Reduced ruminal contractions limit the movements of SCFA from the ruminal fluid/ruminal mat interface to the ventral pool where they can be available for absorption (Storm and Kristensen, 2010). Ruminal acidosis can reduce SCFA absorption across the ruminal epithelium. Steele et al. (2009) reported extensive sloughing of stratum corneum in ruminal papillae and reduced strength of its (startum corneum) cell adhesion in cows that had an acidotic challenge. Therefore, impaired SCFA absorption across the ruminal epithelium in cows having ruminal acidosis is likely due to damaged ruminal epithelium and altered ion transport mechanisms (Penner, 2014). Concentration of SCFA in the rumen also affects the rate of absorption of SCFA. When the ruminal concentration of SCFA

increases, SCFA absorption across the ruminal epithelium also increases (Dijkstra et al., 1993; Schurmann et al., 2014). Increased surface area of the ruminal epithelium has a stimulatory effect on SCFA absorption (Dirksen et al., 1985). Increases in ruminal concentrations of butyrate enhance the growth of ruminal papillae (i.e., increasing the number of cell layers in the epithelium), thus increasing the surface area for SCFA absorption (Malhi et al., 2013). Ruminal epithelial blood flow, which helps to maintain a concentration gradient for SCFA between ruminal fluid and epithelial blood, plays a significant role in SCFA across the ruminal epithelium (Storm et al., 2012).

2.3.4. Short-chain fatty acid metabolism in ruminants

2.3.4.1. Short-chain fatty acid metabolism in ruminal epithelial tissues

Prior to their absorption into the circulation, certain proportions of SCFA are metabolized in ruminal epithelial cells. Once absorbed from the rumen into the cytosol, 70 to 75% of butyrate and 6 to 12% of propionate are metabolized to provide energy for ruminal epithelial function, whereas acetate metabolism in the ruminal epithelium is negligible (Kristensen and Harmon, 2004; Kristensen, 2005). Acetate metabolism in the ruminal epithelium is comparatively small due to low availability of acetyl CoA synthetase enzyme in the cytosol (Anderson and Baird, 1973; van Houtert, 1993). Acetate is metabolized to CO_2 releasing ATP for cellular function. Propionate can be metabolized either to lactate or oxidized to CO_2 in the ruminal epithelium (Pennington and Sutherland, 1956). As indicated above, a substantial amount of butyrate is metabolized to beta-hydroxybutyrate (BHBA) or oxidized to CO_2 in the ruminal epithelium. The oxidation of butyrate within the ruminal epithelium may vary according to ruminal epithelial availability of glucose. In support of this statement, an *in vitro* study conducted by Wiese et al. (2013) reported a decrease in ruminal epithelial oxidation of butyrate as glucose concentration increased (i.e., increased availability of glucose for luminal and serosal uptake) in the incubation medium. The BHBA is subsequently absorbed into the portal circulation. Ruminal epithelial-derived BHBA is a precursor for the milk fatty acid synthesis in the mammary gland (Bauman and Griinari, 2003).

2.3.4.2. Short-chain fatty acid metabolism in the liver and extra-hepatic tissues

Short-chain fatty acids which are absorbed into the circulation are transported to the liver where they are extensively metabolized. Around 80% and 93% of the net portal flux of butyrate and propionate is extracted by the liver, respectively; whereas acetate is usually released by the liver (Kristensen, 2005). Hepatic metabolism of acetate is not significant in comparison to propionate and butyrate (Bergman and Wolff, 1971). Acetate is mainly utilized by extra-hepatic tissues such as muscle, adipose tissue, and mammary gland (van Houtert, 1993). Moreover, In adipose tissue and mammary gland, acetate is used to synthesize long-chain fatty acids (Mayfield et al., 1966). Hepatic metabolism of butyrate produces acetyl CoA which can be utilized in the tricarboxylic acid cycle and ketone bodies such as BHBA (van Houtert, 1993). Even in dairy cows that are not having ketosis, greater amounts of BHBA are released by the liver compared to the hepatic extraction of butyrate; other than butyrate, acetoacetate and fatty acids derived from adipose tissue catabolism can be precursors for hepatic synthesis of BHBA (Kristensen, 2005). As mentioned elsewhere, BHBA is a major source for milk fat synthesis. In addition, BHBA can be an alternative energy source for brain cells and muscles (Berg et al., 2002). Propionate is the major precursor for gluconeogenesis in ruminant liver. Almost 90% of the glucose synthesized in ruminants is derived from propionate. The metabolism of SCFA supplies almost 72% of the whole body energy requirement in cattle (Bergman, 1990).

2.3.5. Effects of partial substitution of sugars on short-chain fatty acid concentration in the rumen

A considerable number of studies (DeFrain et al., 2004, 2006; Chibisa et al., 2015; Oba et al., 2015) have reported increases in ruminal butyrate concentrations when dietary sugar levels were increased as a partial replacement for cereal grain starch. The dietary inclusion of liquid whey or pure lactose as a partial replacement for corn starch linearly increased ruminal butyrate concentration in lactating cows (DeFrain et al., 2004). In that study, molar proportion of ruminal butyrate was increased from 13.9 to 18 mol/100 mol by increased dietary lactose concentration from 0 to 13% (on a DM basis). Partial substitution of corn starch from lactose also increased ruminal concentration of butyrate in transition dairy cows (DeFrain et al., 2006). Recently, Oba et al. (2015) reported an increase in butyrate concentration in the rumen when sucrose replaced corn starch. In that study, they also observed a numerical increase in the expression of genes

related to butyrate metabolism (Oba et al., 2015). Consistent with the above studies, Chibisa et al. (2015) reported an increase in ruminal butyrate concentration when corn or barley starch was partially replaced with lactose. However, the partial replacement of corn starch with sucrose did not affect ruminal concentrations of butyrate in other studies (Broderick, 2003; Broderick and Radloff, 2004; Penner and Oba, 2009).

The effects of feeding sugars on acetate and propionate profiles are also not consistent in the literature. Dietary increase of TS content by adding wheat factory sewage did not alter the molar concentrations of acetate and propionate in cows fed barley-based diets (Khorvash et al., 2014) Similarly, partial substitution of barley or corn starch with lactose inclusion did not affect acetate and propionate concentrations in the rumen (Chibisa et al., 2015). In contrast, DeFrain et al. (2006) reported a decrease in the molar concentration of propionate when dietary lactose content was increased in post-partum cows fed corn-based diets. Dietary inclusion of lactose or sucrose as a partial replacement for corn starch reduced ruminal concentration of acetate in dairy cows (DeFrain et al., 2004; Broderick et al., 2008).

The type of sugar used or the type of feedstuff (i.e., corn or barley) that is partially replaced by sugars might affect SCFA profiles in the rumen (Oba, 2011). Moreover, SCFA concentration in the rumen is affected not only by its rate of production, but also by the rates of absorption and passage, thus giving an impression that differences in those factors related to different sugars may determine the changes in ruminal SCFA concentration (Oba, 2011).

2.4. Sub-acute ruminal acidosis associated with grain-based dairy diets

Although ruminal fermentation of dietary carbohydrates provides SCFA which are an important energy source for dairy cows, the rapid microbial fermentation of carbohydrates can lead to the excess production and ruminal accumulation of SCFA, which can increase the risk of ruminal acidosis (Nocek, 1997). The common forms of ruminal acidosis are acute ruminal acidosis (Owens et al., 1998) and SARA (Krause and Oetzel, 2006). Acute ruminal acidosis occurs when ruminal pH drops below 5.2 (Owens et al., 1998). In high-producing dairy cows, the most prevalent form of ruminal acidosis is SARA (Nocek, 1997; Enemark, 2008). Sub-acute ruminal acidosis is usually defined as periods of depressed ruminal pH below 5.5 (Garrett et al., 1999; Kleen et al., 2003). However, some authors define SARA as episodes of ruminal pH below 5.6 (Cooper et al., 1999) or 5.8 (Beauchemin et al., 2003). The ruminal accumulation of SCFA as

a result of excess production (NRC, 2001), impaired ruminal absorption into the bloodstream (Plaizier et al., 2008), and/or impaired ruminal buffering due to lack of chewing activity (Yang and Beauchemin, 2006) are some of the major reasons for SARA that is associated with cereal grain-based diets. Unlike in acute ruminal acidosis, lactic acid accumulation is not significant in SARA (Oetzel et al., 1999; Krause and Oetzel, 2006).

2.4.1. Factors affecting the severity of sub-acute ruminal acidosis

The source of grain and grain processing influence the severity of ruminal pH depression (Owens et al., 1998). In support of this statement, cows fed diets containing flatly-rolled barley had a lower ruminal pH compared to cows fed diets containing coarsely-rolled barley (Yang et al., 2001). Further, in comparison to corn starch, barley starch is more fermentable in the rumen (e.g. extent of ruminal degradation of steam-rolled barley and steam-rolled corn are 84.6 and 72.1% respectively; Huntington, 1997); thus, cows fed barley-based diets might be at a greater risk of developing ruminal acidosis compared to cows fed corn-based diets. Cows that are not adapted to grain-based diets are more susceptible to develop SARA (Plaizier et al., 2008). Dietary transition may have an effect on the severity of ruminal pH depression. In support of this assertion, Penner et al. (2007) observed extended periods when ruminal pH was below pH thresholds of 5.8, 5.5, and 5.2 in transition (i.e., ± 5 days relative to parturition) dairy cows which were experiencing dietary changes (i.e., transition from high forage dry cow diets to energy-dense early lactating diets)

2.4.2. Prevalence and clinical significance of sub-acute ruminal acidosis in dairy herds

Only a few surveys have been conducted to assess the prevalence of SARA in dairy cows (Plaizier et al., 2008). A study conducted by Oetzel et al. (1999) using 14 Wisconsin dairy farms showed a prevalence of SARA of 20.1% in early lactating cows. Another field survey of 15 dairy farms in the U.S.A. revealed SARA in 19% of early lactating cows and 26% of mid-lactating cows (Garrett et al., 1997). Further, Kleen et al. (2013) detected 20% of lactating cows having SARA in 26 German dairy herds. Even though there are no specific clinical symptoms, researchers use several non-specific clinical signs as diagnostic aids for SARA. Reduction of DMI, laminitis (a delayed response), and diarrhea are visually detectable signs for SARA (Kleen et al., 2003). Decreased fiber digestion due to the impaired activity of cellulolytic bacteria (Plaizier et al., 2001), excess production of propionate that induces satiety (Allen, 2000), and

increased ruminal osmolality (Enemark, 2008) are among the major reasons for reduced DMI reduction in cows afflicted with SARA. Inflammation in the rumen, liver abscesses, and milk fat depression are other important findings related to SARA in cows fed grain-based diets (Kleen et al., 2003; Plaizier et al., 2008).

2.4.3. Changes in the ruminal microbial population in sub-acute ruminal acidosis

Ruminal microbial changes in SARA are not well documented unlike in acute ruminal acidosis (Khafipour et al., 2009). However, SARA creates an unfavorable environment for the survival of cellulolytic microbes in the rumen (Russell and Dombrowski, 1980). When ruminal pH decreased below 5.5, amylolytic microorganisms such as *S. bovis* start to produce lactate instead of producing SCFA (Krause and Oetzel, 2006). Ruminal populations of the lactic acid utilizers such as *M. elsdenii* and *S. ruminantium* also increase (Nagaraja and Titgemeyer, 2007), thus neutralizing the effect of lactate when ruminal pH declines further. Protozoal numbers in the rumen are also reduced at ruminal pH below 5.5. Protozoa have the ability to engulf starch, thus minimizing the exposure of starch granules to other amylolytic ruminal microbes (Nagaraja et al., 1992). Therefore, reduction of the protozoal numbers can subsequently lead to further accumulation of SCFA and aggravation of the condition of SARA (Owens et al., 1998).

2.4.4. Economic losses due to sub-acute ruminal acidosis

In dairy cows, SARA can negatively affect not only the health of the animal but also the profitability of a dairy enterprise. Economic losses due to SARA in North American dairy herds are estimated around 1.3 USD per day per affected animal (Stone, 2004). Lameness that is partly associated with grain-based diets is one of the major reasons for premature culling in dairy herds (Oetzel, 2007). In Canadian dairy herds that are enrolled in DHI programs, lameness ranks third after infertility and mastitis as the major reasons for culling accounting for 6% of culling (http://www.dairyinfo.gc.ca/index_e.php?s1=dff-fcil&s2=mrr-pcle&s3=cr-tr). There is, therefore, a need for replacement heifers to maintain herd size and there are costs that are associated with raising replacement heifers. Also, using replacement heifers to maintain herd size results in a smaller inventory of heifers that are available for sale, thus costing the dairy producer potential income. Reduction of milk fat content and milk production also result in significant financial losses to the producer. The treatment cost for affected animals is another factor that negatively influences profit. Because barley is the major cereal grain in western Canadian dairy

diets and cows fed barley-based diets are at greater risk of SARA than those fed corn as a consequence of the greater rate and extent of barley starch degradation in the rumen, it is important to investigate alternative strategies for feeding cereal grain-based diets in order to reduce the prevalence of SARA in dairy herds. One such strategy that has received little attention with barley-based diets is the replacement of dietary starch with sugars.

2.5. Feeding sugars as an alternative strategy for grain-based dairy diets

As indicated above, it is important to explore alternative strategies to minimize the negative impacts on animal performance associated with cereal grain-based diets. Reducing diet fermentability in the rumen by limiting the intake of dietary starch is a possible approach to minimize SARA (Oetzel, 2007). However, reducing the starch content in the diet can negatively affect dairy cow performance (Fredin, 2014), thus limiting its on-farm implementation. Partial replacement of cereal grain starch with sugars may be an effective alternative approach to mitigate SARA in dairy cows. Over the past few years, a considerable number of studies have been conducted in order to evaluate the performance of dairy cows fed sugar-based diets (Huber et al., 1967; Broderick and Radloff, 2004; Broderick et al., 2008; Chibisa et al., 2015). In comparison to starch, sugars are more rapidly fermented in the rumen; however, the rates of ruminal fermentation differ among the sugar sources and the degree of microbial adaptation to a particular sugar (Oba, 2011). As an example, sucrose is hydrolyzed at a faster rate than lactose in a ruminal environment where microbes are not previously exposed to diets that contain sucrose or lactose (Weisbjerg et al., 1998). However, lactose is hydrolyzed rapidly compared to sucrose when lactose is fed to animals that are adapted to lactose-based diets (Weisbjerg et al., 1998). These differences in the rate and extent of ruminal fermentation of sugars might influence animal responses when sugars replace starch from cereal grains in dairy diets.

2.5.1. Effects of partial substitution of cereal grain starch with sugars on production performance and ruminal pH in dairy cows

Due to rapid ruminal fermentability in comparison to starch, sugars are expected to be more detrimental in terms of depressing ruminal pH (Oba, 2011). However, dietary inclusion of sugars as a partial substitution for dietary starch (without affecting the total dietary NSC concentration) seems to have positive effects on animal performance and ruminal pH. A study conducted by Broderick et al. (2008) observed a linear increase in DMI and milk fat content and

yield when cows were fed sucrose. Further, Broderick and Radloff (2004) showed a linear increase in DMI and quadratic changes in milk yield and solid-not-fat (SNF) yield when corn starch was partially replaced with molasses. Penner and Oba (2009) observed increased DMI and a tendency for increased milk fat yield when sucrose was added (up to 4.7% DM) in corn-based diets. Moreover, DeFrain et al. (2004) observed a tendency for increased DMI when corn starch was replaced with lactose up to 13% inclusion levels on a DM basis. However, some studies (McCormick et al., 2001; Penner et al., 2009b; Chibisa et al., 2015) did not observe improved animal performance (i.e., increase in DMI and milk yield) when cereal grain starch was partially replaced with sucrose or lactose. The reason for this discrepancy is unclear. However, in the literature studies that report any negative effects of feeding sugars as partial replacement for grain starch on DMI and milk yields are uncommon.

Recent studies on partial replacement of cereal grain starch with sugars have shown increased (Khorvash et al., 2014) or tendencies to increase ruminal pH (Penner and Oba, 2009; Penner et al., 2009b) or no depression in ruminal pH (i.e., induction of SARA) (DeFrain et al., 2004; Broderick et al., 2008; Chibisa et al., 2015) when dietary starch was partially replaced with sugars. Therefore, these research findings suggest that there is potential for cereal grain starch to be partially replaced with highly-fermentable sugars in order to improve production performance in high-producing dairy cows without necessarily increasing the risk of SARA. However, these previous studies have used primarily corn-based diets and there are very limited studies with barley-based diets. To my knowledge, there are only two published studies that have investigated the partial replacement of dietary starch with sugars in barley-based diets (Khorvash et al., 2014; Chibisa et al., 2015). Khorvash et al. (2014) explored the effects of partial replacement of barley starch with wheat factory sewage (a source of sucrose) up to 9.3% TS level on production performance and ruminal fermentation characteristics in dairy cows. Dietary increase of wheat factory sewage did not affect milk yield and composition; however, DMI was quadratically increased in cows fed 7.2% TS level compared to those fed control (5.2% TS), 8.3, and 9.3% TS diets (Khorvash et al., 2014). It should be noted that their diets included corn starch at a constant level which could potentially mask the effects of barley on animal performance (Khorvash et al., 2014). Chibisa et al. (2015) compared dietary sugar contents of 3 and 8%, and reported no dietary effects on DMI and milk production. Also, the risk of SARA was not increased when lactose (fed as DWP) partially replaced barley starch (Chibisa et al., 2015). However, in those

studies (Broderick and Radloff, 2004; Broderick et al., 2008) that have reported positive responses to dietary supplementation with sugars, dietary content of sugars has been included up to 10% TS which is greater than the maximum dietary sugar level of 8% that was used in the study by Chibisa et al. (2015). It is important to determine the responses in production (i.e., DMI and milk production) and SARA when cows fed barley-based diets receive total dietary sugar contents >8%.

2.5.2. Why the partial substitution of starch with sugars might improve ruminal pH

As discussed above, feeding sugars as a partial replacement for cereal grain starch seems to be beneficial in terms of rapidly providing energy to dairy cows without depressing ruminal pH (i.e., reduced risk of SARA); however, the underlying mechanisms for this response are not clearly understood. A considerable number of studies (DeFrain et al., 2004, 2006; Chibisa et al., 2015; Oba et al., 2015) have reported increases in ruminal butyrate concentrations when dietary sugar levels were increased as a partial replacement for cereal grain starch. Butyrate has known effects on enhancing the growth and differentiation of epithelial cells in the rumen (Sakata and Yajima, 1984; Feng et al., 1996). Therefore, an increase in the ruminal concentration of butyrate can be expected to potentially increase SCFA absorption across the rumen by increasing the absorptive surface area of the ruminal epithelium (Penner et al., 2011). Recently, Chibisa et al. (2015) reported increases in ruminal concentration of butyrate and Cl^--competitive absorption of acetate and propionate when corn or barley starch was partially replaced with lactose. Chloride-competitive absorption is calculated as the difference between total SCFA absorption and Cl^--insensitive SCFA absorption. The total SCFA absorption across the ruminal epithelium is measured when the experimental buffer medium is free of Cl^- ions. The Cl^--insensitive absorption of SCFA is measured when Cl^- concentration of the experimental buffer is > 40 m*M*. Chloride ions are known to compete with SCFA for their HCO_3^--dependent absorption that is facilitated by anion exchangers (Aschenbach et al., 2009). The Cl^--competitive absorption, therefore, indirectly measures the HCO_3^--dependent absorption of SCFA across the ruminal epithelium (Aschenbach et al., 2009; Chibisa et al., 2015). Based on the findings of Chibisa et al. (2015), they suggested that the reduced risk of SARA when dietary starch is partially replaced with sugars is partly due to increased SCFA absorption across the ruminal epithelium. Moreover, Penner (2015) suggested that the utilization of sugars by ruminal microbes might play a

significant role in reducing the risk of ruminal pH depression when dairy cows are fed sugar-based diets. The incorporation of carbon molecules from dietary sugars into ruminal microbes as reserve carbohydrates (e.g., glycogen) can be a possible mechanism that limits the availability of sugars for microbial fermentation and, consequently, SCFA production. It has been demonstrated that ruminal bacteria and protozoa store sugars as glycogen (Hall, 2011), thus supporting above assertion.

2.5.3. The potential of utilizing whey permeate as a sugar source

Molasses that contains sucrose is the most common sugar source that is utilized for feeding cows at present (Eastridge, 2014). Lactose and lactose-containing byproducts are also potential sugar sources that can be included in lactating cow diets (Huber et al., 1967; DeFrain et al., 2004). Whey as a lactose source has been explored over the past few decades for its impacts on animal performance (Huber et al., 1967; Anderson et al., 1974; Bragg et al., 1986). Whey can be included in animal diets as liquid or dry form (Schingoethe, 1976). These studies demonstrated that whey has similar effects as feeding pure lactose on animal performance and ruminal fermentation, suggesting the potential of utilizing whey in high-producing ruminant diets. In support of this statement, the inclusion of dried whey (at 30% of diet DM) in corn-based diets improved DMI, milk fat concentration and, fat-corrected milk yield (Casper and Schingoethe, 1986).

Whey permeate, which is also known as deproteinized whey, is a byproduct of cheese manufacturing and on average, it contains 75% lactose (Pinchasov et al., 1982). In comparison to pure lactose, whey permeate is cheap; thus, this can encourage feed manufacturers and nutritionists to utilize it in animal feed. Whey permeate can be fed to animals as liquid or dried powder. Inclusion of whey permeate in dairy cow diets is not common due to a lack of information on appropriate inclusion levels, and the impact on production performance and ruminal fermentation characteristics. However, Charbonneau et al. (2006) showed that 10.6% dietary inclusion of DWP as a partial replacement for corn increased DMI and daily milk yield compared to the control diet. Recently, Chibisa et al. (2015) explored the effect of partial replacement of barley or corn starch with whey permeate up to 8% TS. In that study, Chibisa et al. (2015) did not observe positive responses in production performance of dairy cows, suggesting that the dietary inclusion of whey permeate up to 8% TS might not have been

adequate to improve lactation performances. Therefore, it is important to conduct more research on utilization of whey permeate in cereal grain-based dairy diets in order to reveal optimum dietary inclusion levels and to delineate its effects on production performance.

2.6. Nitrogen metabolism in dairy cows

Dietary CP, which includes true protein and non-protein N (NPN), is the major source of N for high-producing dairy cows (NRC, 2001). Based on degradability in the rumen, dietary CP can be categorized into ruminally-degradable (RDP) and ruminally-undegradable protein (RUP) fractions (Bach et al., 2005). Non-protein N (e.g., urea, ammonium salts, peptides, amines, nitrates) are completely rumen degradable, whereas true proteins are either ruminally-degradable or -undegradable in nature (NRC, 2001). Dairy cows meet their AA requirements by utilizing microbial protein, RUP, and endogenous protein, collectively referred to as metabolizable protein, that are available for enzymatic digestion in the small intestine (Leng and Nolan, 1984; NRC, 2001). Microbial protein provides 50 to 80% of the metabolizable protein requirements of dairy cows (Storm and Ørskov, 1983; Bach et al., 2005). Besides being a major component of metabolizable protein reaching the small intestine, microbial protein also has an excellent essential AA profile that closely matches the essential AA profile of milk true protein (NRC, 2001). Ruminally-degradable proteins are hydrolyzed to peptides and AA by the proteolytic enzymes that are secreted by ruminal microbes. Almost 30 to 50% of the ruminal microbes that get attached to feed particles have extracellular proteolytic enzymes (Prins et al., 1983). Subsequently, peptides and AA that arise from extracellular protein degradation are transported into microbial cells. Peptides are further hydrolyzed to AA by the intracellular peptidase activity. If availability of energy in the rumen is not limited, AA are either used for microbial protein synthesis or are further metabolized. In dietary situations where ruminal microbes are energy-deficient, AA are further deaminated to SCFA, CO_2, and NH_3, thus providing ATP for microbial growth (Bach et al., 2005). Ruminal microbes can synthesize their own cellular proteins by utilizing NH_3 (that arises from AA deamination and hydrolysis of NPN sources), carbon skeletons, and energy (Bach et al., 2005) . Bacteria, such as *P. ruminicola, B. fibrisolvens,* and *S. bovis*, ciliate protozoa, and some species of ruminal fungi are responsible for N metabolism in the rumen (Wallace, 1996). Type of dietary protein, other available nutrients in the diet,

predominant microbial population, rate of passage, and ruminal pH are some of the major factors that determine the rate and extent of protein degradation in the rumen (Bach et al., 2005).

2.6.1. Impact of pH and high-concentrate diets on ruminal nitrogen metabolism

As mentioned above, ruminal pH has a considerable impact on N metabolism in the rumen. The maximum proteolytic enzyme activity in ruminal microbes exist within the pH range of 5.5 to 7 (Kopecny and Wallace, 1982), suggesting that SARA associated with grain-based diets may depress proteolysis in the rumen. A study conducted by Calsamiglia et al. (2002) using a dual-flow continuous culture system observed a significant reduction in protein degradation at pH 5.7 compared to pH 6.4; however, total microbial N flow and the efficiency of microbial protein synthesis were unaffected. In that study, the reason for reduced protein degradation with low pH treatment could be partly due to reduced fiber digestion (Calsamiglia et al., 2002). Ruminal pH depression associated with grain-based diets also reduces the number of fibrolytic microbes, which play a significant role in fiber digestion in the rumen (Russell and Dombrowski, 1980). Ruminal microbes have synergistic relationships among each other in terms of nutrient digestion and utilization (Russell and Rychlik, 2001). Therefore, a decrease in fiber digestion when ruminal pH is depressed (e.g., SARA) may minimize the access of proteolytic bacteria towards plant protein structures, thus indirectly reducing protein degradation (Bach et al., 2005). In the rumen, the fermentation of dietary carbohydrates provides energy to support microbial growth (Bach et al., 2005); however, carbohydrate fermentation also produces SCFA that can result in depressions in ruminal pH that might negatively affect microbial activities. Therefore, feeding cereal-grain based diets (i.e., high energy supply) can result in a reduction in protein degradation and post-ruminal microbial N flow when ruminal pH is depressed (Calsamiglia et al., 2008). Amylolytic microbes, that are predominant on high concentrate diets, also have proteolytic activity; however, at low ruminal pH they utilize a greater portion of available energy to maintain the proton-motive force across the cell membrane (Stewart et al., 1997).

2.7. Efficiency of nitrogen utilization in dairy cows

Pollution associated with emission of greenhouse gases and nitrogenous waste materials has become a serious threat to environmental integrity. As indicated above, rapid expansion and development of the livestock industry negatively affect the environment by emitting greenhouse gasses (i.e., CH_4 and CO_2) and excreting excess N (i.e., NH_3 and NO_3^-) that can potentially

pollute air, water and soil (Tamminga, 1992). Dairy cows excrete N to the environment mainly in the form of urea via urine. In addition, undigested dietary N, undigested microbial N and endogenous N are also excreted to the environment via feces (Tamminga, 1992). On average, 69% of the total urinary N excreted by dairy cows is present as urea (Bristow et al., 1992). Once excreted, urinary urea is hydrolyzed to NH_3 by urease enzymes which are usually present in feces (VandeHaar and St-Pierre, 2006) and soil. Including dairy, livestock contributes to almost 50% of the total NH_3 emission to the atmosphere (VandeHaar and St-Pierre, 2006).

Inefficient N utilization in high-producing ruminants (Castillo et al., 2000) is one of the important factors that can contribute to excess N excretion. Nitrogen is an important constituent of milk protein. However, the efficiency of incorporating N from dietary CP into milk is relatively low (Hristov et al., 2004). As mentioned by Sinclair et al. (2014), dairy cows capture around 25 to 35% of dietary CP as milk N. Therefore, as much as 72 to 73% of dietary N is excreted via urine and feces (Tomlinson et al., 1996). Feeding excess dietary CP can contribute to poor efficiency of N utilization in dairy cows. In support of this statement, increased dietary CP concentration from 15.1 to 18.4% resulted in decreased milk N efficiency (MNE; i.e., milk N/N intake) from 31 to 25% and an increase in urinary N excretion from 23 to 35% (when expressed as a proportion of N intake) (Broderick, 2003). In addition, Kebreab et al. (2001) predicted a 30% increase in urinary N excretion when dietary N intake increases from 400 to 500 g of N per day. When dietary N intake increases, then the extent of ruminal protein degradation increases (Ørskov and McDonald, 1979). Besides dietary N intake, dietary level of RDP can also reduce the efficiency in N utilization in high-producing cows. A study conducted by Hristov et al. (2004) revealed significantly increased urinary and fecal N excretion and decreased MNE in cows fed 11.6% RDP compared to those fed 9.4% RDP on a DM basis. Inadequate energy availability in the rumen that might limit the microbial sequestration of NH_3-N for protein synthesis may be another driving force for inefficient N utilization (Mutsvangwa, 2011). Broderick (2003) demonstrated a linear increase in MNE and a linear decrease in urinary N excretion when dietary NDF concentrations were reduced from 36 to 28% by increasing dietary starch levels (i.e., by increasing dietary energy levels).

In diets for high-producing cows, protein is the most expensive nutrient component compared to other dietary nutrients (Muller, 2016). Therefore, inefficient N utilization in dairy

cows not only affects the environment but also increases feed costs. Reducing dietary CP concentration can be a possible approach to minimize excess N losses and feed costs; however, its application in high-producing dairy cows is limited because some studies have demonstrated negative effects on milk production when dietary CP concentration is reduced (Kalscheur et al., 1999; Bach et al., 2000; Olmos Colmenero and Broderick, 2006; Cabrita et al., 2007). Kalscheur et al. (1999) compared dietary CP levels of 15.2 and 17.4% in early lactating cows fed diets that contained corn and barley grain. In that study, they observed significantly lower milk yield and composition in cows fed 15.2% dietary CP compared to those fed 17.4% dietary CP (Kalscheur et al., 1999). Similar results in milk yield and composition were observed when early lactating cows were fed diets contained 14% CP and 15% starch, compared to those fed 16% CP and 25% starch on a DM basis (Cabrita et al., 2007). In addition, Olmos Colmenero and Broderick (2006) reported a tendency for a quadratic change in milk yield when dietary CP concentration was increased from 13.5 to 19.4%. In that study the peak milk yield was observed in cows fed diets that contained 16.5% CP. Therefore, it is necessary to explore other feeding strategies to feed dairy cows in order to improve their N utilization efficiency in a cost-effective manner without compromising animal performance.

2.7.1. Nutrient synchrony and its effects on nitrogen utilization efficiency

Maximizing the efficiency of converting RDP into microbial protein is one of the key objectives of feeding high-producing dairy cows (Cabrita et al., 2006). Ruminal availability of energy is a driving force for microbial protein synthesis, thus stressing the importance of nutrient synchrony on enhancing the efficiency of N utilization. Nutrient synchrony is the concept of synchronizing the rates of RDP and carbohydrate degradation in the rumen (NRC, 2001). Carbohydrates and proteins are interdependent for their utilization by ruminal microbes (Nocek and Russell, 1988). A lack of energy availability in the rumen may result in RDP being utilized as an energy source (i.e., amino acids derived from RDP would be deaminated to provide ATP to support maintenance and growth requirements of ruminal microbes) (Bach et al., 2005). Daily variations in the pattern of energy and protein supply to the ruminal microbes may be a considerable reason for nutrient asynchrony in most of the dairy feeding protocols (Cabrita et al., 2006). Matching the rates of carbohydrate and protein degradation in the rumen may enhance the

capture of NH_3-N for microbial protein synthesis, thus increasing the availability of AA for the milk protein production (Mutsvangwa, 2011).

Nutrient synchrony can be achieved not only by synchronizing the rates of carbohydrate and protein degradation, but also by changing the ruminal degradation of a carbohydrate source or feeding carbohydrate sources that vary in ruminal degradability (Cabrita et al., 2006). Yang et al. (1997) evaluated the effects of feeding different carbohydrates sources (steam-rolled barley, steam-rolled hull-less barley or steam-rolled corn) that have different rates of ruminal degradability on production performance and ruminal fermentation characteristics in dairy cows. Feeding steam-rolled barley that had the highest rate of ruminal degradation decreased ruminal concentration of NH_3-N and increased the flow of microbial N to the duodenum, suggesting that increased rate of carbohydrate degradation in the rumen could improve the efficiency of N utilization. Moreover, a recently conducted *in vitro* study reported a decrease in NH_3-N concentration when the degradability of corn was improved by an enzyme treatment, indicating more efficient capture of NH_3-N into microbial cells as protein (Seo et al., 2013). These results indicate that increased rates of carbohydrate degradation in the rumen could improve the efficiency of microbial protein synthesis by supplying adequate energy to capture AA and NH_3-N that are available in the rumen.

2.7.2. Effects of feeding sugars on nitrogen utilization efficiency in dairy cows

Since the range in degradability for sugars is generally greater than that of starch in the rumen (e.g., sugars: 40-60 %/h; starch: 20-40 %/h; Amburgh et al., 2015), substitution of starch with sugars may rapidly provide energy for microbial protein synthesis. However, the effects of feeding sugars on the efficiency of N utilization in dairy cows are inconsistent in the literature. Partial substitution of barley starch with dextrose in forage-based diets resulted in increased ruminal microbial N outflow (Piwonka et al., 1994). Khezri et al. (2009) explored the effects of substituting corn starch with sucrose on ruminal fermentation characteristics, nitrogen metabolism, and production performance of lactating cows fed alfalfa and corn silage-based diets. In that study, dietary inclusion of sucrose reduced concentrations of ruminal NH_3-N and milk urea-N (MUN), and tended to increase milk protein content (Khezri et al., 2009). Furthermore, Sannes et al. (2002) reported a tendency for decreased ruminal NH_3-N and MUN concentrations without any effects on the efficiency of N utilization when 3% sucrose was added

to diets that contained corn. Increasing dietary sucrose levels from 0 to 7.5% as a partial replacement for corn starch decreased ruminal NH_3-N concentrations from 13.9 to 11.5 mg/dL; however, MUN concentrations and efficiency of microbial N utilization were not affected (Broderick et al., 2008). Recent findings of Chibisa et al. (2015), who substituted corn or barley starch with lactose up to 8% total dietary sugars levels, were consistent with the reduction in ruminal NH_3-N concentrations with sugar supplementation that has been reported in previous studies. Several authors reported that dietary inclusion of sugars did not affect the efficiency of N utilization (i.e., ruminal NH_3-N concentrations were not affected) in lactating cows (DeFrain et al., 2004, 2006; Penner and Oba, 2009); however, microbial protein synthesis was reduced in *in vitro* culture studies (Hall and Herejk, 2001). As explained by Oba (2011), the type of sugar and its dietary inclusion levels could be partly responsible for these inconsistent findings in terms of N utilization efficiency in dairy cows when dietary starch is partially substituted with sugars.

2.8. Urea-N recycling in ruminants

2.8.1. Formation of ammonia and transportation to the liver

Urea is the end-product of protein catabolism in mammals. Ammonia is synthesized as an intermediate product of AA catabolism; however, excess accumulation of NH_3 at tissue level is highly toxic (Stewart and Smith, 2005). Excess NH_3 in the blood can cause irreversible central nervous system damage in mammals (Braissant et al., 2013). In order to prevent the accumulation of NH_3 that is generated from extra-hepatic tissue protein catabolism, NH_3 is transported to liver using several biochemical mechanisms. The glucose-alanine pathway is the primary mechanism of NH_3 transport to the liver from muscle tissues. Ammonia that is produced in tissues other than muscles is transported via the glutamine synthase/glutaminase system (Newsholme et al., 2003). Apart from that, in ruminants, NH_3 that is produced from deamination of AA, endogenous sources such as urea and other NPN, is absorbed across the gastro-intestinal tract (GIT) epithelium into portal circulation and subsequently transported to the liver (Abdoun et al., 2006). The rumen contributes up to 77% of the total NH_3-N absorbed into portal blood, whereas 33% NH_3-N is absorbed across the intestines (Reynolds and Huntington, 1988); however, these proportions may vary among different diets. Ruminal NH_3 is lipid-soluble in nature, thus getting absorbed across the ruminal epithelium via simple diffusion (Abdoun et al., 2006). Between pH 6 and 7, ruminal NH_3 exists mainly as NH_4^+ (Abdoun et al., 2006), which is

less soluble in lipids, and in this form it is transported across the ruminal wall using K^+ ion transport system (Abdoun et al., 2005). Outflow of SCFA as the dissociated form (i.e., $SCFA^-$) through anion protein channels that are located in the basolateral membrane of the ruminal epithelium facilitates the efflux of NH_4^+ thorough K^+ channels (Aschenbach et al., 2011).

2.8.2. Detoxification of ammonia in urea cycle

Ammonia that enters the liver is detoxified by periportal cells using a series of enzyme catalytic reactions in the urea cycle which is also known as the ornithine cycle (Figure 2.2). Synthesis of carbamoyl-phosphate using NH_3 and intra-mitochondrial HCO_3^- is the first step of the urea cycle. This reaction is catalyzed by carbamoyl-phosphate synthase enzyme. Carbamoyl-phosphate is the first N donor for urea synthesis. The second step of the urea cycle is the synthesis of citrulline from carbamoyl phosphate and ornithine. Ornithine carbamoyltranferase catalyzes the formation of citrulline. Citrulline is then transferred from the mitochondria into the cytosol where it reacts with aspartate, which is the second N donor in the urea cycle, to form argininosuccinate. The synthesis of argininosuccinate is catalyzed by the enzyme argininosuccinate synthase. Cleavage of argininosuccinate into arginine and fumarate is the next step which is catalyzed by argininosuccinate lyase enzyme. Hydrolysis of arginine by arginase to ornithine and urea is the final step of the urea cycle. In dairy cows, urea-N synthesis in the liver can range from 43 to 123% of digestible N intake (Lapierre and Lobley, 2001).

2.8.3. Recycling of urea-N

Urea that is produced in the liver is either excreted in the urine or recycled to the GIT. In mammals, urea that returns to the GIT is broken down to NH_3 and CO_2 by microbial urease activity (Stewart and Smith, 2005). In comparison to mono-gastric animals and humans, greater proportion of endogenous urea produced in the liver returns to the GIT in ruminants (Lapierre and Lobley, 2001; Stewart and Smith, 2005). Even in ruminants fed diets that contain high CP concentrations, endogenous production of urea-N often exceeds the digestible N intake, thus leading animal to be in negative N balance. In such situations, recycling of urea-N plays a vital role in compensating the N deficit and subsequently to aid the host animal to maintain a positive N balance (Lapierre and Lobley, 2001). Around 40 to 80% of endogenous urea-N returns to

various regions in the GIT via several mechanisms in ruminants (Lapierre and Lobley, 2001). Urea-N can be secreted into the GIT via saliva (Huntington, 1989). The mechanisms involved

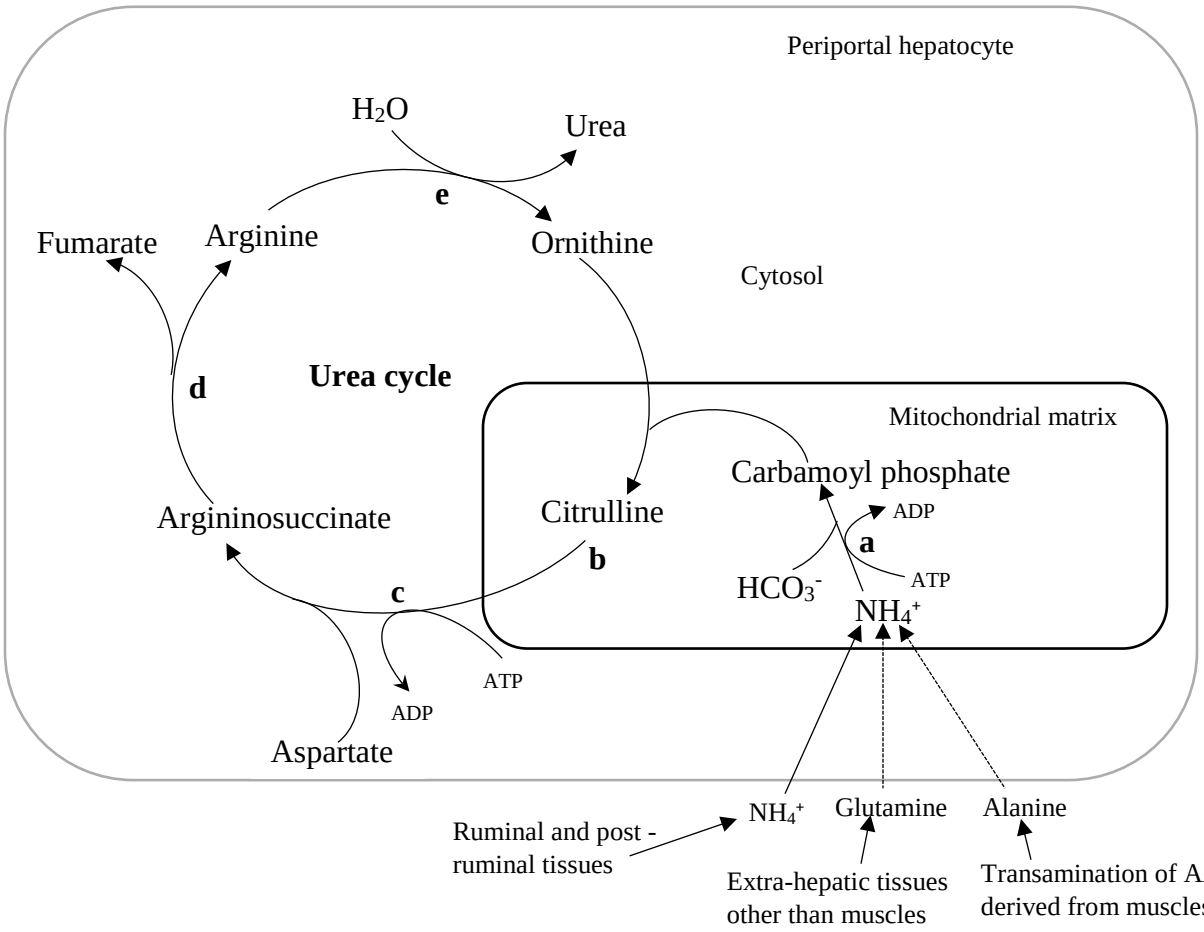

Figure 2.2 Detoxification of ammonia (NH_4^+) via urea cycle in ruminants (adapted from Meijer et al., 1990; Newsholme et al., 2003; Abdoun et al., 2006). Synthesis of urea in periportal hepatocytes is an energy dependent process. Carbamoyl phosphate derived from NH_4^+ and cytosolic aspartate are the nitrogen donors for the urea cycle. Each chemical reaction in the urea cycle is catalyzed by a specific enzyme; a. carbamoyl-phosphate synthase, b. ornithine carbamoyltranferase, c. argininosuccinate synthase, d. argininosuccinate lyase, e. arginase.

in urea-N secretion from the bloodstream into the rumen are simple diffusion (Houpt and Houpt, 1968), carrier-mediated facilitated diffusion by urea transporter-B proteins (Abdoun et al., 2010) and aquaporin-mediated transport (Walpole et al., 2015).

Ammonia that is produced from the hydrolysis of recycled urea is utilized by ruminal microbes to synthesize microbial protein. As has been reported, 12 to 38% of N that is recovered from ruminal bacteria originates from recycled urea-N (Al-Dehneh et al., 1997); however, these proportions can vary among diets (Stewart and Smith, 2005). As has been reviewed by Lapierre and Lobley (2001), 50% of recycled urea-N is utilized by the animal for anabolic processes, whereas up to 40% of recycled urea is reabsorbed across the GIT epithelium as NH_3 and subsequently detoxified in urea cycle. The remaining 10% of recycled urea is excreted in feces. The extent of anabolic utilization (i.e., primarily via microbial protein synthesis) of recycled urea-N depends largely on the availability of RFC. (Huntington et al., 2009; Davies et al., 2013).

2.8.4. Factors that regulate urea-N recycling in ruminants

2.8.4.1. Ruminal factors

Ruminal concentration of NH_3 has an inverse relationship with urea-N entry to the rumen (Kennedy and Milligan, 1980; Doranalli et al., 2011; Recktenwald et al., 2014). Physiological adaptation of the ruminal epithelium that can be mediated by the changes in ruminal NH_3 concentration is likely be the reason for the inhibitory effects of NH_3 on the secretion of urea-N from the blood stream into the rumen (Doranalli et al., 2011). Microbial urease activity has been reported to have a stimulatory effect on trans-epithelial migration of urea into the rumen by hydrolyzing urea to NH_3, thus maintaining a favorable concentration gradient for urea-N flux (Rémond et al., 1996; Muscher et al., 2010). Ruminal concentration of SCFA can influence urea-N recycling in ruminants. Norton et al. (1982) reported an increase in urea entry to the rumen during intra-ruminal butyrate infusion for two consecutive weeks. Simmons et al. (2009) suggested that increased concentration of ruminal butyrate may upregulate urea transporter UT-B protein, thus enhancing urea-N recycling to the rumen. Secretion of urea-N to the GIT can be influenced by ruminal pH. A study conducted by Abdoun et al. (2010) using ruminal epithelia mounted in Ussing chambers reported a bell-shaped change (with peak urea flux attained at pH 6.2) in net flux of urea across the ruminal epithelium when SCFA were present in the incubation medium. Moreover, Abdoun et al. (2010) demonstrated an increase in trans-epithelial flux of

urea-N when CO_2 concentration was elevated in the *in vitro* buffer medium at pH 6 to 6.4. These observations suggest that the changes in ruminal factors (such as SCFA, HCO_3^-/ CO_2, etc.) can improve the urea-N recycling to the rumen when ruminal pH is within the range of 6 to 6.4 that would be indicative an actively-growing microbial populations (Abdoun et al., 2010).

2.8.4.2. Dietary factors and plasma urea-N concentration

Dietary N intake has a positive correlation with endogenous production of urea (UER) in ruminants (Lapierre and Lobley, 2001; Stewart and Smith, 2005). In support of this statement, UER increased by 25% when dietary CP levels increased from 15.3% to 16.7% in dairy cows fed corn-based diets without affecting the proportions of recycled urea used for anabolism (Recktenwald et al., 2014). Another study conducted by Recktenwald (2010) reported a 12% increase in urea-N recycled to the GIT when dairy cows were fed diets that were formulated to have inadequate levels of CP for optimum microbial protein synthesis, suggesting that repartitioning of UER more towards recycling to the GIT in order to provide adequate N for microbial growth. The extent of protein degradability in the rumen can influence urea-N recycling to the GIT. In support of this statement, progressively increasing RUP supply by continuous abomasal infusion of casein in beef steers fed hay that contained low CP content linearly increased the amount of urea-N recycled to the GIT (Wickersham et al., 2009b). Urea-N recycling to the GIT can be influenced by the RFC concentration in the diet. Increase in dietary RFC that increases the availability of energy for ruminal microbes can decrease the ruminal concentration of NH_3-N by enhancing the microbial capturing of NH_3-N as microbial protein which, in turn, improves urea-N recycling to the GIT (Kennedy and Milligan, 1980). Moreover, RFC can enhance the microbial capturing of recycled N that is utilized for further anabolic process (Huntington et al., 2009; Davies et al., 2013). Plasma urea-N (PUN) concentration is another factor that can modulate urea-N recycling in ruminants. Sunny et al. (2007) reported increased UER, gut urea entry rate and urea-N utilized for anabolism when PUN concentrations were increased from 1.27 to 5.93 m*M* via intra-jugular infusion of urea in sheep fed isonitrogenous diets. However, PUN concentrations above 6 m*M* in sheep and 4 m*M* in cattle do not exhibit any relationship with urea-N recycling (Lapierre and Lobley, 2001).

2.8.5. Effects of dietary carbohydrates on urea-N recycling in ruminants

Enhancing the sequestration of recycled urea-N into microbial protein may be an effective way of improving the N utilization efficiency of ruminal microbes. Urea-N recycling facilitates ruminal microbes to overcome short term N deficiencies associated with asynchronized diets (Reynolds and Kristensen, 2008). As discussed above, diet (i.e., dietary protein, dietary carbohydrate) has a significant impact on urea-N recycling in ruminants. Rapidly-degradable dietary carbohydrates can not only improve microbial protein flow to the intestine, but also enhance the utilization of recycled urea-N in the gut (Theurer et al., 1999). Increasing the energy availability in the rumen, either by increasing the amounts of carbohydrate supply (Kennedy and Milligan, 1980; Huntington, 1989) or by increasing the proportions of carbohydrates fermented in the rumen (Gozho et al., 2008) may enhance the capture of NH_3-N that is derived from recycled urea-N by providing ATP and carbon skeleton for microbial protein synthesis. A reduction in NH_3-N concentration in the rumen could further increase the urea-N entry to the gut as high ruminal NH_3 concentrations have been reported to have inhibitory effects on urea-N secretion into the rumen (Doranalli et al., 2011). Several studies have reported positive responses in terms of urea-N recycling in sheep and beef cattle fed highly-fermentable carbohydrate diets (Kennedy and Milligan, 1980; Huntington, 1989; Davies et al., 2013); however, studies in dairy cows are limited. As reviewed by Theurer et al. (1999), GIT entry rate of urea-N in dairy cows was increased when steam-flaked corn was fed to dairy cows as a carbohydrate source compared to steam- or dry-rolled corn. Gozho et al. (2008) compared the effects of barley grain processing methods on urea-N recycling kinetics in dairy cows fed alfalfa and barley silage-based diets. In that study, they found that urea-N entry rate to the GIT tended to be greater in cows fed dry-rolled barley compared to those fed pelleted barley. Pelleted barley induced a lower ruminal pH compared to dry-rolled barley, which could have resulted in a reduction in urea-N secretion into the rumen in that study (Gozho et al., 2008).

2.8.5.1. Effects of feeding sugars on urea-N recycling in ruminants

Feeding sugars increases the rate of ruminal carbohydrate degradation and energy supply to ruminal microbes; thus, this could potentially improve urea-N recycling to the GIT. A study conducted by Kennedy (1980) provided an evidence for improved urea-N recycling and subsequent utilization for the anabolic purposes when beef steers were fed diets containing

sucrose as the sugar source compared to those fed diets contained pasture hay. In that study, the amount of urea-N recycled to the GIT was not measured; however, the amount of recycled urea-N that was metabolized in the rumen was measured using continuous jugular infusions of ^{14}C-labeled urea (Kennedy, 1980). The ruminal degradation of urea-N was 11.7% greater in diets that contained sucrose compared to diets that contained pasture hay as the major source of carbohydrate. Moreover, a 4% numerical increase in post-ruminal microbial protein flow was also detected when steers were fed pasture hay, fresh alfalfa, and sucrose mixture compared to those fed pasture hay and fresh alfalfa only (Kennedy, 1980).

In the literature, it is difficult to find direct evidence for the effects of feeding sugars on urea-N recycling kinetics in dairy cows. However, results of a few studies provide indirect evidence for improved urea-N recycling to the GIT when dairy cows are fed sugars as a partial replacement for grain starch. Increased urea-N recycling to the gut reduces urinary urea-N and fecal urea-N excretion (Huntington, 1989; Kiran and Mutsvangwa, 2010; Recktenwald, 2010). Hence, a reduction in urinary urea-N excretion may provide indirect evidence for repartitioning of blood urea away from urinary excretion to secretion into the gut in ruminants. In support of this assertion, partial replacement of corn starch with dried molasses up to 7.2% total dietary sugar resulted in a 17% reduction of urinary urea-N excretion (Broderick and Radloff, 2004), therefore suggesting that a greater proportion of endogenous urea-N was recycled. Moreover, Broderick et al. (2008) observed a significant reduction in urinary urea-N concentration from 174 to 137 g/d when total dietary sugar concentration was increased from 2.6 to 10% by substituting corn starch with sucrose. As indicated above, increases in ruminal concentration of butyrate can increase urea-N recycling to the rumen (Norton et al., 1982; Simmons et al., 2009). Several studies have reported increased butyrate concentrations in the rumen when dairy cows were fed diets containing lactose as a sugar source (DeFrain et al., 2004, 2006; Chibisa et al., 2015), but the effects of sugar supplementation on urea recycling to the GIT remain unclear. Therefore, a major focus of the study reported in this thesis was to delineate the effects of partial substitution of dietary starch with sugars on whole-body urea kinetics in dairy cows.

2.9. Effects of partial substitution of dietary starch with sugars on nutrient digestibility

Increased dietary sucrose concentration from 0 to 7.5% as a partial replacement for corn starch quadratically changed apparent NDF digestibility without affecting the digestibility of

DM, CP, and NSC in a continuous culture system (Vallimont et al., 2004). Broderick and Radloff (2004) also reported a linear increase in NDF and ADF digestibility when high-moisture shelled corn was partially substituted with dried molasses, whereas quadratic changes were observed when liquid molasses were added to the diet. Also, in that study, dietary inclusion of dried molasses up to 7.2% total dietary sugar linearly increased DM and OM digestibility, whereas dietary inclusion of liquid molasses up to 10% total dietary sugar was unable to elicit a similar response (Broderick and Radloff, 2004). In contrast, Broderick et al. (2008) reported that apparent nutrient digestibility were not altered when dairy cows were fed sucrose as a partial replacement for corn starch. Moreover, Partial replacement of corn starch with sucrose up to 8.7% total dietary sugar content did not affect NDF digestibility in dairy cows (Penner and Oba, 2009; Penner et al., 2009). Recently, Chibisa et al. (2015) also reported no sugar effect on NDF, ADF, and DM apparent total-tract digestibility when corn or barley starch was partially substituted with lactose, even though ether extract (EE) digestibility was greater in cows fed supplemental sugar. The reasons for the discrepancies in reported observations on digestibility of NDF and other nutrients when sugars are fed as a partial substitution for cereal grain starch are not clear, but could be related to, among other factors, differences in basal dietary composition, levels of sugar supplementation, and dietary DMI.

2.10. Summary

Sub-acute ruminal acidosis and inefficient N utilization negatively affect the production performance of high-producing dairy cows. Cereal grain-based diets that contain readily-fermentable starch are an excellent source of energy for high-producing dairy cows; however, excessive production of SCFA when high levels of dietary starch are fed can lead to SARA. Inefficient capture of dietary N as productive N in dairy cows not only reduces their lactational performance, but also can potentially contribute to environmental pollution due to excessive N excretion. Reducing dietary starch and CP concentrations can potentially reduce the risk of SARA and excess N excretion; however, the practical application of this approach is limited due to potential negative influence on animal performance. Nitrogen utilization efficiency in dairy cows can be improved by enhancing the efficiency of microbial protein synthesis and urea-N recycling to the GIT. A considerable number of studies have shown that the partial substitution of corn starch with sugars is beneficial in terms of improving production performance and

microbial utilization of N in dairy cows without putting them at a risk of SARA; however, research with dairy cows fed barley-based diets is limited. Barley is the major dietary starch source in western Canadian dairy diets and dairy cows fed barley-based diets are at a greater risk of SARA than those fed corn based-diets. Partial replacement of barley starch with lactose (fed as DWP) up to 8% TS did not increase the risk of SARA; however, production performance was unaffected (Chibisa et al., 2015). It is important to determine the responses in production (i.e., DMI and milk production) and SARA when cows fed barley-based diets receive total dietary sugar contents >8%.

2.11. Hypothesis

The hypothesis for this thesis research was that partial replacement of barley starch with dried whey permeate (DWP) in diets for dairy cows (graded levels up to 12.5% total dietary sugar level) would reduce the risk of ruminal acidosis and optimize N utilization in dairy cows.

2.12. Objective

The major objective of this thesis research was to delineate the effects of partial replacement of barley starch with DWP (a source of lactose; graded levels up to 12.5% total dietary sugar level) on ruminal fermentation characteristics (i.e., ruminal concentrations of NH_3-N and SCFA and ruminal pH) transport of SCFA across the ruminal epithelium, urea-N recycling to the digestive tract, ruminal microbial protein production, omasal nutrient flow, whole-body N balance, and production performance in lactating cows.

3. MATERIALS AND METHODS

3.1. Animals and experimental design

Eight multiparous lactating Holstein cows (mean DIM = 97 ± 10; mean BW = 733 ± 47 kg at the start of the experiment) were used in a replicated 4 × 4 Latin square design with four dietary treatments. Each experimental period was 28 days in length, consisting of 18 days of dietary adaptation and 10 days of data and sample collection. Four cows in one Latin square were ruminally-cannulated and they were used to determine dietary effects on ruminal fermentation, ruminal N metabolism, omasal nutrient flow, SCFA absorption, and urea-N recycling kinetics. All cows were housed in individual tie-stalls at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan). In order to use these cows in this experiment, University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048) approval was obtained. Care and handling of all experimental cows were conducted according to the guidelines of Canadian Council of Animal Care (1993).

3.2. Experimental treatments and feeding management

Animals were fed a standard barley-based diet as a control [3.5% total sugar (TS)] or diets that were formulated to contain 6.5, 9.5 or 12.5% TS, with DWP (contained 83% lactose) added as a partial replacement for barley starch. The inclusion levels of DWP were based on previous studies showing improved animal performance with total dietary sugar levels up to 10% (Broderick et al., 2008). Also, a previous study at the University of Saskatchewan (Chibisa et al., 2015) noted no effects of the partial replacement of barley starch with DWP when total dietary sugar levels were increased from 3% (basal diet) to 8%; consequently, a primary objective for the current study was to determine if dietary sugar levels > 8% would influence production responses. Diets were formulated to be isonitrogenous (17.2 to 17.4% CP; Table 3.1). Dried whey permeate was included in a pellet which was then mixed in the appropriate proportions with steam-rolled barley to make a concentrate mixture. Cows were fed a TMR twice daily at 0830 and 1630 h for ad libitum intake. The TMR was composed of the concentrate mix plus forage, with a forage:concentrate ratio of 50:50 on a DM basis. The forage component of the TMR was a mixture of barley silage (~71% of forage DM) and chopped alfalfa hay (~29% of forage DM).

Table 3.1. Ingredient and chemical composition of experimental diets

	Experimental diets			
	Control	6.5% sugar	9.5% sugar	12.5% sugar
Ingredient composition, % of diet DM				
Alfalfa hay	14.28	14.28	14.28	14.28
Barley silage	35.34	35.34	35.34	35.34
Barley grain	27.85	24.10	20.24	16.35
Pelleted ingredients[1]				
Dried whey permeate	-	3.75	7.60	11.50
Soy bean hulls	2.53	2.14	1.79	1.43
Wheat DDGS[2]	5.53	3.93	2.86	1.43
Canola meal	5.00	6.78	7.14	8.21
Corn gluten meal	2.32	2.86	3.93	5.00
Soy bean meal	3.00	2.68	2.68	2.32
Tallow	0.61	0.61	0.61	0.61
Dairy premix[3]	1.80	1.80	1.80	1.80
Sodium bicarbonate	0.97	0.97	0.97	0.97
Salt	0.37	0.37	0.37	0.37
Limestone	0.23	0.23	0.23	0.23
Dynamate[4]	0.17	0.17	0.17	0.17
Chemical composition				
DM, %	57.3	57.5	57.8	58.1
CP, % of DM	17.3	17.4	17.3	17.2
NDF, % of DM	34.4	34.6	33.5	32.5
ADF, % of DM	24.6	24.9	24.1	23.1
Starch, % of DM	24.3	22.2	21.2	19.1
Total WSC[5], % of DM	3.64	6.59	9.59	12.62
Ether extract, % of DM	2.51	2.43	2.19	2.23
Ash, % of DM	9.03	9.96	9.76	11.05
Calcium, % of DM	1.02	1.17	1.17	1.52
Phosphorous, % of DM	0.58	0.58	0.55	0.57
NFC[6], % of DM	36.8	35.7	37.2	37.1
NSC[7], % of DM	28.0	28.8	30.8	31.7
NE_L[8] Mcal/kg of DM	1.65	1.66	1.66	1.64

[1]Ingredients were pelleted (pellet size: 4 mm) and then mixed in the appropriate proportions with steam-rolled barley to prepare the concentrate mixture at Canadian Feed Research Centre (North Battleford, SK, Canada).

[2]Dried distillers grains with solubles

[3]Dairy premix (Masterfeeds LP, Saskatoon, SK, Canada) contained (/kg of premix; DM basis): 250,000 IU of vitamin A, 80,000 IU of vitamin D3, 2,000 IU of vitamin E, 16%, Ca,

6.5% P, 6.3% Na, 7.0% Mg, 2,500 mg Zn, 1,500 mg Mn, 675 mg Cu, 20 mg Se, 80 mg I, and 5.52% ground wheat as the premix carrier

[4]Dynamate (The Mosaic Company, Plymouth, MN) contained 18% K, 11% Mg, and 22% S

[5]Water-soluble carbohydrates; determined according to (Hall, 2014) using lactose as a standard

[6]Non-fiber carbohydrates = 100 – (%NDF + %CP + %ether extract + %ash)

[7]Non-structural carbohydrates = %WSC + %Starch

[8]Net energy of lactation; calculated from NRC (2001)

3.3. Data collection and sampling

On day 18 (0900 h) of each experimental period, temporary vinyl catheters (0.86 mm I.D. × 1.32 mm O.D.; Scientific Commodities Inc., Lake Havasu City, AZ) were placed in the right and left jugular veins of the 4 ruminally-cannulated cows in order to facilitate continuous isotope infusion and blood sampling. Urea transfer to the GIT, microbial protein production in the rumen, whole-body N balance, and apparent total-tract nutrient digestibilities were determined from day 19 to day 23 of the experimental period (Lobley et al., 2000; Plaizier et al., 2000). Briefly, background samples of urine and feces were collected prior to the initiation of isotope infusion on day 19 to measure ^{15}N natural abundance. Jugular infusions of double-labeled urea ([$^{15}N^{15}N$]-urea) (99.8 atom % ^{15}N; Cambridge Isotope Laboratories, Andover, MA) prepared in 0.9 % sterile saline were initiated at 1100 h and continued until 0600 h on day 25 at the rate of 1 L/day/cow. The daily dosage of [$^{15}N^{15}N$]-urea was determined based on the average individual dietary N intake of cows from day 10 to day 15 of the experimental period. For this calculation, daily endogenous production of urea was predicted as 100% of the daily dietary N intake. Targeted isotope enrichment in urine was 0.03% of the predicted daily urea production. Total collections of urine and feces were conducted from 1100 h on day 19 to 1100 h on day 23. Feces were collected into large steel trays that were placed over the gutter behind each tie-stall. Total daily fecal output of each cow was thoroughly mixed inside the steel tray, quantitatively transferred into a pre-weighed plastic container and weighed. A 2.5% sub-sample of daily fecal output was collected and stored at -20°C for later chemical analysis. In order to facilitate the collection of daily urine output of each cannulated cows, indwelling Bardex Foley urinary bladder catheters (26 Fr, 75cc ribbed balloon, lubricious-coated; C.R. Bard Inc., Covington, GA) were inserted at 0900 h on day 18. To secure the catheter inside the urinary bladder, the ribbed balloon was infused with 80 mL of double-distilled water (ddH_2O) after inserting the catheter into the urinary bladder. Urinary bladder catheters were connected to urine collection tubing at the initiation of isotope infusion on day 19 at 1100 h. Urine was collected into 20-L pre-weighed carboy polyethylene containers. To prevent microbial growth and volatilization of NH_3-N in urine, 150 mL of 14.4 *M* HCl was added daily into each empty carboy container such that urinary pH was maintained below 3. The weight of total daily urine output was recorded (the weight of added HCl was considered negligible) and a 5% sub-sample that was pooled by cow for each period was collected and stored at -20°C for later N analysis. A sub-sample of 50 mL

was collected (prior to pooling) and stored at -20°C to determine urinary urea-N enrichment. In addition, a 2-mL sub-sample was collected into a 15-mL centrifuge tube containing 8 mL of ddH_2O and stored at -20°C for later analysis of urea-N.

In order to quantify omasal nutrient flow, indigestible NDF (iNDF; Huhtanen et al., 1994), ytterbium chloride ($YbCl_3$; Siddons et al., 1985), and Cr-EDTA (Udén et al., 1980) were used as digesta markers as described by Reynal et al. (2005). Indigestible NDF, $YbCl_3$, and Cr-EDTA were markers for large particle phase (LPP), small particle phase (SPP), and fluid phase (FP) of omasal digesta, respectively. On day 14 of each experimental period, ruminal infusion solutions of $YbCl_3$ and Cr-EDTA were prepared in ddH_2O and filled into pre-weighed plastic containers. A 45-mL sample of infusion solution was taken from each container and stored at room temperature for later analysis of Cr and Yb concentrations. To determine the background concentrations of Cr, Yb and ^{15}N, a 500-mL sample of omasal digesta was collected on day 19, just before the marker infusion started at 1100 h. Thereafter, a priming dose that was equivalent to half of the daily dose of Cr-EDTA and $YbCl_3$ was first administered into the rumen via the ruminal cannula. Subsequently, Cr-EDTA and $YbCl_3$ were infused continuously until 0600 h on day 25, at a constant rate of 1 L/day using a peristaltic pump (Model:205U, Watson and Marlow, Cornwall, UK). The infusion rate of 1 L/day rate ensured the supply of 2.2 g of Yb and 2.7 g of Cr/cow/day. The weight of marker solution infused each day was measured and recorded. The sampling of omasal digesta was conducted form day 23 to day 25. Omasal samples were taken at 0900, 1500, and 2100 h on d 23, 0300, 1200, and 1800 on day 24, and 0000 and 0600 h on d 25, to represent the 24 h-feeding cycle, according to the technique described by Huhtanen et al. (1997). Briefly, the reticulo-omasal orifice was located by inserting the hand into the reticulum through the ruminal cannula and a sampling tube was inserted into the omasal canal. The sampling tube was removed from the omasal canal after each sample collection and reinserted at each sampling in order to minimize potential negative effects (i.e., impaired normal digestive functions and digesta passage) of keeping the tube inside the omasal canal in-between samplings. At each collection, a 525-mL of sample was collected from each cow. From this sample, a 300-mL sub-sample was pooled for each cow in each period to yield a 2.4-L composite sample that was stored at -20°C for later analysis. A sub-sample of 100 mL was also pooled by cow by period and kept frozen at -20°C as a spare sample. The remaining 125-mL of sub-sample was transfered into a 250-mL centrifuge bottle was kept in ice. Two 125-mL sub-samples from

consecutive samplings were then mixed and used to isolate particle- and fluid-associated bacteria using differential centrifugation (Reynal et al., 2005). The omasal digesta sample was filtered through two layers of cheesecloth and the remaining particulate matter was further washed with 250 mL of chilled 0.9% (w/v) NaCl. The filtrate and the saline wash were collected into a 1,000-mL centrifuge bottle and used to isolate fluid-associated bacteria (FAB). The particulate matter that was retained on the cheesecloth was transferred into another 1,000-mL centrifuge bottle that contained 175 mL of chilled 0.1% Tween-80 solution that was prepared in 0.9% (w/v) NaCl and kept for particle-associated bacteria (PAB) isolation. In order to isolate FAB, the filtrate was centrifuged (1,000 × *g*, 5°C, 5 min), and the supernatant was carefully decanted and centrifuged again (11,300 × *g*, 5°C, 30 min). The supernatant that was obtained after the second centrifugation was decanted and discarded. The remaining pellet was re-suspended in 50 mL of McDougall's buffer and re-centrifuged (11,300 × *g*, 5°C, 30 min). The resulting FAB pellets after each isolation were pooled by cow per period and stored at -20°C for later analysis. The pellet obtained from the centrifugation at 1,000 × *g* was mixed with the particulate matter in 0.1% Tween-80 solution and homogenized for 20 sec using a blender (NuBlend, Waring Commercial, Torrington, CT) to dislodge PAB. The homogenized contents were transferred back to the centrifuge bottle and kept in a refrigerator for 24 hours to facilitate the detachment of PAB. After 24 hours, the homogenized contents were filtered through two layers of cheesecloth and the remaining pellet was discarded. The filtrate was centrifuged (1,000 × *g*, 5°C, 5 min), and the supernatant was carefully decanted. The decanted supernatant was centrifuged again (11,300 × *g*, 5°C, 30 min). The supernatant that was obtained after the second centrifugation was decanted and discarded. The remaining pellet was re-suspended in 50 mL of McDougall's buffer and re-centrifuged (11,300 × *g*, 5°C, 30 min). The resulting pellet after the final centrifugation was PAB. The PAB pellets were also pooled by cow per period and stored at -20°C for later analysis.

Ruminal digesta samples were collected at the same time points of omasal sampling. Approximately 1,000 mL of ruminal digesta was collected from the cranio-ventral, central, caudo-ventral, and dorsal sacs of the rumen by collecting a sample of 250 mL from each location using a graduated plastic cup. The ruminal digesta was then strained through four layers of cheesecloth into a collection pail and the pH of the filtrate was immediately measured using a portable pH meter (Orion Research Inc., Beverly, MA). Two 10-mL sub-samples of strained

ruminal fluid were collected into 15-mL centrifuge tubes that contained either 25% (w/v) meta-phosphoric acid or 1% H_2SO_4 acid and stored at -20°C for the later analysis of ruminal SCFA and NH_3 concentrations, respectively.

Starting on day 19 of each experimental period, ruminal pH was measured continuously for 3 consecutive days using the Lethbridge Research Center Ruminal pH Measurement System (Dascor, Escondido, CA) as described by Penner et al. (2006).

Jugular blood samples were collected via the contralateral jugular catheter, from each cannulated cow at 1100 h from day 20 to 23. Blood samples were also collected from day 23 to 25 at each omasal sampling. Blood samples were collected into heparinized 10-mL vacutainer tubes and immediately centrifuged (1,500 × *g*, 4°C, 15 min) to separate plasma. Plasma samples were stored at -20°C for later analysis of PUN, glucose, and BHBA concentrations.

Feed offered and orts were recoded daily over the entire experimental period. From day 19 to day 23, TMR and orts samples were collected and stored at -20°C for later analysis. Daily milk yields for all 8 cows were recorded from day 19 to day 28. Milk samples were collected from all three milkings at 0430, 1230, and 1900 h on day 19 to 23 into vials that contained 2-bromo-2-nitropropane-1-2-diol as a preservative. Samples were pooled per cow per day proportionally based on milk yield and submitted to the CanWest DHI Laboratory (Edmonton, AB, Canada) for CP, fat, lactose, and MUN analysis using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerod, Denmark) according to AOAC (1990). On day 1 of each experimental period and on next day after the end of period 4, all cows were weighed and the weights were recorded.

In order to determine ruminal SCFA absorption, the temporarily isolated and washed reticulo-rumen (WRR) technique was conducted on day 28 of each experimental period as described by Care et al. (1984). In brief, the reticulo-ruminal contents of each cow were manually evacuated through the ruminal cannula. The evacuated reticulo-ruminal contents were stored in pre-weighed insulated plastic containers and covered with lids. After the evacuation, the reticulo-rumen was washed with lukewarm water 3 times (10 L/wash, 39°C) followed by 3 washes with a pre-heated wash buffer solution (8 L/wash, 39°C). The chemical composition of the wash buffer solution was 100 m*M* NaCl, 25 m*M* $NaHCO_3$ and 30 m*M* Na-acetate. The pH of the wash buffer was adjusted to 6.2 using 14.4 *M* HCl. Osmolality of the final wash buffer

solution was 280 ± 8 mOsmol/kg. In order to isolate the reticulo-rumen, a custom-made esophageal-occluding device (University of Leipzig, Leipzig, Germany) was placed in the distal esophagus. A tube through the nose of the cow secured the position of the occluding device in the esophagus and the inflated cuff prevented the entry of saliva into the reticulum. The saliva produced by the animal was sucked using a suction pump and diverted into a pre-weighed 10-L container through a tube attached to the esophageal occluding device. An indwelling Bardex Foley urinary bladder catheter (26 Fr, 75cc ribbed balloon, lubricious-coated; C.R. Bard Inc., Covington, GA) was inserted into the omasal canal and the reticulo-omasal orifice was occluded by inflating the balloon of the catheter which prevented the passage of experimental buffer into the omasum. After the isolation, the reticulo-rumen was washed again using 8 L of wash buffer. A pre-heated experimental buffer (20 L, 39°C) that contained Cr-EDTA as a volume marker was introduced into the reticulo-rumen and incubated for 65 minutes. Osmolality of the final experimental buffer solutions were 325 ± 6 mOsmol/kg; whereas, the pH of the experimental buffer was adjusted to pH 6.2 by adding gluconic acid. The experimental buffers were either high Cl^- or low Cl^- (Table 3.2). Both experimental buffer types were subsequently incubated inside the reticulo-rumen, according to a sequentially randomized order that was balanced for residual effects. Before introducing the second experimental buffer, the first experimental buffer was completely vacuumed out and the reticulo-rumen was thoroughly washed with 8 L of wash buffer to prevent buffer carryover effects. To ensure thorough mixing of the incubation buffer in the reticulo-rumen, the buffer was continuously gassed with CO_2 through a tube that had an air stone attached on the end. Samples (35 mL) of the experimental buffers were taken into 50-mL centrifuge tubes that contained 7 mL of 25% (w/v) chilled meta-phosphoric acid at 0 min (prior to incubation), and at 5 min and 65 min after the introduction of the incubation buffer into the rumen. Samples were stored at -20°C for later analysis of SCFA and Cr concentrations. Weights of the evacuated reticulo-rumen digesta were measured, digesta samples were taken and stored at -20°C for later DM analysis. Following the collection of the last experimental buffer sample, residual buffers were vacuumed out of the reticulo-rumen and the digesta was transferred back. During the WRR procedure, feed and water were removed.

Table 3.2. Composition of experimental buffers

	Buffer	
	Low chloride	High chloride
Ingredient, m*M*		
Calcium gluconate	2	-
Magnesium gluconate	2	-
Sodium gluconate	5	-
Calcium chloride	-	2
Magnesium chloride	-	2
Sodium chloride	-	5
Potassium acetate	20	20
Sodium acetate	40	40
Mannitol	84	-
Choline chloride	0	40
Sodium propionate	25	25
Butyric acid	15	15
Sodium bicarbonate	25	25
Chromium EDTA	2	2
Chloride concentration, m*M*	-	49
Osmolality, mOsmol/kg	325 ± 6	325 ± 6
pH	6.2	6.2

3.4. Sample analysis

At the end of each experimental period, TMR, orts, fecal, and reticulo-rumen digesta samples that were collected during WRR procedure, were thawed overnight at room temperature and subsequently analyzed for DM by drying to constant weight in a forced-air oven at 55°C (AOAC, 1990; method 930.15). Dried TMR and orts samples were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England), and fecal samples were ground through a 1-mm screen using a Retsch ZM100 ultra centrifuge mill (Retsch-Allee 1-5, 42781 Haan, Germany). Following grinding, TMR, orts, and fecal samples were pooled per collection period for each cow. Prior to the pooling of fecal samples, daily individual sub-samples were taken in order to analyze for ^{15}N enrichment. Pooled samples were submitted to Cumberland Valley Analytical Services (Hagerstown, MD) for compositional analysis. Samples were analyzed for ash (AOAC, 2000; method 942.05), CP (AOAC, 2000; method 990.03), EE (AOAC, 2006; method 2003.05), ADF (AOAC, 2000; method 973.18 with modifications), NDF (Van Soest et al., 1991 with modifications), starch (Hall, 2009), and minerals (AOAC, 2000; method 985.01); whereas, total water-soluble carbohydrates (WSC) were determined using the method described by Hall (2014).

Milk samples were analyzed for fat, CP, lactose, and MUN using infrared spectroscopy (MilkoScan 605; Foss Electric, Hillerød, Denmark; AOAC, 1990; method 972.16) at CanWest DHI Laboratory. Frozen urine samples were thawed at room temperature and subsequently analyzed for total N using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05). Plasma glucose was determined using the glucose oxidase/peroxidase enzyme (No. P7119; Sigma, St. Louis, MO) and dianisidine dihydrochloride (No. F5803; Sigma) assay, with absorbance being determined at 450 nm using a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Urea-N in plasma and diluted urine samples was quantified using the assay described by Fawcett and Scott (1960), with absorbance being determined at 600 nm using a spectrophotometer (Model: 80-2097-62, Pharmacia LKB Biochrom, UK). Beta-hydroxybutyrate concentrations in deproteinized plasma samples were determined by enzymatic oxidation of BHBA to acetoacetate with 3-hydroxybutrate dehydrogenase (H6501; Roche, Mississauga, Ontario, Canada), and concomitant reduction of NAD to NADH was determined using the plate reader at 340 nm wavelength.

The 2.4-L composite omasal digesta samples were separated into three digesta phases (i.e., LPP, SPP, and FP) according to the method described by Reynal and Broderick. (2005). Briefly, the composite digesta samples were thawed at room temperature and carefully squeezed through one layer of cheesecloth. The solid material that was retained on the cheesecloth was defined as the LPP. The filtrate was centrifuged at 1,000 × *g* (5°C, 5 min) and the resulting supernatant was defined as the FP. The FP was carefully decanted from the pellet which was defined as the SPP. Three omasal digesta phases were carefully transferred into aluminum trays, frozen at -20°C and subsequently freeze-dried. Freeze-dried LPP samples were ground through a 1-mm screen using the Retsch ZM100 ultra centrifuge mill, whereas SPP and FP samples were ground using a coffee grinder prior to the determination of Yb, Cr and iNDF concentrations which were later used to reconstitute omasal true digesta (OTD).

Indigestible NDF concentrations in TMR, orts, LPP, and SPP were determined according to the method describes by Ahvenjärvi et al. (2000). In brief, 1.5 g of LPP, 3.5 g of SPP, and 3.0 g of TMR and ort samples were weighed into 5- × 10-cm nylon mesh bags (6 µm pore size; part no. 03-6/5, Sefar America Inc., Depew, NY). Samples of TMR, orts, and LPP were weighed in duplicate, whereas SPP samples were weighed in triplicate. Nylon bags were incubated in 5 ruminally-cannulated cows for 12 consecutive days, such that bags with TMR, orts, LPP, and SPP were evenly distributed across cows. Following incubation, nylon bags were removed from the rumen and immediately soaked in cold water to prevent further microbial degradation. Nylon bags were then rinsed with cold water until rinse water was clear, and then soaked in water for 30 min. Thereafter, bags were dried at 55°C for 48 hours in a forced-air oven and then weighed. Dried samples were analyzed for iNDF content according to the ANKOM method 6 for A200 and A2001 series. To measure the Cr and Yb concentrations, approximately 1-g of LPP, SPP, and FP were combusted at 550°C overnight in a muffle furnace (Lindberg/Blue M Box Furnace, Lindberg/Blue M, Asheville, NC). Combusted samples were digested using 1.5 *M* HNO_3 acid solution containing 2 g/L KCl (Vicente et al., 2004), before being analyzed for Cr and Yb concentrations using an atomic absorption spectrophotometry (Perkin Elmer 2300, Perkin-Elmer Corp, Norwalk, CT). The mean Cr concentration of FP (545.0 mg/kg) was 14 and 20 times greater compared to that of SPP (39.1 mg/kg) and LPP (27.3 mg/kg), respectively. Moreover, the mean Yb concentration in SPP (311 mg/kg) was 2.5 and 3 times greater than in LPP (124.4 mg/kg) and FP (100.8 mg/kg), respectively. The iNDF concentration of LPP (392.3 mg/kg) was

7.2 times greater than that of SPP (54.6 mg/kg). Because Cr, Yb and iNDF concentrations were significantly greater in their respective digesta phases, marker concentrations were used to reconstitute OTD as described by France and Siddons (1986). Furthermore, marker concentrations in SPP and LPP were used to reconstitute 2-g samples of particle phase (PP) (Reynal and Broderick, 2005). Reconstituted OTD was analyzed for OM (AOAC, 1990; method 942.05), CP using macro-Kjeldahl procedure (AOAC, 1990; method 984.13), ADF (ANKOM method 5 for A200 and A2001 series), NDF (ANKOM method 6 for A200 and A2001 series), EE (AOAC, 1990; method 920.39), total starch using Megazyme Total Starch Assay Kit (McCleary et al., 1997; Megazyme International Ireland Ltd., Wicklow, Ireland), and WSC (Hall, 2014). Organic matter content in bacterial pellets was analyzed by combustion in a muffle furnace at 600°C overnight.

To quantify ^{15}N and non-ammonia nitrogen (NAN) concentrations, frozen bacterial pellets (i.e., PAB and FAB including background samples) were freeze-dried and finely ground using a mortar and pestle. Daily individual and background fecal samples, FP, PP, and OTD samples were pulverized using a ball mill. Ground bacterial pellets and pulverized fecal, FP, PP, and OTD samples were prepared for the analysis of ^{15}N in NAN as described by Brito et al. (2006). Briefly, 2-mg samples were weighed into 8- × 12-mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK) and placed in 96-well microtiter plates. In order to volatilize NH_3-N, 50 μL of 72 m*M* K_2CO_3 was added to each tin capsule and samples were incubated for 24 h at 60°C in a forced-air oven. Following incubation, samples were combusted to N_2 gas in a Costech ECS4010 elemental analyzer (Costech Analytical, Valencia, CA) coupled with a continuous flow isotope ratio-mass spectrometry (Delta V Advantage mass spectrometer, Thermo Scientific, Bremen, Germany) and analyzed for ^{15}N natural abundance and enrichment. Ammonia-N concentrations in OTD samples were also determined. In brief, a 0.5-g sample was weighed into a 15-mL centrifuge tube, and then 10 mL of sodium citrate (77.5 m*M*, pH 2.2) was added and the mixture was thoroughly vortexed. The mixture was then incubated at 39°C for 30 minutes in a forced-air oven before being centrifuged at 12,000 × *g* at 4°C for 15 minutes. The supernatant was sub-sampled into 2-mL micro-centrifuged tubes and re-centrifuged (16,000 × *g*, 10 min, 4°C). Subsequently, the supernatant was analyzed for NH_3-N concentration using the phenol-hypochlorite assay (Broderick and Kang, 1980).

In preparation for the analysis of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea enrichment, background and daily urine samples were thawed overnight in a refrigerator. To isolate urea-N, a urine sample that contained 1.5 mg of urea-N was passed through a pre-packed ion exchange column (Poly-Prep® Columns, AG® 50W-X8, hydrogen form #7316213; Biorad, Richmonds, CA) as described by Archibeque et al. (2001). Following the application of urine, the column was washed with 7 mL of ddH_2O. Urea was eluted into test tubes by passing 20 mL ddH_2O through the column. Samples were then air-dried at 60°C. Following air drying, samples were quantitatively transferred into 17- × 60-mm borosilicate glass tubes by rinsing with 1 mL of ddH_2O three times. Subsequently, samples were freeze-dried and then analyzed for [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea enrichment by isotope ratio-mass spectrometry (IRMS; N-15 Analysis Laboratory, University of Illinois, Urbana-Champaign) as described by Lobley et al. (2000). Conditions that were used in this IRMS assay should facilitate [$^{14}N^{14}N$]- [$^{14}N^{15}N$]- and [$^{15}N^{15}N$]-urea to produce ions with mass/charge (m/z) values of 28, 29 and 30, respectively (Sarraseca et al., 1998). Values that were obtained for the analysis of standards that were prepared using [$^{15}N^{15}N$]-urea (99.8 atom % ^{15}N) and [$^{14}N^{14}N$]-urea (natural abundance urea; 0.368 atom% ^{15}N) were used to make necessary corrections for [$^{14}N^{15}N$]-urea produced by non-monomolecular reactions (Lobley et al., 2000).

In order to determine NH_3-N concentrations, ruminal fluid samples were thawed overnight in a refrigerator. Thawed samples were vortexed and then centrifuged (12,000 × *g*, 10 min, 4°C). A 1-mL sub-sample of the supernatant was transferred into a micro-centrifuge tube and re-centrifuged (16,000 × *g*, 10 min, 4°C). The resulting supernatant was analyzed for NH_3-N concentration using the phenol hypochlorite-assay as described by Broderick and Kang (1980). Ruminal fluid and buffer samples from the WRR procedure were analyzed for SCFA using gas chromatography (GC) according to the method described by Khorasani et al. (1996), with slight modifications. Briefly, samples were thawed overnight in a refrigerator and centrifuged at 12,000 × *g* at 4°C for 10 min until a clear supernatant was obtained. From the supernatant, 1.5 mL was transferred into micro-centrifuge tubes and centrifuged (16,000 × *g*, 10 min, 4°C). Thereafter, 1 mL of the resulting supernatant was mixed with 0.2 mL of isocaproic acid (which was used as an internal standard) in a GC vial. Separation of SCFA was conducted on an Agilent GC system (Agilent 6890 Series, 254 Agilent Technologies, Waldbronn, Germany) using a column (30.0 m

× 320 µm × 0.25 µm; 255 model 7HM-G009-11, Zebron, Phenomenex, Torrance, CA). The column temperature was held at 90°C for 0.1 min, then increased to a final temperature of 170°C at a rate of 10°C/min. Column flow rate was 35 mL/min. The injector temperature was 170°C and the detector temperature was 250°C.

3.5. Calculations and statistical analysis

Actual WRR buffer volumes at 5 min and 65 min of incubation were calculated using the Cr concentrations of buffer samples that were collected at 0, 5 and 65 min of incubation according to following formulas: $V_{5min} = (C_{0min} \times V_{0min}) / C_{5min}$ and $V_{65min} = (C_{0min} \times V_{0min}) / C_{65min}$ where V = actual volume of buffer and C = Cr concentrations of buffer samples. Subsequently, absorption rates of acetate, propionate, butyrate, and total SCFA, were calculated using the following formula; absolute SCFA absorption rate (mmol/h) = $[(V_{5min} \times A_{5min}) – (V_{65min} \times A_{65min})]$; and fractional SCFA absorption rate (%/h) = $[(V_{5min} \times A_{5min}) – (V_{65min} \times A_{65min})] / (V_{5min} \times A_{5min}) \times 100$ where, A = SCFA concentrations of buffer samples at the respective time of sampling (Gäbel et al., 1993).

Daily nutrient intakes (kg/d) were determined as [DM offered (kg/d) × % of nutrient in TMR] – [DM refused (kg/d) × % of nutrient in orts]. For this calculation, DMI of cannulated cows during total collection (i.e., DMI from day 19 to day 23 of the experimental period) were used. Apparent total-tract nutrient digestibility was calculated as [nutrient intake (kg/d) – fecal nutrient output (kg/d)] ÷ nutrient intake] × 100. Omasal flow of nutrients, apparent and true nutrient digestibility in the rumen were calculated as described by Reynal and Broderick (2005). Non-ammonia N in OTD was calculated as the difference between total N and NH_3-N. Total NAN flow at the omasal canal was assumed to contain NAN in FAB, NAN in PAB and, NAN of non-bacterial origin (NANBN) as described by Reynal and Broderick (2005). Based on the findings of Ahvenjärvi et al. (2002) (i.e., similar back ground ^{15}N atom% in rumen microbes and digesta), mean background ^{15}N atom% of 0.3681 ± 0.0002 in bacterial pellets was assumed to be similar that of in rumen and omasal digesta for necessary calculations. Following this assumption, ^{15}N enrichment (^{15}N APE) in FAB, PAB, and omasal fractions were calculated as the difference between ^{15}N atom% in the sample and the mean background ^{15}N atom% (i.e., ^{15}N APE = ^{15}N atom% in the sample - 0.3681). Omasal flows of FAB NAN, PAB NAN, total bacterial NAN, NANBN, RDP supply, bacterial OM, and OM truly digested in the rumen

(OMTDR) were calculated as described by Brito et al. (2009) as follows; FAB NAN flow = FP NAN flow × (FP ^{15}N APE ÷ FAB ^{15}N APE); PAB NAN flow = PP NAN flow × (PP ^{15}N APE ÷ PAB ^{15}N APE); total bacterial NAN flow = FAB NAN flow + PAB NAN flow; NANBN flow = total NAN flow − total bacterial NAN flow; RDP supply = total CP intake − (NANBN flow × 6.25); and OMTDR = OM intake − (omasal OM flow − total bacterial OM flow). The omasal flow of NAN that was derived from recycled urea-N (NANRN) was computed using the following equation; NANRN = bacterial NAN flow × (bacterial ^{15}N APE ÷ urinary ^{15}N APE) (Wickersham et al., 2009a), with urinary ^{15}N APE being calculated by taking the sum of (^{15}N^{15}N)-urea enrichment and half of the (^{14}N^{15}N)-urea enrichment (Wickersham et al., 2009a). Efficiency of microbial protein synthesis was calculated as [microbial NAN (g) ÷ OMTDR (kg)] (Brito et al., 2009). Flows were expressed as grams or kilograms per day.

Whole body urea-N kinetics were calculated according to the model described by Lobley et al. (2000), using urinary enrichment of (^{15}N^{15}N)-urea, and (^{14}N^{15}N)-urea, and total fecal ^{15}N excretion.

For unknown reasons, a pH data logger which was used for continuous ruminal pH measurements in cows that were fed the 12.5% TS diet for three experimental periods malfunctioned and this resulted in the collection of unusable ruminal pH data. Thus, due to limited replication it was not possible to conduct meaningful statistical analysis on the data on continuous ruminal pH measurements (i.e., daily minimum and maximum pH, mean pH, and duration [min/d] and total area [pH × min] that ruminal pH was <5.8).

Production data from all 8 cows were analyzed by Proc Mixed procedure of SAS (SAS version 9.4; Sas INC, CARY, NC) as a replicated 4 × 4 Latin square design using the following model: $Y_{ijkl} = \mu + S_i + P_{j(i)} + C_{k(i)} + T_l + ST_{il} + E_{ijkl}$, where, Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of ith square, $P_{j(i)}$ is the fixed effect of jth period (within square i), $C_{k(i)}$ is the random effect of kth cow (within square i), T_l is the fixed effect of lth dietary treatment, ST_{il} is the interaction between ith square and lth, and E_{ijkl} is the residual error. All data on ruminal fermentation characteristics (i.e., pH, and NH_3-N and SCFA concentrations), apparent ruminal and total-tract nutrient digestibilities, nitrogen balance, whole body urea-N kinetics, blood parameters, ruminal SCFA absorption rates, microbial protein production, and omasal nutrient flows were analyzed using the Proc Mixed procedure of SAS as a 4 × 4 Latin

square design according to the fallowing model: $Y_{jkl} = \mu + P_j + C_k + T_l + E_{jkl}$ where, Y_{jkl} is the dependent variable, μ is the overall mean, P_j is the fixed effect of the j^{th} period, C_k is the random effect of the k^{th} cow, T_l is the fixed effect of the l^{th} treatment and E_{jkl} is the residual error. The Kenward-Roger method was used to approximate degrees of freedom. Orthogonal polynomial contrasts were used to test for linear, quadratic and cubic effects of dietary sugar levels. Significance was declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$. All reported values were least squares means, which were separated using the PDIFF test in SAS.

4. RESULTS

4.1. Dietary characteristics

The inclusion levels of 0, 3.75, 7.6, and 11.5% of DWP as a partial replacement for barley starch targeted TS concentrations of 3.5, 6.5, 9.5, and 12.5% in experimental diets, respectively. The actual dietary sugar levels based on chemical analysis of experimental diets were 3.64, 6.59, 9.59 and 12.62%, respectively (Table 3.1). As expected, the partial replacement of barley grain with DWP in equivalent amounts resulted in decreases in dietary starch contents from 24.3% to 19.1%. Total NSC and NFC concentrations ranged from 28.0 to 31.7%, and 35.7 to 37.2%, respectively, across experimental diets.

4.2. Production parameters and blood metabolites

Dry matter intake, milk yield, energy-corrected milk (ECM), and feed efficiency were not affected ($P > 0.05$) by dietary inclusion DWP as a partial replacement for barley starch (Table 4.1). Milk fat and protein content and yield were also not different ($P > 0.05$) among diets. Milk lactose content increased quadratically ($P = 0.04$) from 4.40 for the control to 4.46% for 9.5% TS, with a slight decrease to 4.40% for the 12.5% TS diet; however, milk lactose yield was unaffected ($P > 0.05$) by dietary addition of DWP. Milk urea-N concentration tended to change cubically ($P = 0.07$) as dietary content of DWP increased. There was no effect ($P > 0.05$) of partial substitution of barley starch with DWP on PUN concentration. However, plasma BHBA concentration increased cubically ($P = 0.04$) in cows fed the 9.5% TS diet compared to those fed the control diet and 6.5% TS, then decreased in cows fed 12.5% TS compared to those fed the 9.5% TS diet. Partial replacement of barley starch with DWP cubically increased ($P = 0.02$)

Table 4.1. The effects of partial replacement of barley starch with whey permeate on dry matter intake (DMI), body weight, milk yield, milk composition (*n* = 8), and blood metabolites in cows (*n* = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
DMI, kg/d	26.1	26.4	26.3	25.8	0.81	0.71	0.29	0.71
Body weight, kg	745	740	745	734	18.8	0.08	0.50	0.89
Body weight change, kg/d	0.391	0.094	0.294	0.297	0.2450	0.06	0.84	0.70
Milk yield, kg/d	34.3	35.0	35.6	34.6	1.63	0.95	0.16	0.41
ECM[1], kg/d	35.4	35.9	36.6	36.1	1.07	0.62	0.38	0.32
Feed efficiency[2]	1.35	1.36	1.40	1.40	0.026	0.32	0.95	0.15
Milk fat, %	3.66	3.66	3.67	3.81	0.176	0.18	0.34	0.58
Milk fat yield, kg/d	1.25	1.27	1.30	1.30	0.042	0.33	0.81	0.30
Milk protein, %	3.22	3.21	3.20	3.20	0.074	0.72	0.83	0.70
Milk protein yield, kg/d	1.10	1.12	1.14	1.10	0.032	0.82	0.13	0.39
Milk lactose, %	4.40	4.42	4.46	4.40	0.049	0.78	0.04	0.16
Milk lactose yield, kg/d	1.51	1.54	1.58	1.53	0.067	0.98	0.15	0.31
Milk urea-N, mg/dL	14.1	15.1	14.0	13.7	0.43	0.88	0.11	0.07
Plasma urea-N, mg/dL	14.2	15.3	14.6	14.2	0.59	0.77	0.16	0.41
Plasma glucose, mg/dL	60.6	62.3	59.6	58.9	0.80	0.48	0.12	0.02
Plasma ß-hydroxybutyrate, mg/dL	9.97	9.04	11.34	10.18	0.626	0.57	0.86	0.04

[1]Energy-corrected milk = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)] (Orth, 1992)
[2]Feed efficiency = ECM/DM intake

plasma glucose concentration from 60.6 for the control diet to 62.3 mg/dL for 6.5% TS, then decreased to 59.6 mg/dL for 12.5% TS. There was a tendency for a linear decrease in body weight ($P = 0.08$) and daily body weight change ($P = 0.06$) of cows as dietary content of DWP increased.

4.3. Ruminal fermentation characteristics

Ruminal concentrations of total SCFA, acetate, propionate, and isobutyrate were not affected ($P > 0.05$) by dietary inclusion of DWP (Table 4.2). Ruminal concentration of NH_3-N increased cubically ($P = 0.03$) from 12.1 for the control diet to 12.3 mg/dL for 6.5% TS and then decreased to 10.9 mg/dL for the 9.5% TS diet fallowed by a decrease to 9.4 mg/dL for the 12.5% TS diet. Partial replacement of barley starch with DWP cubically increased ($P = 0.04$) ruminal concentration of butyrate (12.8, 13.0, 14.1, and 14.9 m*M* for the control, 6.5, 9.5, and 12.5% TS diets, respectively). There was a tendency for a linear decrease ($P = 0.10$) in ruminal isovalerate concentration across diets; however, total branched-chain fatty acid (BCFA) concentration was unaffected ($P > 0.05$) by dietary addition of DWP. The ratio of acetate:propionate was not affected ($P > 0.05$) by partial substitution of barley starch with DWP. Ruminal valerate concentration increased in a cubic manner ($P = 0.01$) from the control diet to 12.5% TS diet. The mean ruminal pH tended to change quadratically ($P = 0.10$) as dietary content of DWP increased.

4.4. Ruminal short-chain fatty absorption

There was no effect ($P > 0.05$) of partial replacement of barley starch with DWP on Cl^--insensitive absolute absorption rates (mmol/h) of acetate, propionate, butyrate, and total SCFA (Table 4.3). Similarly, Cl^-- insensitive fractional absorption rates (%/h) of acetate, propionate, butyrate, and total SCFA were not affected ($P > 0.05$) by dietary inclusion of DWP (Table 4.4). In terms of total absolute and fractional absorption rates, acetate, propionate, and total SCFA decreased cubically ($P \leq 0.05$) in cows fed 9.5% TS compared to those fed the control diet and the 6.5% TS diet, then increased in cows fed the 12.5% TS diet compared to those fed the 9.5% TS diet. Total absolute ($P = 0.10$) and fractional ($P = 0.09$) absorption rates of butyrate tended to change cubically as level of dietary TS increased. Dietary inclusion of DWP did not affect ($P > 0.05$) Cl^-- competitive absolute absorption rates of acetate, butyrate, and total SCFA; however, a tendency for a cubic change ($P = 0.09$) was observed in Cl^-- competitive absolute absorption rate

Table 4.2. The effects of partial replacement of barley starch with whey permeate on ruminal fermentation characteristic in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
Ruminal SCFA[1], mmol/L								
Acetate	74.5	73.5	75.5	72.0	1.73	0.24	0.46	0.66
Propionate	23.4	22.6	24.1	22.8	1.05	0.52	0.82	0.45
Butyrate	12.8	13.0	14.1	14.9	0.43	0.04	0.51	0.04
Isobutyrate	0.90	0.89	0.85	0.82	0.040	0.35	0.96	0.32
Valerate	1.55	1.55	1.77	1.80	0.054	0.09	0.82	0.01
Isovalerate	1.47	1.42	1.37	1.29	0.055	0.10	0.79	0.20
Acetate:Propionate	3.14	3.22	3.17	3.25	0.142	0.61	0.99	0.95
Total SCFA	115	114	117	113	2.8	0.60	0.67	0.59
Total BCFA[2]	2.38	2.32	2.20	2.10	0.089	0.14	0.81	0.18
Ammonia-N, mg/dL	12.1	12.3	10.9	9.40	0.567	0.03	0.15	0.03
Ruminal pH	6.32	6.31	6.34	6.22	0.053	0.06	0.10	0.95

[1]Short-chain fatty acids
[2]Branched-chain fatty acids

Table 4.3. The effects of partial replacement of barley starch with whey permeate on absolute ruminal absorption rates of short-chain fatty acids (SCFA) in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
Total absorption[1], mmol/h								
Acetate	478	490	378	435	36.5	0.90	0.45	0.05
Propionate	227	223	178	201	15.2	0.46	0.24	0.02
Butyrate	129	128	100	111	8.6	0.51	0.51	0.10
Total SCFA[2]	834	844	655	748	58.4	0.71	0.38	0.03
Cl^--insensitive absorption[3], mmol/h								
Acetate	397	361	372	371	28.0	0.34	0.39	0.94
Propionate	183	178	181	185	11.7	0.99	0.59	0.76
Butyrate	105	107	100	108	9.3	0.66	0.68	0.57
Total SCFA	686	646	653	664	47.4	0.63	0.46	1.00
Cl^--competitive absorption[4], mmol/h								
Acetate	112.9	105.7	20.9	40.5	41.87	0.54	0.76	0.18
Propionate	56.2	33.4	6.5	6.4	13.15	0.11	0.44	0.09
Butyrate	29.6	25.7	6.3	-1.9	10.91	0.19	0.85	0.14
Total SCFA	199.4	164.1	33.5	44.3	61.08	0.30	0.72	0.12

[1]Total absorption = SCFA absorption measured with low chloride buffer
[2]Total SCFA = acetate + propionate + butyrate
[3]Cl^--insensitive absorption = SCFA absorption measured with high chloride buffer
[4]Cl^--competitive absorption = Total absorption - Cl^--insensitive absorption

Table 4.4. The effects of partial replacement of barley starch with whey permeate on fractional ruminal absorption rates of short-chain fatty acids (SCFA) in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
Total absorption[1], %/h								
Acetate	44.1	46.7	34.2	40.7	3.52	0.79	0.40	0.01
Propionate	50.1	50.8	40.5	47.0	3.74	0.91	0.28	0.03
Butyrate	48.1	48.9	38.0	43.7	3.26	0.87	0.51	0.09
Total SCFA	46.1	48.2	36.1	42.7	3.42	0.86	0.39	0.02
Cl^--insensitive absorption[3], %/h								
Acetate	36.0	33.9	33.1	32.3	2.96	0.35	0.77	0.58
Propionate	41.4	41.5	38.9	39.4	3.19	0.78	0.95	0.44
Butyrate	40.7	42.2	36.4	38.4	4.10	0.94	0.96	0.22
Total SCFA	38.0	37.0	35.0	35.0	3.15	0.55	0.85	0.46
Cl^--competitive absorption[4], %/h								
Acetate	22.5	24.7	3.7	9.4	8.60	0.68	0.85	0.13
Propionate	22.9	17.5	2.8	3.4	6.13	0.19	0.65	0.08
Butyrate	21.8	20.4	5.6	-1.7	8.47	0.21	0.74	0.15
Total SCFA	22.3	22.3	3.8	6.3	7.45	0.43	0.87	0.10

[1]Total absorption = % SCFA absorption measured with low chloride buffer
[2]Total SCFA = acetate + propionate + butyrate
[3]Cl^--insensitive absorption = % SCFA absorption measured with high chloride buffer
[4]Cl^--competitive absorption = Total absorption - Cl^--insensitive absorption expressed as a % of total absorption

of propionate. There was no diet effect ($P > 0.05$) on Cl^-- competitive fractional absorption rates of acetate and butyrate. The Cl^-- competitive fractional absorption rates of propionate ($P = 0.08$) and total SCFA ($P = 0.10$) tended to decrease cubically in cows fed 9.5% TS in comparison to those fed the control and 6.5% TS diets, then tended to increase slightly in cows fed 12.5% TS compared to those fed the 9.5% TS diet.

4.5. Nutrient intakes, ruminal digestibilities and omasal nutrient flows

Dietary inclusion of DWP did not affect DMI ($P > 0.05$); however, a tendency for a linear decrease ($P = 0.08$) was observed for omasal flows of the fluid and particle phases (Table 4.5). Omasal DM flow tended to decrease linearly ($P = 0.07$) as dietary TS content increased. Apparent ruminal DM digestion when expressed as kilograms per day or as a percentage of total DMI linearly increased ($P = 0.02$) across diets. Similar to DMI, OM intake was not affected ($P > 0.05$) by increased dietary content of DWP. However, partial substation of barley starch with DWP resulted in a linear decrease ($P = 0.04$) in omasal OM flow. There was a tendency for a linear increase ($P = 0.06$) in apparent ruminal OM digestion when expressed as kilograms per day as dietary content of DWP increased. Apparent OM digestion in the rumen when expressed as a percentage of OM intake linearly increased ($P = 0.03$) across diets. However, diets did not affect ($P > 0.05$) OM truly digested in the rumen. Intake, omasal flow and apparent ruminal digestibility of ADF and NDF were not affected ($P > 0.05$) by partial replacement of barley starch with DWP. Respectively, EE intake and apparent ruminal EE digestion expressed as a percentage of EE intake decreased ($P = 0.04$) and tended to decrease ($P = 0.06$) in a cubic manner in cows fed 9.5% TS compared to those fed the control and 6.5% TS diets, followed by an increased in cows fed the 12.5% TS diet compared to those fed the 9.5% TS diet. There was no diet effect ($P > 0.05$) on omasal flow and apparent ruminal digestion of EE expressed as kilograms per day. Starch intake decreased linearly ($P = 0.01$) when dietary content of DWP increased. Starch flow at the omasal canal ($P = 0.03$) and apparent ruminal starch digestion expressed as kilograms per day ($P = 0.02$) were decreased linearly by diets. However, apparent ruminal starch digestion when expressed as a percentage of starch intake was not affected ($P > 0.05$) by dietary inclusion of DWP. Partial replacement of barley starch with DWP cubically increased ($P < 0.01$) WSC intake and apparent ruminal digestion when expressed as kilograms per day or as a percentage of WSC intake.

Table 4.5. The effects of partial replacement of barley starch with whey permeate on ruminal nutrient digestion and omasal nutrient flows in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
Omasal fluid flow, kg/d	7.83	7.65	7.73	6.52	0.560	0.08	0.26	0.59
Omasal particle flow, kg/d	12.6	12.4	13.0	11.8	0.47	0.08	0.19	0.50
DM								
Intake, kg/d	25.3	25.7	25.5	24.8	0.99	0.46	0.23	0.59
Omasal flow, kg/d	20.4	20.0	20.7	18.4	1.00	0.07	0.21	0.97
Apparent digestion, kg/d	4.85	5.65	4.80	6.37	0.387	0.02	0.37	0.59
Apparent digestion, % of DM intake	19.2	21.9	18.6	26.1	1.65	0.02	0.24	0.72
OM								
Intake, kg/d	23.1	23.2	23.0	22.1	0.93	0.17	0.26	0.48
Omasal flow, kg/d	16.9	16.3	17.1	15.0	0.82	0.04	0.22	0.89
Apparent digestion, kg/d	6.23	6.88	5.98	7.05	0.362	0.06	0.53	0.24
Apparent digestion, % of OM intake	26.8	29.5	25.8	32.3	1.53	0.03	0.29	0.46
True digestion, kg/d	12.4	13.0	12.2	12.4	0.67	0.64	0.60	0.27
True digestion, % of OM intake	53.3	56.0	53.0	56.4	1.77	0.20	0.84	0.52
NDF								
Intake, kg/d	8.38	8.55	8.18	7.73	0.399	0.23	0.28	0.18
Omasal flow, kg/d	4.73	4.68	4.50	4.10	0.211	0.14	0.46	0.29
Apparent digestion, kg/d	3.68	3.88	3.65	3.60	0.341	1.00	0.61	0.49
Apparent digestion, % of NDF intake	44.0	45.1	44.7	46.1	2.58	0.48	0.96	0.90
ADF								
Intake, kg/d	5.95	6.18	5.85	5.43	0.335	0.29	0.23	0.22
Omasal flow, kg/d	3.55	3.65	3.30	2.98	0.195	0.18	0.33	0.12
Apparent digestion, kg/d	2.40	2.55	2.55	2.45	0.256	0.87	0.56	0.96

Table 4.5 (cont'd). The effects of partial replacement of barley starch with whey permeate on ruminal nutrient digestion and omasal nutrient flows in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
Apparent digestion, % of ADF intake	40.2	40.7	43.3	44.8	2.85	0.38	0.88	0.33
Ether extract								
Intake, kg/d	0.650	0.650	0.550	0.575	0.0439	0.41	0.70	0.04
Omasal flow, kg/d	0.425	0.450	0.525	0.400	0.0239	0.23	0.22	0.12
Apparent digestion, g/d	0.200	0.200	0.075	0.200	0.0408	0.56	0.22	0.11
Apparent digestion, % of ether extract intake	27.5	29.5	9.3	29.6	5.28	0.33	0.16	0.06
Starch								
Intake, kg/d	6.33	5.85	5.53	4.83	0.328	0.01	0.68	0.08
Omasal flow, kg/d	1.13	0.95	0.98	0.83	0.079	0.03	0.87	0.50
Apparent digestion, kg/d	5.20	4.90	4.55	3.98	0.270	0.02	0.57	0.07
Apparent digestion, % of starch intake	82.2	83.6	82.4	82.5	0.91	0.66	0.56	0.49
WSC[1]								
Intake, kg/d	0.98	1.81	2.68	3.43	0.086	<0.01	0.29	<0.01
Omasal flow, kg/d	0.475	0.475	0.525	0.425	0.0573	0.34	0.29	0.62
Apparent digestion, kg/d	0.50	1.33	2.10	2.98	0.084	<0.01	0.70	<0.01
Apparent digestion, % of WSC intake	51.5	74.1	80.1	88.2	2.27	<0.01	0.01	<0.01

[1]Water-soluble carbohydrates

4.6. Omasal flows of nitrogen fractions and microbial protein synthesis

Nitrogen intake was unaffected ($P > 0.05$) by dietary inclusion of DWP (Table 4.6). Nitrogen apparently digested in the rumen expressed as grams per day increased cubically ($P < 0.01$) from -54.9 for the control diet to -39.7 for the 6.5% TS diet, then decreased to -94.3 for the 9.5% TS diet followed by an increase to -43.4 for the 12.5% TS diet. Similarly, nitrogen apparently digested in the rumen expressed as a percentage of N intake increased cubically ($P < 0.01$) from -8.05 for the control diet to -5.58 for the 6.5% TS diet, then decreased to -13.5 for the 9.5% TS diet followed by an increase to -5.88 for the 12.5% TS diet. Nitrogen truly digested in the rumen (when expressed as grams per day or as a percentage of N intake) and RDP supply expressed as grams per day tended to change cubically ($P = 0.06$) as dietary TS content increased. The RDP supply when expressed as a percentage of DMI increased cubically ($P = 0.01$) in cows fed the 6.5% TS diet compared to those fed the control diet, then decreased in cows fed both 9.5 and 12.5% TS diets compared to those fed the control diet. Omasal flows of N expressed as grams per day increased quadratically ($P = 0.05$) from 765 for the control diet to 809 for 9.5% TS, followed by a decrease to 729 for the 12.5% TS diet. Moreover, omasal flow of NH_3-N expressed as grams per day increased quadratically ($P = 0.05$) from 27.6 for control diet to 31.8 g/d for 6.5% TS diet with a decrease to 25.5 g/d for 12.5% TS diet. Omasal flow of NAN expressed as grams per day tended to change quadratically ($P = 0.07$) as level of DWP increased. Omasal flows of N and NAN expressed as a percentage of N intake were increased cubically ($P < 0.01$) by the 9.5% TS diet compared to the control and 6.5% TS diets, then decreased by 12.5% TS compared to the control diet. Dietary inclusion of DWP did not affect ($P > 0.05$) NANBN expressed as grams per day or as a percentage of NAN flow or as a percentage of DMI. However, NANBN expressed as a percentage of N intake was decreased cubically ($P = 0.05$) by 6.5% TS compared to the control diet, and then increased by both 9.5 and 12.5% diets compared to the control diet. There was no diet effect ($P > 0.05$) on RUP supply expressed as grams per day or as a percentage of DMI. Dietary inclusion of DWP quadratically decreased ($P = 0.04$) FAB NAN flow express as grams per day (258, 251, 247, and 193 g/d for the control, 6.5, 9.5, 12.5% TS diets, respectively). Moreover, FAB NAN flow expressed as a percentage of NAN flow cubically decreased ($P = 0.02$) across diets. The PAB NAN expressed as grams per

Table 4.6. The effects of partial replacement of barley starch with whey permeate on intake, digestibility, and omasal flow of nitrogen constituents in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
N intake, g/d	710	723	715	686	31.8	0.37	0.21	0.48
N apparently digested in the rumen								
g/d	-54.9	-39.7	-94.3	-43.4	9.09	0.03	0.05	<0.01
% of N intake	-8.05	-5.58	-13.5	-5.88	1.250	0.03	0.07	<0.01
N truly digested in the rumen								
g/d	457	473	445	424	24.3	0.20	0.16	0.06
% of N intake	64.2	65.3	62.1	61.9	1.35	0.51	0.58	0.06
RDP supply								
g/d	2,859	2,954	2,780	2,651	151.8	0.20	0.16	0.06
% of DM intake	11.2	11.5	10.9	10.7	0.24	0.18	0.19	0.01
Flow at omasal canal								
N								
g/d	765	763	809	729	36.5	0.08	0.05	0.20
% of N intake	108	106	114	106	1.3	0.03	0.07	<0.01
NH_3-N, g/d	27.6	31.8	30.1	25.5	2.87	0.59	0.05	0.41
NAN[1]								
g/d	738	731	779	704	34.3	0.08	0.07	0.17
% of N intake	104	101	109	102	1.20	0.03	0.11	<0.01
NANBN[2]								
g/d	225	218	240	237	12.4	0.78	0.86	0.16
% of NAN flow	30.7	29.9	30.8	33.7	1.35	0.19	0.18	0.35
% of N intake	32.0	30.4	33.7	34.4	1.39	0.45	0.34	0.05
% of DMI	0.90	0.85	0.93	0.95	0.037	0.65	0.32	0.13

Table 4.6 (cont'd). The effects of partial replacement of barley starch with whey permeate on intake, digestibility, and omasal flow of nitrogen constituents in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
RUP								
g/d	1,580	1,563	1,686	1,638	86.0	0.87	0.83	0.20
% of DM intake	6.30	6.13	6.63	6.60	0.253	0.72	0.76	0.14
FAB[3] NAN								
g/d	258	251	247	193	15.9	<0.01	0.04	0.11
% of NAN	35.0	34.3	31.7	27.1	1.30	<0.01	0.13	0.02
PAB[4] NAN								
g/d	255	261	293	275	15.5	0.56	0.30	0.05
% of NAN	34.4	35.8	37.5	39.3	1.24	0.09	0.89	0.16
Total bacterial NAN								
g/d	513	517	539	468	28.2	0.04	0.04	0.58
% of NAN	69.3	70.1	69.3	66.3	1.35	0.19	0.18	0.35
Microbial efficiency								
g of microbial N/kg OMTDR[5]	41.8	39.8	44.4	37.7	1.14	0.02	0.11	0.13

[1]Non-NH_3-N
[2]Non-NH_3-nonbacterial N
[3]Fluid-associated bacteria
[4]Particle-associated bacteria
[5]OM truly digested in the rumen

day cubically increased ($P = 0.05$) from 255 for the control diet to 293 for the 9.5% TS diet, followed by a decrease to 275 for the 12.5% TS diet. However, PAB NAN expressed as a percentage of NAN tended to increased linearly ($P = 0.09$) as diet content of DWP increased. Total bacterial NAN flow expressed as grams per day quadratically increased ($P = 0.04$) in cows fed 9.5% TS diet compared to those fed control and 6.5% TS diets, then decreased in cows fed 12.5% TS diets compared to those fed control and 6.5% TS diets, whereas total bacterial NAN flow expressed as a percentage of NAN flow was unaffected ($P > 0.05$) by diets. Dietary inclusion of DWP as a partial replacement for barley starch linearly decreased ($P = 0.02$) microbial N efficiency expressed as grams of microbial N per kg of OMTDR.

4.7. Apparent nitrogen balance

Total N intake, apparent N balance, and productive N were not influenced ($P > 0.05$) by partial replacement of barley starch with DWP (Table 4.7). There was a linear increase ($P = 0.03$) in total daily urine output as dietary TS content increased. Total urinary N excretion expressed as grams per day and total urinary N excretion expressed as a percentage of N intake quadratically increased ($P = 0.02$) and tended to change in a quadratic manner ($P = 0.07$), respectively in cows fed the 6.5% TS diet compared to those fed the control diet, then decreased in cows fed 9.5 and 12.5% TS diets compared to those fed the 6.5% TS diet. Urinary urea-N excretion (UUE) expressed as grams per day quadratically increased ($P = 0.05$) from 160 for the control diet to 181 g/d for the 9.5% TS diet, followed by a decreased to 163 g/d for the 12.5% TS diet. However, dietary addition of DWP did not affect ($P > 0.05$) urinary urea-N excretion when expressed as a percentage of total urinary-N excretion. Daily fecal output cubically increased ($P = 0.05$) from 8.64 for the control diet to 9.01 kg for the 6.5% TS diet, then decreased to 8.01 kg for 9.5% TS, followed by a slight increased to 8.69 kg for the 12.5% TS diet. However, fecal N excretion expressed as kilograms per day or as a percentage of N intake were unaffected ($P > 0.05$) by diets. Total N excretion expressed as grams per day cubically increased ($P = 0.05$) in cows fed the 6.5% TS diet compared to those fed control diet, then decreased in cows fed 9.5 and 12.5% TS diets compared to those fed the 6.5% TS diet. There was no diet effect ($P > 0.05$) on total N excretion expressed as a percentage of N intake. Partial replacement of barley starch with DWP did not affect ($P > 0.05$) Milk N secretion when expressed as grams per day or as a percentage of N intake.

Table 4.7. The effects of partial replacement of barley starch with whey permeate on apparent nitrogen balance in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
N intake, g/d	710	723	715	688	31.8	0.37	0.21	0.48
Urinary Excretion								
Total, kg/d	27.8	33.6	33.0	33.2	2.17	0.03	0.08	0.56
Total N, g/d	223	255	238	223	7.1	0.67	0.02	0.19
Total N, % of N intake	31.9	35.4	33.5	32.2	1.25	0.58	0.07	0.29
Urea-N, g/d	160	177	181	163	8.0	0.84	0.05	0.68
Urea-N, % of urinary N	70.1	70.0	75.6	74.8	3.00	0.56	0.92	0.16
Fecal Excretion								
DM, kg/d	8.64	9.01	8.01	8.69	0.283	0.36	0.56	0.05
N, g/d	209	226	209	232	5.9	0.01	0.52	0.22
N, % of N intake	29.7	31.3	29.5	34.2	1.80	0.04	0.30	0.86
Total N Excretion								
g/d	434	482	446	453	8.3	0.04	0.03	0.05
% of N intake	61.7	66.7	62.9	66.3	2.77	0.11	0.71	0.50
Milk N								
g/d	165	165	171	163	6.9	0.50	0.33	0.39
% of N intake	23.2	22.7	24.2	23.8	0.74	0.93	0.91	0.20
Apparent N balance, g/d	111	77.6	94.3	72.2	23.43	0.16	0.77	0.89
Productive N[1], g/d	275	243	265	236	27.5	0.16	0.94	0.74

[1]Calculated as N secreted in the milk + N apparently retained by the cow

4.8. Apparent total-tract nutrient digestibility

Apparent total-tract digestibility of DM, OM, EE, ADF, and NDF were not affected ($P > 0.05$) by experimental diets (Table 4.8). There was a linear decrease ($P = 0.05$) in CP digestibility as dietary DWP content increased. Apparent total-tract digestibility of starch was cubically increased ($P = 0.04$) for 9.5% TS compared to the control and 6.5% TS, then decreased for 12.5% TS compared to the 9.5% TS diet. Apparent total-tract digestibility of WSC increased in a cubic manner ($P < 0.01$) when dietary TS content increased.

4.9. Whole-body urea kinetics

Urea-N loss to feces (UFE) and urea-N utilized for anabolic purposes (UUA) were not affected ($P > 0.05$) by partial replacement of barley starch with DWP (Table 4.9). Endogenous production of urea (UER) quadratically increased ($P = 0.02$) from 467 for the control diet to 531 g/d for 6.5% TS followed by a decreased to 472 g/d for the 12.5% TS diet. Urea-N recycled to the GIT (GER) tended to change quadratically ($P = 0.07$) as dietary content of DWP increased. Dietary inclusion of DWP as a partial replacement for barley starch tended to change ($P = 0.09$) urea-N returned to the ornithine cycle (ROC) in a quadratic manner. Fractions of UER eliminated in urine and recycled to the GIT were not affected by diets ($P > 0.05$). Fraction of GER excreted in feces tended to change quadratically ($P = 0.07$) in cows fed experimental diets. However, fractions of GER return to ornithine cycle and utilized for anabolism were similar across diets ($P > 0.05$). Partial substitution of barley starch with DWP did not affect ($P > 0.05$) ruminal microbial capturing of recycled N when expressed as grams per day; however, ruminal microbial capturing of recycled N when expressed as a percentage of total microbial NAN, tended to change in a cubic manner ($P = 0.10$) among diets.

Table 4.8. The effects of partial replacement of barley starch with whey permeate on apparent total-tract nutrient digestibility in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
Nutrient digestibility, %								
DM	65.4	64.9	68.2	64.6	1.98	0.41	0.30	0.19
OM	67.7	66.9	70.1	67.3	1.87	0.46	0.44	0.16
CP	70.3	68.7	70.5	65.8	1.80	0.05	0.29	0.87
Ether extract	73.3	71.3	75.9	73.0	3.64	0.74	0.90	0.44
ADF	43.4	42.0	42.9	36.6	5.06	0.33	0.60	0.85
NDF	45.5	45.1	47.0	41.3	3.96	0.38	0.46	0.93
Starch	92.7	92.7	94.3	93.4	0.66	0.82	0.36	0.04
WSC[1]	89.6	95.6	97.1	97.5	0.83	<0.01	0.01	0.01

[1]Water-soluble carbohydrates

Table 4.9. The effects of partial replacement of barley starch with whey permeate on urea-N recycling kinetics in cows (n = 4)

Variable	Experimental diets				SEM	*P* value		
	Control	6.5% sugar	9.5% sugar	12.5% sugar		Linear	Quadratic	Cubic
Urea-N kinetics[1], g/d								
UER	467	531	522	472	22.3	0.78	0.02	0.79
GER	307	354	340	309	20.7	0.84	0.07	0.65
ROC	261	295	295	271	17.0	0.65	0.09	0.88
UUE	160	177	181	163	8.0	0.84	0.05	0.68
UFE	11.1	11.5	11.4	12.4	0.76	0.24	0.66	0.80
UUA	34.6	47.6	33.2	25.0	7.36	0.67	0.18	0.15
Fractional Urea-N transfers								
UER to urine	0.341	0.335	0.350	0.354	0.0179	0.76	0.74	0.47
UER to GIT	0.659	0.665	0.650	0.646	0.0179	0.76	0.74	0.47
GER to ROC	0.850	0.833	0.873	0.886	0.0199	0.46	0.46	0.12
GER to feces	0.036	0.033	0.032	0.042	0.0028	0.20	0.07	0.84
GER to UUA	0.114	0.134	0.093	0.072	0.0205	0.39	0.34	0.12
Ruminal microbial capture of recycled N								
g N/d	81.3	69.5	84.5	75.0	5.76	0.15	0.82	0.11
% of total microbial NAN[2]	15.8	13.7	15.8	15.9	0.65	0.59	0.17	0.10

[1]UER = urea-N entry rate; GER = gastro-intestinal entry rate; ROC = return to ornithine cycle; UUE = urinary urea-N excretion; UFE = urea-N loss to feces; UUA = urea-N utilized for anabolism

[2]Non-NH_3-N

5. DISCUSSION

Adding sugars or sugar-containing byproducts to high-producing ruminant diets (mainly as a sweetener) has been an age-old practice. However, utilizing sugars to improve the palatability of feed has taken a new perspective in recent years as sugars have been viewed as a potential alternative energy source to partially replace cereal grains in the diet. In the recent past, numerous studies have explored the effects of partial substitution of cereal grain starch with sugars on production performance and ruminal fermentation characteristics of lactating cows (DeFrain et al., 2004; Broderick et al., 2008; Chibisa et al., 2015). The majority of these studies were conducted with corn-based lactating cow diets; however, barley is the major cereal grain used in western Canadian dairy diets and research on partial substitution of barley starch with sugars on dairy cattle performance is limited. The current study was an effort to fill this research gap of exploring the effects of partial substitution of barley starch with DWP up to 12.5% TS levels on production performance, ruminal fermentation characteristics, N utilization, and urea-N recycling in dairy cows.

Experimental diets that were used in the current study were formulated (CPM-Dairy, v 3.0.8) to have a CP concentration of 17.0% on a DM basis. However, chemical analysis showed that dietary TMR contained 17.2 to 17.4% CP, which differed slightly from the target CP of 17%. These minor deviations in dietary CP from the target CP could largely be attributed to variations in forage CP content. Total WSC concentrations for the control, 6.5, 9.5, and 12.5% TS diets were 3.64, 6.59, 9.59, and 12.62%, respectively, indicating only minor variations from the target dietary WSC values. As expected, dietary inclusion of DWP as a partial substitution for barley starch resulted in dietary starch contents that progressively decreased from 24.3% for the control diet to 22.2, 21.2, and 19.1% (on a DM basis) for the 6.5, 9.5, and 12.5% TS diets, respectively. Dietary content of NFC (mean = 36.7 ± 0.69%) was within the suggested range of 35 to 40% for sugar and cereal grain-based lactating diets that have been suggested as optimum in order to reduce the risk of SARA (Beauchemin and Penner, 2014). Slight decreases in NDF and ADF contents were observed as TS content increased across diets. Chibisa et al. (2015) also observed decreases in dietary NDF and ADF contents when they partially replaced barley or corn starch with lactose. Average NDF and ADF contents of barley grain are 20.8 and 7.2%, respectively (NRC, 2001), whereas DWP is free of structural fibers. Therefore, it is not

surprising to observe a decrease in NDF and ADF contents of diets when barley starch was partially substituted with DWP.

One of the major objectives of this study was to determine the effects of dietary inclusion of DWP on DMI, milk yield and milk composition of lactating cows. Partial substitution of barley starch with DWP up to 12.5% TS content did not affect DMI (mean = 26.7 kg/d). Ruminal NDF digestibility is one of the factors that partly influence DMI in dairy cows; increased ruminal NDF digestibility reduces rumen fill, thus increasing the DMI of the animal (Oba and Allen, 1999). However, in the present study, there was no difference in apparent ruminal NDF digestibility across diets and this could partly explain why DMI was similar in cows fed experimental diets. This is in agreement with the findings of Chibisa et al. (2015) who reported that DMI was unaffected when DWP was included (up to 8% TS) in barley- or corn-based diets. Dietary inclusion of pure lactose up to 15.7% in corn-based diets did not affect DMI in early post-partum cows (DeFrain et al., 2006). In contrast, dietary inclusion of sucrose (Broderick and Radloff, 2004; Broderick et al., 2008; Penner and Oba, 2009) or lactose (DeFrain et al., 2004) as a partial replacement for corn starch improved DMI in dairy cows. Moreover, partial substitution of barley starch with wheat factory sewage up to 9.3% TS quadratically altered DMI in dairy cows (Khorvash et al., 2014). Reasons for observed discrepancies in DMI when feeding sugars as a partial substitution for cereal grain starch are not clearly understood (Penner, 2015).

Daily milk yields (mean = 34.9 kg/d) were similar across all experimental diets. Dry matter intake is one of the most important factors that determine milk yield in lactating cows. Dry matter intake has a positive correlation with milk production (NRC, 2001); therefore, it was not surprising to observe similar milk yields in the present study as DMI was unaffected by the dietary inclusion of DWP. Ruminal and post-ruminal supply of nutrients can also influence milk production (Reynolds, 2006). In the present study, ruminal and apparent total-tract OM digestibility was not affected by the dietary inclusion of DWP, thereby suggesting that post-absorptive nutrient supply to support milk production might have been similar across diets. This could partly be another reason for similar milk yields across dietary treatments. In agreement with results of the present study, Chibisa et al. (2015) reported that milk yields were unaffected when barley starch was partially replaced with lactose. Moreover, dietary inclusion of wheat

factory sewage (a source of sucrose) up to 9.3% TS level as a partial replacement for barley starch did not affect milk yield in early lactating cows (Khorvash et al., 2014). Similarly, other studies reported no effects of dietary inclusion of sucrose (Ordway et al., 2002; Broderick et al., 2008; Penner et al., 2009b) or lactose (DeFrain et al., 2004) on milk yields in corn- or barley-based lactating cow diets. However, Broderick and Radloff (2004) observed quadratic changes in milk yield when dietary sucrose level was increased by adding liquid molasses as a partial replacement for corn starch up to 10% TS level. In that study, DMI was also quadratically changed by increased dietary TS content, so it is likely that the changes in milk yield with supplemental sucrose could partly be attributed to changes in DMI.

In the present study, milk fat content and yield were unaffected by increased TS content in diets. Similarly, dietary addition of sucrose or lactose did not affect milk fat content or fat yield in cows fed cereal grain-based diets (Ordway et al., 2002; DeFrain et al., 2004; Penner et al., 2009b; Khorvash et al., 2014; Chibisa et al., 2015). Contrary to these results, Broderick et al. (2008) reported a linear increase in milk fat content and fat yield when dietary sucrose content was increased up to 10% TS level in corn-based diets. Moreover, Broderick and Radloff (2004) also observed quadratic increase in milk fat yield peaking at 4.2% TS level, when liquid molasses partially substituted corn starch. Dietary inclusion of sucrose by partially replacing corn starch increased milk fat content when barley concentration was constant across diets (Khezri et al., 2009). Elevated concentrations of plasma BHBA can increase milk fat content in dairy cows (Kessel et al., 2008; Melendez et al., 2016). In a recent study that was conducted to explore the effects of partial replacement of corn starch with sucrose on transition cow performance, milk fat yield tended to increase and plasma BHBA concentration increased in cows fed high-sugar diets compared to those fed low-sugar diets (Penner and Oba, 2009). However, the cubic increase in plasma BHBA levels that was observed in the present study did not affect milk fat content. It should be noted that, besides plasma BHBA, ruminally-derived acetate and long-chain fatty acids arising from adipose tissue catabolism are also major precursors for milk fat synthesis in the mammary gland (Glascock et al., 1956; Bauman and Griinari, 2003; Månsson, 2008).

In the present study, dietary increase of DWP did not affect milk protein content or yield. In agreement with our findings, increased TS content in corn- or barley-based diets did not

change milk protein content or yield in lactating cows (DeFrain et al., 2004, 2006; Penner et al., 2009b; Khorvash et al., 2014; Chibisa et al., 2015). Amino acids to support milk protein synthesis are primarily derived from the post-ruminal digestion of microbial protein that is synthesized in the rumen. In the present study, total microbial NAN flow was quadratically changed by the dietary inclusion of DWP, but this had no influence on milk protein content or yield. It should be noted that AA that are derived from post-ruminal digestion of microbial protein can be directed towards other uses within the body (e.g., growth) other than milk protein synthesis, and this could partly explain why the changes in microbial NAN flow were not accompanied by similar changes in milk protein content or yield. Others (Broderick and Radloff, 2004) have reported that the partial substitution of corn starch with liquid molasses quadratically changed milk protein yield.

Milk lactose content quadratically increased from 4.40% for the control diet to 4.46% for the 9.5% TS diet with a slight decrease to 4.40% for the 12.5% TS diet; however, milk lactose yield was unaffected by adding DWP although changes in milk lactose yield followed numerical trends that were similar to those of milk lactose content. Chibisa et al. (2015) observed a tendency for a decrease in milk lactose content, without affecting lactose yield when they explored the effects of partial replacement of barley or corn starch with DWP up to 8% TS level. In other studies, the inclusion of sucrose in corn-based diets increased (Cherney et al., 2003) or decreased (Sannes et al., 2002) milk lactose content, so responses in milk lactose content or yield when dietary starch is placed with sugars have been inconsistent.

Plasma urea-N and milk urea-N are useful indicators of protein status of dairy cows (Hammond, 1997). Plasma urea-N is derived from urea that is synthesized in the liver as a consequence of NH_3 detoxification via the ornithine cycle. In ruminants, PUN is mostly derived from NH_3 absorbed into portal blood from the rumen (Reynolds and Huntington, 1988). In the present study, ruminal NH_3-N concentration was cubically changed by dietary treatments; however, PUN concentration was unaffected by dietary inclusion of DWP. It should be noted that NH_3 derived from microbial catabolism of AA in the hind gut and extra-hepatic tissue catabolism are also substrates for hepatic ureagenesis (Stewart and Smith, 2005). The concentration of MUN tended to increase cubically from 14.1 for control diet to 15.1 mg/dL for 6.5% TS, and then decreased to 14.0 mg/dL for 9.5% TS followed by a slight decrease to 13.7

mg/dL for the 12.5% TS diet. This observation is somewhat surprising as MUN is known to be highly correlated with PUN concentration (Roseler et al., 1993). Urea is an uncharged molecule; therefore, plasma urea rapidly equilibrates with body fluids including milk (Roseler et al., 1993; Broderick and Clayton, 1997). Plasma urea-N and MUN typically respond similarly to dietary manipulation. That was not the case in the present study, and the reasons for that are unclear. However, even though the observed responses in PUN and MUN concentrations were not congruent from a statistical standpoint, it should be noted that numerical trends were similar.

Another major objective of this study was to determine the effects of partial replacement of barley starch with DWP on the efficiency of N utilization in dairy cows. The extent of ruminal protein degradation has a major influence on the efficiency of dietary N utilization in dairy cows (NRC, 2001). Extensive ruminal degradation of dietary CP can elevate ruminal NH_3-N concentrations, particularly in dietary situations in which the supply of RFC is limiting. Because the absorption of ruminal NH_3-N into portal blood is a concentration-dependent process (Rémond et al., 1996), the excessive portal uptake of NH_3-N when ruminal NH_3-N concentrations are elevated can result in significant wastage of dietary N. Ruminal NH_3-N concentration, therefore, is often used as an indicator of ruminal N efficiency. In the present study, ruminal NH_3-N concentration increased cubically from 12.1 for the control diet to 12.3 mg/dL for the 6.5% TS diet, and then decreased to 10.9 mg/dL for the 9.5% TS diet followed by a slight decrease to 9.4 mg/dL for the 12.5% TS diet. The reason for increase in ruminal NH_3-N concentration for 6.5% TS is not clear; however, these observations tend to suggest an improvement in ruminal N efficiency when diets were formulated to have progressively greater TS levels. Increasing diet fermentability by adding lactose (Charbonneau et al., 2006; DeFrain et al., 2006; Chibisa et al., 2015) or sucrose (Broderick et al., 2008) decreased ruminal NH_3-N concentration in lactating cows fed corn-based diets. Increasing the rate of carbohydrate degradation (i.e., increasing the rate of energy supply to ruminal microbes) in the rumen reduces ruminal NH_3-N concentration by providing adequate energy for microbial sequestration of peptides, AA, and NH_3-N that are derived from dietary and endogenous N sources (Nocek and Russell, 1988; Russell et al., 1992). In addition, increasing the rate of ruminal energy availability enhances the growth of amylolytic microbes that utilize preformed AA and peptides as preferred N sources for protein synthesis; as a consequence, the greater microbial use of preformed peptides and AA would reduce their ruminal catabolism which, in turn, would reduce ruminal

NH_3-N concentration (Oh et al., 1999; Hristov et al., 2005). Also, RDP supply has a positive relationship with ruminal NH_3-N concentration (Devant et al., 2000). In the present study, RDP supply cubically increased (the reason is unclear) for the 6.5% TS diet compared to the control diet, then decreased for the 9.5% and 12.5% TS diets compared to the control diet, thus reflecting the changes observed in ruminal NH_3-N concentrations. According to NRC (2001) recommendations, dietary RDP supply should range from 9.5 to 10.5% (as a % of DMI) in order to provide adequate nitrogenous substrates (i.e., NH_3-N, AA, etc.) for microbial growth. In the present study, all experimental diets exceeded the 10.5% RDP, suggesting that diets were not deficient in RDP supply. In order to ensure that ruminal N availability does not limit microbial protein synthesis, Satter and Slyter (1974) suggested that ruminal NH_3-N concentration should be above a minimum threshold concentration of 5 mg/dL, In the present study, mean ruminal NH_3-N concentrations that were observed ranged from 9.40 to 12.3 mg/dL across dietary treatments, which would suggest that ruminal N availability did not limit microbial growth.

Together with ruminal NH_3-N concentration, another common measure of N efficiency in ruminants is microbial N flow to the duodenum. Microbial N is a major component of the metabolizable protein reaching the small intestine (NRC, 2001). Chamberlain et al. (1993) and Kim et al. (1999) observed a decrease in ruminal NH_3-N concentration and an increase in duodenal flow of microbial N when sugars were added to grass silage-based diets that were fed to lactating cows. In the present study, total microbial NAN flow quadratically increased in cows fed the 9.5% TS diet compared to those fed the control and 6.5% TS diets, followed by a decrease in cows fed the 12.5% TS diet compared to those fed the control diet; however, these changes did not follow the cubic changes that were observed in ruminal NH_3-N concentrations. Dietary inclusion of sugars as a partial substitution of cereal grain starch does not always improve microbial N flow to the duodenum. In support of this assertion, Chibisa et al. (2015) reported a decrease in ruminal NH_3-N concentration when dairy cows were fed DWP as a partial substitution for barley or corn starch; however, total microbial N flow was unaffected by dietary treatments (Chibisa, 2013). Similar results were obtained when Broderick et al. (2008) partially replaced corn starch with sucrose. Moreover, Sannes et al. (2002) reported a decrease in both ruminal NH_3-N concentration and microbial protein synthesis when 3% sucrose was added to corn-based lactating diets. In that study, mean ruminal NH_3-N concentration of diets that contained sucrose was 3.89 mg/dL, which was below the suggested minimum threshold

concentration of 5 mg/dL for optimum microbial synthesis (Satter and Slyter, 1974), and this could partly explain the reduction in microbial protein synthesis.

Microbial efficiency, expressed as grams of N per kilogram of OM truly digested in the rumen, was linearly decreased when dietary content of DWP increased. The mean microbial efficiency for all experimental diets was 40.9, which is greater than the mean value (33.1) that was reported by Chibisa (2013) in a study in which barley or corn starch were partially replaced with DWP up to 8% TS. Moreover, Broderick et al, (2008) reported a mean value of 31 for microbial efficiency when dietary TS content was increased up to 10% on DM basis. One of the major determinants of microbial efficiency is the retention time of DM in the rumen (Russell et al., 1992). Greater DM flows can reduce the retention time of microbes in the rumen, thus improving microbial efficiency by reducing their maintenance energy requirements (Russell et al., 1992). In comparison to studies of Chibisa (2013; 18.8 kg/d) and Broderick et al. (2008; 18.5 kg/d), mean omasal DM flow was numerically greater in the present study (19.9 kg/d) and could be a possible reason for the relatively higher microbial efficiency that was observed in the present study. In support of findings from the present study that suggest greater microbial efficiency as ruminal DM outflow increases, Chibisa and Mutsvangwa (2013) also reported a mean microbial efficiency of 44.2 when mean omasal DM flow was 23.1 kg/d.

The present study was also focused on determining the effects of partial substitution of barley starch with DWP on whole-body urea-N kinetics in dairy cows. Repartitioning of endogenous urea-N from urinary excretion to secretion into the GIT, particularly the rumen where urea-N can provide NH_3-N to support microbial growth, can improve N utilization. In the present study, endogenous production of urea (UER) quadratically increased when barley starch was partially replaced with DWP. A major factor that will influence endogenous urea production is the level of N intake. Numerous studies have demonstrated that there is a positive correlation between N intake and endogenous urea production (Harmeyer and Martens, 1980; Huntington, 1989; Reynolds et al., 1994; Lapierre et al., 2000; Marini and Van Amburgh, 2003). However, in the present study, N intake was not influenced by the addition of DWP as a source of supplemental sugar; therefore, factors other than N intake could be responsible for the differences in UER that we observed. In ruminants, ruminally-derived NH_3-N is the major contributor of N for the endogenous production of urea (Reynolds and Huntington, 1988), so

NH_3-N concentration in the rumen has a positive correlation with hepatic synthesis of urea. However, in the present study the quadratic changes in UER that were observed did not mirror the cubic changes that were observed in ruminal NH_3-N concentration. The reasons for this discrepancy are unclear but it is noteworthy that the numerical trends in changes in N intake and UER as dietary level of TS increased were similar. In ruminants, UER often exceeds apparently digestible N intake, therefore; all the endogenously-derived urea was lost in urine, the animal would be in a negative N balance (Lapierre and Lobley, 2001). In this situation, urea recycling to the GIT plays a vital role in providing adequate N to maintain a positive N balance in the animal. The GER represents the amount of endogenous urea-N output that enters the GIT and can, therefore, be potentially used as a source of N for microbial protein synthesis. Various studies have demonstrated a positive correlation between UER and GER (Archibeque et al., 2001b; Marini et al., 2004; Recktenwald et al., 2014) In the present study, GER tended to increase quadratically as dietary content of TS increased, reflecting the quadratic changes observed in UER. Level of dietary N and RFC, ruminal concentrations of NH_3-N and SCFA, and ruminal pH are some of the major factors that regulate transepithelial movement of urea-N from the bloodstream into the rumen (Kennedy, 1980; Huntington, 1989; Rémond et al., 1996; Marini and Van Amburgh, 2003; Simmons et al., 2009; Abdoun et al., 2010). Increasing the rate of carbohydrate fermentation in the rumen has been reported to stimulate urea secretion into the rumen (Kennedy, 1980; Kennedy and Milligan, 1980a; Huntington, 1989). In the present study, increasing the rate of ruminal carbohydrate fermentation by increasing the dietary level of DWP stimulated GER up to 9.5% TS in the diet, before GER decreased at 12.5% TS to levels similar to the control diet. The reason(s) for the decrease in GER at 12.5% TS are unclear, but could be related to changes in ruminal pH as dietary NSC content increased. Changes in ruminal pH in the presence of SCFA and/or CO_2 have been demonstrated to have an impact on urea transport across the rumen epithelium in a Ussing chamber model (Abdoun et al., 2010). Abdoun et al. (2010) varied mucosal pH in Ussing chambers from 5.4 to 7.4 in the presence of SCFA and observed a bell-shaped response in net urea flux, with peak urea flux attained at a mucosal pH of 6.2. Such a pH would be indicative of active fermentative processes in the rumen that would ensure that there is an actively-growing microbial population that could use recycled urea-N for microbial protein synthesis (Abdoun et al., 2010). In the present study, mean ruminal pH values for the control, 6.5, 9.5, and 12.5% TS diets were 6.32, 6.31, 6.34, and 6.22, respectively, which

indicates that the greatest urea flux into the GIT was attained at a ruminal pH of 6.31 to 6.34. These results might suggest that the optimum pH for maximal urea secretion from the bloodstream into the rumen might be different than that suggested based on in vitro studies. Also, these data need to be interpreted somewhat cautiously because ruminal pH measurements were based on spot sampling (rather than continuous pH measurements) and might not be fully representative of diet-induced changes in ruminal pH. It should be noted that, when GER was expressed as a proportion of UER (i.e., UER to GIT), there were no differences across diets. Dietary inclusion of DWP did not affect the amount of recycled urea-N that was utilized for anabolic processes (i.e., UUA, mean = 35.1 g/d). Microbial capture of NH_3-N derived from urea-N is the primary mechanism of utilizing recycled urea-N for anabolic processes (Lobley et al., 2000). In the present study, microbial capture of recycled urea-N (mean = 77.6 g/d) was similar across diets. It should be noted that microbial capture of recycled N was greater than UUA. In the model described by Lobley et al. (2000), UUA is calculated as the difference between urea-N recycled to the GIT and sum of urea-N return to ornithine cycle (ROC) and the fraction of recycled urea-N that is eliminated via feces (UFE; Lobley et al., 2000). However, the fraction of microbial AA that is not digested and absorbed by the animal, and the microbial AA that were absorbed and subsequently catabolized without being utilized by the host for protein deposition are not included in the calculation of UUA in that model. Therefore, this is likely to be the reason for the difference between microbial capture of recycled N and UUA (Wickersham et al., 2009a). However, ruminal microbial capture of recycled N when expressed as a percentage of total microbial NAN tended to change cubically (15.8, 13.7, 15.8, and 15.9% for the control, 6.5, 9.5, and 12.5% TS diets, respectively), thus suggesting that increased rate of energy supply with progressively greater TS levels could potentially improve microbial utilization of recycled N for microbial protein synthesis. In the present study, microbial capture of recycle N ranged from 13.7 to 15.9%. According to estimates in recently-published studies, the capture of recycled N as microbial NAN in dairy cows can vary within the range of 8.5 to 25% of total microbial NAN (Ouellet et al., 2002, 2010; Lapierre et al., 2008; Chibisa and Mutsvangwa, 2013).

Similar to DMI, partial replacement of barley starch with DWP did not affect N intake of cows in the present study; however, apparent ruminal N digestibility cubically increased as dietary TS content increased. Negative values in apparent ruminal N digestion as was observed in the present study have also been reported by others (Broderick et al., 2008; Chibisa and

Mutsvangwa, 2013), and this can be attributed to nitrogenous input into the rumen as recycled urea-N. In the present study, the excretion of urinary urea-N (UUE) increased quadratically as dietary TS content increased; however, UUE when expressed as a percentage of total urine N excretion (mean = 72.6%) was not affected by dietary treatment. In contrast, UUE was unaffected (mean = 73.9% of urine N) when corn or barley starch was partially substituted with lactose (Chibisa et al., 2015). However, dietary inclusion of sucrose as a partial substitution for corn-starch decreased UUE (Sannes et al., 2002; Broderick and Radloff, 2004; Broderick et al., 2008), suggesting that a greater proportion of UER was recycled to the GIT; it should be noted that urea recycling to the GIT was not measured in those previous studies. In the present study, I did not observe a significant change in the fraction of UER excreted in urine when TS content increased across diets. In terms of fecal N excretion, there was a linear increase as diet content of DWP increased, whereas others observed no changes when they partially replaced dietary starch with sucrose or lactose (Cherney et al., 2003; Broderick and Radloff, 2004; Chibisa et al., 2015). Even though the dietary addition of DWP altered urinary and fecal N excretion, total N excretion was unaffected by partial replacement of barley starch with DWP up to 12.5% TS level. Similarly, milk N and apparent N balance were also unaffected by diets, thus leaving productive N to be unchanged with increased dietary TS content in the present study.

Ruminal pH is one of the important physiological parameters that indicates ruminal activity (e.g., microbial fermentation, ruminal buffering, SCFA production and absorption, etc.). In the present study, a major objective was to determine the effects of partial substitution of barley starch with lactose on ruminal pH and severity of ruminal acidosis (RA) in dairy cows. In order to make definitive conclusions on the severity of RA, it is important that ruminal pH is measured continuously using indwelling pH probes such that the duration and area where minimum ruminal pH is below each threshold [i.e., mild RA = 5.8 > ruminal pH> 5.5, moderate RA = 5.5 > ruminal pH > 5.2, and acute RA =ruminal pH < 5.2; Penner et al., (2007)] can be calculated. Unfortunately, the malfunction of the pH measurement system in cows fed the 12.5% TS diet over three measurement periods rendered the continuous pH measurement data unusable due to inadequate replication. Therefore, only pH data obtained from spot ruminal fluid sampling via ruminal cannula are available, but these data are limited in their ability to assess the severity of RA as they only measure ruminal pH at one point in time (Penner et al., 2006). Moreover, pH is not homogenously distributed throughout the rumen (Aschenbach et al., 2011), such that spot

pH sampling is not always representative. However, despite these limitations, other studies have also used spot ruminal samples to measure ruminal pH (Broderick and Radloff, 2004; Broderick et al., 2008; Khorvash et al., 2014). In the present study, mean ruminal pH tended to change quadratically (6.32, 6.31, 6.34, and 6.22 for the control, 6.5, 9.5, and 12.5% TS diets, respectively) as dietary TS content increased. The reason(s) for the tendency for decrease in ruminal pH in cows fed 12.5% TS diet are not clear, but could be partly related to greater NSC content compared to other experimental diets. Increases in dietary NSC content can potentially increase the rate of SCFA production and, thereby, decrease ruminal pH. However, as mentioned elsewhere, these data should be interpreted somewhat cautiously as spot ruminal pH measurements are not always representative due to limitations already discussed. Khorvash et al. (2014) reported a linear increase in mean ruminal pH when they examined the effects of partial substitution of barley starch with wheat factory sewage up to 9.3% TS level. Moreover, Penner and Oba (2009) and Penner et al. (2009) reported tendencies for increased mean ruminal pH when sucrose was added to corn-based diets. Others have reported that mean ruminal pH was unaffected when dietary starch was partially substituted with sucrose (McCormick et al., 2001; Broderick and Radloff, 2004; Broderick et al., 2008) or lactose (DeFrain et al., 2004; Chibisa et al., 2015). Sugars are more rapidly degraded in the rumen when compared to starch (Amburgh et al., 2015), so the partial substitution of starch with sugars could potentially induce SARA due to a more rapid accumulation of SCFA; therefore, the lack of effect of supplemental sugars as a partial substitution for starch on RA as has been observed in previous studies is desirable from the standpoint of animal health.

Short-chain fatty acid absorption and its relationship with regulation of rumen pH has been well demonstrated (Gäbel et al., 1991; Dijkstra et al., 1993; Penner et al., 2009c; Chibisa et al., 2015). The contribution of Cl^--competitive SCFA absorption in reducing the risk of SARA in dairy cows fed lactose up to 8% TS as a partial replacement for starch in barley- or corn-based diets was recently demonstrated (Chibisa et al., 2015). In the present study, we also determined the effects of dietary inclusion of DWP up to 12.5% TS as a partial replacement for barley starch on SCFA absorption across the ruminal epithelium using the temporarily isolated and washed reticulo-rumen (WRR) technique. As described by Aschenbach et al. (2009), two experimental buffers that were different in chloride concentrations (i.e., a low Cl^- buffer and a high Cl^- buffer that contained 0 m*M* Cl^- and 49 m*M* Cl^-, respectively) were used to quantify the total SCFA

absorption and anion ($SCFA^-$, Cl^-, HCO_3^-) exchange system-dependent SCFA absorption. In the present study, total and Cl^--insensitive absorption of SCFA were not affected by increasing TS content in diets. Similar results in total and Cl^--insensitive absorption of SCFA were observed by Chibisa et al. (2015).

An increase in ruminal butyrate concentration with the dietary inclusion of sugars (especially when feeding lactose) as a partial replacement for cereal grain starch is well-documented in the literature (DeFrain et al., 2004, 2006; Chibisa et al., 2015), although other studies have reported that butyrate concentration was unaffected by the dietary addition of sugars (Broderick et al., 2008; Penner and Oba, 2009; Penner et al., 2009b). In the present study, the dietary inclusion of DWP up to 12.5% TS resulted in a cubic increase in ruminal butyrate concentration. Because butyrate is a potent modulator for cellular growth and differentiation (Sakata and Yajima, 1984; Feng et al., 1996), an increase in ruminal butyrate concentration is expected to increase ruminal epithelial absorptive surface area which, in turn, would increase SCFA absorption (Penner et al., 2011b). However, changes in ruminal butyrate concentration in the present study were not reflected in changes in total absorption rates (i.e., passive diffusion and transporter-mediated absorption) of SCFA. The present results showed that Cl^--competitive absorption of SCFA (i.e., HCO_3^- dependent absorption of SCFA) was largely unaffected by partial replacement of barley starch with DWP, although there was a tendency for a cubic decrease in propionate absorption. Contrary to our results, Chibisa et al. (2015) reported an increase in Cl^--competitive acetate and propionate absorption in cows fed DWP as a partial replacement for corn or barley starch up to 8% TS level; however, their results on butyrate absorption were similar to our findings. Butyrate is highly lipid soluble and may primarily depend on passive diffusion for its transport across the ruminal epithelium (Aschenbach et al., 2009). An increase in dietary sugar level was suggested to upregulate HCO_3^- dependent absorption of ionized acetate and propionate (Chibisa et al., 2015); however, our results do not support this suggestion. The reason(s) for this discrepancy is not clear; however, results should be cautiously interpreted as the variation is high (i.e., high standard error of means) in terms of Cl^--competitive absorption rates of SCFA within treatments. As suggested by Chibisa et al. (2015), upregulation of SCFA absorption across the ruminal epithelium is one of the mechanisms that could partly be involved in the regulation of ruminal pH in dairy cows fed diets that contain sugars as a partial replacement for cereal grain starch. However, a few limitations (i.e.,

unavailability of more representative pH measurements and large variation in the data on SCFA absorption rates within treatments) in the present study restricted the potential ability of making more definitive conclusions on the regulation of ruminal pH and its relationship to SCFA absorption across ruminal epithelium in cows fed increasing amounts of DWP as a partial replacement for barley starch.

As discussed elsewhere, ruminal concentration of butyrate cubically increased as dietary content of DWP increased. Butyrate is the primary energy source for ruminal epithelial cells (Bergman, 1990). Ruminal epithelial metabolism of butyrate produces BHBA which is subsequently absorbed into the blood circulation. Several studies have reported increases in both butyrate and plasma BHBA concentrations when corn starch was partially replaced with lactose (DeFrain et al., 2004, 2006). In the present study, dietary inclusion of DWP cubically altered plasma BHBA concentration. However, changes in ruminal butyrate concentration in the present study were not consistent with the observed changes in plasma BHBA concentration. Other than ruminal epithelial metabolism of butyrate, hepatic metabolism of both acetoacetate and fatty acids derived from adipose tissue catabolism can also contribute to plasma BHBA pools (Kristensen, 2005). It is noteworthy that plasma BHBA concentrations of cows in the present study were below 12.5 mg/dL, which has been suggested as the threshold concentration that is indicative of sub-clinical ketosis (Nielsen et al., 1994); therefore, it can be surmised that cows in the present study were not in a state of sub-clinical ketosis (Nielen et al., 1994). Penner and Oba (2009) detected an increase in plasma BHBA concentration without a change in ruminal butyrate concentration in transition cows fed sucrose, suggesting that ruminal butyrate and plasma BHBA concentrations do not always change simultaneously. Also, Chibisa et al. (2015) did not observe an increase in plasma BHBA levels with increased ruminal butyrate concentration when cows were fed lactose in corn- or barley-based diets.

In addition to the cubic changes observed in ruminal concentration of butyrate, there were no significant changes in acetate and propionate concentrations across diets. Further, total ruminal SCFA concentration was also unaffected by increased dietary sugar levels. The effects of feeding sugars on SCFA profiles are not consistent in the literature. Type of sugar or the type of feedstuff that is partially replaced by the particular sugar might affect SCFA profiles in the rumen (Oba, 2011). In agreement with our findings, dietary inclusion of lactose did not affect

acetate and propionate concentration in the rumen (Chibisa et al., 2015). However, dietary inclusion of lactose or sucrose as a partial replacement for corn starch reduced ruminal concentration of acetate in dairy cows (DeFrain et al., 2004; Broderick et al., 2008). Ultimately, the concentrations of SCFA in the rumen are a function of the relative rates of SCFA production, absorption, and passage from the rumen. As reviewed by Dijkstra et al. (2012), 20 to 40% of total SCFA produced in the rumen pass to the duodenum. Therefore, similar SCFA concentration across diets in the present study may be partly due to the balance between production, absorption, and passage of SCFA.

Apparent ruminal DM digestibility was linearly increased by dietary inclusion of DWP, thus suggesting an efficient microbial utilization of nutrients with increased rate of energy availability in the rumen. In contrast, Chibisa (2013) and Broderick et al. (2008) did not observe any effects of dietary inclusion of sugars on apparent DM digestion. The mean apparent ruminal DM digestibility in the present study was 21.5%, which was relatively lower when compared to values reported in other studies (Brito et al., 2006, 2009; Chibisa, 2013) that used the triple marker method (as in the present study) to estimate omasal DM flows. However, in agreement with the present results, Broderick et al. (2008) reported a mean apparent ruminal DM digestibility of 22.8% using the triple marker method to estimate omasal outflow. The intake, omasal flow and apparent digestion of OM reflected the results of ruminal DM digestion. Moreover, OM truly digested in the rumen was not affected by dietary addition of DWP.

Partial substitution of corn starch with lactose up to 10% TS resulted in a quadratic change in apparent ruminal NDF digestibility with a peak at 5.1% TS levels (Broderick et al., 2008). However, partial replacement of barley starch with DWP up to 12.5% TS level did not affect the apparent ruminal NDF digestibility in the present study. Similarly, Chibisa (2013) reported that apparent ruminal NDF digestibility was unaffected in response to the increase of DWP in diets. Similar to the apparent ruminal NDF digestibility, apparent total-tract NDF digestibility was not influenced by experimental diets. In agreement with our findings partial replacement of barley starch with sucrose or lactose did not affect apparent total-tract NDF digestibility in dairy cows (Penner and Oba, 2009; Chibisa et al., 2015). However, feeding early lactating cows with dried molasses as a partial substitution for corn starch linearly increased apparent total-tract NDF digestibility (Broderick and Radloff, 2004). In the present study,

apparent ruminal digestibility of ADF was not affected by dietary addition of DWP. Similar results were observed by Broderick et al. (2008) in cows fed sucrose (up to 10% TS level) as a partial replacement for corn starch. Apparent total-tract ADF digestibility was also unaffected by the manipulation of TS content in experimental diets. Dietary increase of TS content did not affect apparent total-tract digestibility of ADF in cows fed cereal-grain based diets (Chibisa et al., 2015), whereas a few studies have reported increased or quadratically changed apparent total-tract ADF digestibility when TS content was increased in corn- or barley-based diets (Broderick and Radloff, 2004; Khorvash et al., 2014).

Dietary intake of WSC cubically increased as dietary TS content increased. Similarly, both apparent ruminal digestibility and apparent total-tract digestibility of WSC were cubically increased by dietary inclusion of DWP. Water-soluble carbohydrates are mainly associated with the fluid phase of omasal digesta. In the present study, there was a tendency for a linear decrease in omasal fluid flow as dietary content of DWP increased. Therefore, a greater proportion of dietary sugars are expected to be digested in the rumen; however, results of the present study do not support this assertion. It is difficult to find published data from previous studies that have reported ruminal degradability of WSC. Recently, Sniffen and Tucker (2015) reported that only 50 to 70% of naturally-available dietary sugars are fermented by rumen microbes. Furthermore, 84% of sugars can be degraded in the rumen when dietary sugar content is increased by adding an external sugar source (Emanuele and Sniffen, 2016). Sugars and fructans are the major components of WSC (McDonald and Henderson, 1964). In contrast to pentose sugars (i.e., arabinose, xylose, ribose), hexose sugars (i.e., glucose, fructose, galactose) are highly fermentable in the rumen (Emanuele and Sniffen, 2016). During the ensiling process, hexose sugars are utilized by the fermenting bacteria, whereas pentose sugars that are more slowly degradable remain in silage (Emanuele and Sniffen, 2016). Our experimental diets contained 35% barley silage on a DM basis. As there were no added sugars in the control diet, barley silage and alfalfa hay were the major sources of WSC in that diet. Therefore, the 5-carbon sugars that are contained in barley silage could be the likely reason why ruminal digestion of WSC was depressed in the control diet when compared to diets that contained added sugar. In addition, bacteria and protozoa can store sugars and fructans as glycogen (Thomas, 2009), thus reducing the digestibility of WSC in the rumen.

6. GENERAL DISCUSSION

It is important to explore feasible feeding strategies to overcome potential negative effects of cereal grain-based lactating diets (i.e., SARA) and to reduce excess N losses to the environment. Dietary inclusion of sugars (mainly sucrose) as a partial substitution for dietary starch in corn-based diets have been demonstrated to have positive effects on production performance (i.e., DMI and milk yield) and N utilization efficiency in dairy cows without compromising the ruminal health (Broderick and Radloff, 2004; Broderick et al., 2008).

Effects of feeding sugars as a partial substitution for cereal grain starch on production performance in dairy cows are inconsistent in the literature. Type of sugars and their fermentation characteristics may partly attribute to this inability of sugars to elicit positive responses (Hall, 2010; Penner, 2015). The rate and extent of ruminal degradation of cereal grain that is replaced by sugars can also influence the ultimate outcome of feeding sugars in terms of DMI and milk yield. This could partly be a reason why I did not observe an improvement in DMI and milk yield with barley based-diets, when others were detecting positive responses with corn-based diets (Broderick and Radloff, 2004; DeFrain et al., 2004; Broderick et al., 2008). Given the fact that carbohydrates and proteins are interdependent for their utilization by ruminal microbes (Mutsvangwa, 2011), it can be suggested that the rate of ruminal degradability of dietary crude protein source might be a factor that potentially influence the impact of sugars on production performance in dairy cows; however, further research is needed to check the validity of this presumption. In general, it is recommended to keep the total ruminally-fermentable carbohydrate content constant when dietary TS content is increased as a partial replacement for cereal grain starch. Given that, it is questionable that the suggestion of increased productive outcomes in lactating cows with feeding sugars (as total energy availability to the host animal might not change with increasing dietary TS content) although the rate of ruminal fermentation of carbohydrate increases. On the other hand, microbial utilization of sugars as reserved carbohydrates such as glycogen has been reported in several studies. Although this is beneficial from the stand point of ruminal pH regulation, reduced microbial fermentation of sugars in the rumen may limit the production of SCFA (i.e., limit energy availability to the host animal) that can ultimately affect the production performance of the animal to a certain extent.

As discussed elsewhere, it is not reasonable to expect increased productive outcomes with keeping the total ruminally-fermentable carbohydrate concentration in the diet constant when sugars partially replace cereal grain starch. However, benefits of manipulating the rates of carbohydrate degradation in the rumen by adding sugars are likely related to altering the ruminal fermentation characteristics. One such example is shift in SCFA profiles in the rumen. Increase in ruminal concentration of butyrate is fairly consistent with feeding dairy diets that contain lactose. Although I did not observe significant effects, increases in plasma BHBA concentration with increased ruminal concentration of butyrate potentially increase milk fat content in dairy cows.

One of the major objectives of my study was to determine the effects of partial substitution of barley starch with DWP on ruminal fermentation characteristics and SCFA absorption across the ruminal epithelium. Increased ruminal concentration of butyrate was expected to enhance the growth of ruminal epithelium and subsequently to increase the SCFA absorption; however, I did not observe significant changes in total absorption rates of SCFA across the rumen epithelium. Bicarbonate dependent absorption of SCFA was also largely unaffected by increased dietary TS content. The results of the present study suggest the need of exploring other mechanisms that can influence SCFA absorption and regulation of ruminal pH (e.g., microbial utilization of sugars for glycogen synthesis, uptake of glucose by ruminal epithelial cells, salivary buffering, etc.) in cows fed sugars as a partial substitution for cereal-grain starch.

Due to some limitations, it was unable to make definitive conclusions about ruminal pH changes and severity of ruminal acidosis in cows fed DWP as a partial replacement for barley starch up to 12.5% TS levels. However, DMI, milk yield, and milk fat content were unaffected by increased dietary TS content, partly suggesting that animals were not affected by prolong periods of depressed ruminal pH below the thresholds defined for SARA.

In the present study, ruminal NH_3-N concentration decreased in cows fed 9.5% and 12.5% TS diets compared to those fed the control and 6.5% TS diets, thus suggesting an improvement in ruminal N utilization with progressively greater TS levels. Moreover, quadratic changes in bacterial NAN flows revealed a peak in cows fed 9.5% TS diets. Based on these results, it can be suggested that the 9.5% TS level might be the optimum for microbial protein

synthesis in cows fed DWP as a partial substitution for barley starch. However, this improvement in the efficiency of microbial N utilization was not mirrored by the production performance in cows fed experimental diets.

According to the literature, my study was the first attempt to explore the effects of feeding sugars as a partial replacement for dietary starch on urea-N recycling in dairy cows. Contrary to my expectations, the proportion of UER that was recycled to the GIT was unaffected by increased dietary TS content, although changes were observed in absolute urea-N transfers. However, total N excretion (expressed as a % of N intake) was not altered by experimental diets. Given the fact that excess N losses from livestock industry has become an environmental burden, results of my study suggest that the strategy of feeding DWP up to 12.5% TS levels would not be an additional pressure to the environmental pollution.

7. CONCLUSION

In general, I can summarize that partial replacement of barley starch with DWP up to 12.5% TS would not compromise the productive outcomes in dairy cows; therefore, DWP can be included up to 12.5% dietary TS in barley-based lactating diets. Partial replacement of barley starch with DWP up to 12.5% TS levels does not affect the production performance in dairy cows. Although, the proportion of endogenously derived urea-N that was recycled to the GIT was unaffected, dietary TS level of 9.5 and 12.5% seems to be beneficial for capturing recycled N as microbial protein. Dietary TS level of 9.5% potentially improve ruminal N efficiency by decreasing NH_3-N concentration and increasing omasal microbial N flow.

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