OPTIMIZING THE EFFICIENCY OF NUTRIENT UTILIZATION IN DAIRY COWS

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By

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ABSTRACT

A series of experiments were conducted to determine nutritional strategies to improve the efficiency of N utilization in dairy cows when feeding co-products including wheat-based (W-DDGS) and corn-wheat blend distillers grains with solubles (B-DDGS), and dried whey permeate (DWP). In Experiment 1, the objective was to determine the effects of replacing canola meal (CM) as the major protein source with W-DDGS on ruminal fermentation, microbial protein production, omasal nutrient flow, and animal performance. Cows were fed either a standard barley silage-based total mixed ration containing CM as the major protein supplement (0% W-DDGS, control) or diets formulated to contain 10, 15 and 20% W-DDGS (dry matter [DM] basis), with W-DDGS replacing primarily CM. Diets were isonitrogenous (18.9% crude protein [CP]). Inclusion of W-DDGS to the diet did not negatively affect ruminal fermentation, microbial protein production, and omasal nutrient flow. However, there was a 0.7- to 2.4-kg increase in DM intake, and a 1.2- to 1.8-kg increase in milk yield after the addition of W-DDGS in place of CM. In Experiment 2, the objective was to delineate the effects of including either W-DDGS or B-DDGS dried distillers grains with solubles as the major protein source in low or high CP diets fed to dairy cows on ruminal function, microbial protein synthesis, omasal nutrient flows, urea-N recycling, and milk production. The treatment factors were type of distillers co-product (W-DDGS vs. B-DDGS) and dietary CP content (15.2 vs. 17.3%; DM basis). The B-DDGS was produced from a mixture of 15% wheat and 85% corn grain. All diets were formulated to contain 10% W-DDGS or B-DDGS on a DM basis. Feeding up to 10% of dietary DM as B-DDGS or W-DDGS as the major source of protein did not have negative effects on metabolizable protein (MP) supply and milk production in dairy cows. However, reducing dietary CP content from 17.3 to 15.2% decreased milk production. This response was attributed to an insufficient supply of ruminally degradable protein (RDP) that suppressed microbial nonammonia N (NAN) synthesis in the rumen, thus decreasing intestinal MP supply. In Experiment 3, the objective was to determine the effects of replacing barley or corn starch with lactose (as DWP) in diets containing 10% W-DDGS on ruminal function, omasal nutrient flow, and lactation performance. The treatment factors were source of starch (barley vs. corn) and dietary inclusion level of DWP (0 vs. 6%; DM basis) as a partial replacement for starch. Diets were isonitrogenous (18% CP) and contained 3 or 8% total sugar. The starch content of the low

sugar diet was 24% compared to 20% for the high sugar diet. Dry matter intake, and milk and milk component yields did not differ with diet. However, partially replacing dietary corn or barley starch with sugar up-regulated ruminal acetate and propionate absorption, and reduced ruminal NH_3-N concentration, but had no effect on ruminal pH, microbial protein synthesis, omasal nutrient flow and production in dairy cows. In summary, data presented in this thesis indicate that W-DDGS and B-DDGS can be included as the major source of protein in dairy cow diets without compromising ruminal function, nutrient supply and milk production in dairy cows. Feeding medium to low CP diets, and partial replacement of starch with sugar in diets containing W-DDGS and B-DDGS can improve N utilization efficiency in dairy cows. Additionally, an upregulation of facilitated transport of acetate and propionate across epithelial cells possibly prevents the occurrence of ruminal acidosis when lactose partially replaces starch in cow diets.

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DEDICATION

To My Late Mom Marble Chibisa

For Teaching Me To Dream

&

To Aspire For Life's Olympus.

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LIST OF ABBREVIATIONS

$(^{15}NH_4)_2SO_4$	Ammonium sulfate
^{15}NB	Background ^{15}N abundance
$[^{15}N^{15}N]$-urea	Double-labeled urea
AA	Amino acid
ADF	Acid detergent fiber
AOAC	Association of Official Analytical Chemists
BCAA	Branched-chain AA
BCFA	Branched-chain FA
B-DDGS	Corn-wheat DDGS
BH	Biohydrogenation
BHBA	β-hydroxybutyrate
BW	Body weight
CLA	Conjugated linoleic acid
CP	Crude protein
CM	Canola meal
Cr-EDTA	Chromium- Ethylenediaminetetraacetic acid
DDGS	Dried distillers grains with solubles
DM	Dry matter
EAA	Essential AA
ECM	Energy corrected milk

FA	Fatty acid
FAB	Fluid-associated bacteria
FP	Fluid phase
GC	Gas chromatography
GER	GIT entry rate (amount of recycled urea-N entering the GIT)
GIT	Gastrointestinal tract
GLC	Gas liquid chromatography
INDF	Indigestible NDF
LP	Large particle phase
MFD	Milk fat depression
MP	Metabolizable protein
MPS	Microbial protein synthesis
MUFA	Monounsaturated FA
MUN	Milk urea-N
N	Nitrogen
NAN	Non-NH_3-N
NANBN	Non-NH_3 nonbacterial N
NDF	Neutral detergent fiber
NEAA	Nonessential AA
NE_L	Net energy of lactation
NH_3	Ammonia

NPN	Non-protein N
NSC	Nonstructural carbohydrate
n-3	Omega-3
n-6	Omega-6
OM	Organic matter
OMTDR	OM truly digested in the rumen
OTD	Omasal true digesta
PAB	Particle-associated bacteria
PF	Particulate phase
PUFA	Polyunsaturated fatty acid
PUN	Plasma urea-N
RDP	Ruminally degradable protein
RFC	Ruminally fermentable carbohydrate
ROC	Urea-N re-entering the ornithine cycle in the liver
RUP	Ruminally undegradable protein
SCFA	Short-chain fatty acid
SFA	Saturated FA
SP	Small particle phase
TMR	Total mixed ration
UER	Urea-N entry rate (total endogenous urea-N production)
UFE	Urea-N in feces

UT	Urea transporter(s)
UUA	Urea-N utilized for anabolism
UUE	Urine urea-N excretion
UUN	Urine urea-N
VFA	Volatile fatty acid
W-DDGS	Wheat-based DDGS
$YbCl_3$	Ytterbium chloride

1. GENERAL INTRODUCTION

As a result of a low efficiency in N utilization, dairy cows excrete over 70% of dietary N in urine and feces (VandeHaar and St-Pierre, 2006; Ryan et al., 2011). Urea-N is the major form of N that is excreted in urine and it readily undergoes microbial transformation in the environment. Following volatilization, denitrification and leaching of urea-N, there is production of compounds including NH_3-N, ammonium ions (NH_4^+), nitrous oxide (N_2O) and nitrates (NO_3^-) that contribute to atmospheric, ground and surface water pollution (Tamminga, 1992; VandeHaar and St-Pierre, 2006; Hristov, 2011a). This has led to increased public advocacy on the reduction of the harmful effects animal agriculture can have on ecological health. Therefore, dairy farmers are increasingly required to be accountable for environmental pollution associated with milk production, as governments implement various policies and regulations (Ryan et al., 2011; Higgs et al., 2012; Moraes et al., 2012). Although farmers in North America do not currently face direct costs associated with N loss, the implementation of taxes on N excretion on farms in some European countries including the Netherlands is a harbinger of future changes in environmental policy (Higgs et al., 2012). Therefore, there is urgent need for research to address the inefficient use of N, especially in the rumen, that leads to the excretion of dietary N as urinary urea-N (UUN; Calsamiglia et al., 2010).

The indiscriminate degradation of dietary protein by ruminal microbes contributes to the low efficiency in N use by dairy cows (Calsamiglia et al., 2010; Hristov et al., 2011a). Peptides, AA and NH_3-N that are produced from protein degradation are all used for microbial protein synthesis (MPS), a process that is highly dependent on the availability of fermentable energy (Russell et al., 1983; Hristov et al., 2005). Excess peptides and AA are degraded to NH_3-N, and the bulk of ruminal NH_3-N not sequestered into microbial protein is lost through absorption into portal circulation. Given that NH_3-N is neurotoxic, it is converted to urea-N in the liver (Marini and Van Amburgh, 2006; Hristov et al., 2011a). After its release into blood, a proportion of urea-N (BUN) is recycled to the gastrointestinal tract (GIT), whereas the remaining BUN is irreversibly lost as UUN. Therefore, manipulation of NH_3- and urea-N transactions across the portal drained viscera offers an opportunity to improve the efficiency of N usage in dairy cows (Calsamiglia et al., 2010). Feeding medium to low CP diets can reduce the detrimental loss of ruminal NH_3-N, and the subsequent synthesis and excretion of urea-N in urine (Marini and Van

Amburgh, 2006; Reynolds and Kristensen, 2008; Hristov et al., 2011a). Additionally, restricting N intake up-regulates urea-N recycling to the rumen. Besides compensating for the limited supply of dietary N to support MPS, this increase in urea-N transfer to the rumen also reduces urinary excretion of urea-N (Røjen et al., 2011b). Similarly, efficiency of N use in the rumen can be improved by increasing dietary fermentable carbohydrate (CHO) supply, as a result of enhanced capture of amino- and NH_3-N for MPS (Reynolds and Kristensen, 2008; Hristov et al., 2011a). The subsequent decrease in ruminal NH_3-N concentration as energy supply increases also reduces urea-N synthesis in the liver, and is favorable for transfer of a greater amount of urea-N to the rumen.

Besides the increasing need to integrate environmental costs as key determinants of production decisions, dairy farmers also face shrinking profit margins due to rising feed costs (Martel et al., 2011; Bradford and Mullins, 2012). Feeds account for up to 50% of total operational expenses of dairy farms, with protein being one of the most expensive components of dairy rations (VandeHaar and St-Pierre, 2006). Therefore, capture of only less than 30% of dietary N as milk protein has huge economic implications on profitability. This has resulted in farmers and nutritionists exploring the use of cheaper non-traditional ingredients in dairy diets, including co-products from the bioethanol and agricultural processing industries (Golombeski et al., 2006; Martel et al., 2011; Bradford and Mullins, 2012). In recent years, the dramatic expansion of the bioethanol industry in western Canada has led to large quantities of wheat-based (W-DDGS) and blend dried distillers grains with solubles (B-DDGS) being available for use as ruminant feed. Since these co-products are usually cheaper than commonly-used traditional protein supplements like canola meal (CM), their inclusion in cow diets could reduce feed costs. However, the nutritional characteristics and feeding value of W-DDGS and B-DDGS as protein supplements for lactating cows are yet to be fully established (Schingoethe et al., 2009; Abdelqader and Oba, 2012). Therefore, this information gap will curtail the ability of farmers to feed these widely available high-CP ingredients. Although it might be economically prudent to include W-DDGS and B-DDGS in cow diets, the bulk of N they supply is irreversibly lost as urine- and fecal-N (Schmit et al., 2009). Additionally, use of distillers grains could result in overfeeding of N and increase UUN excretion, especially when replacing high energy, low CP ingredients like barley grain and silage (Schmit et al., 2009; Gehman and Kononoff, 2010).

Therefore, there is need for research to ensure the judicious use of these co-products on dairy farms. Therefore, the aim of my thesis research was to determine strategies to improve the efficiency of N use in dairy cows when feeding the widely available and cost-effective W-DDGS and B-DDGS.

2. REVIEW OF LITERATURE

2.1 Sustainable Agriculture

Traditionally, the main goal of animal agriculture has been to maximize production of the various primary products, including milk, at the lowest possible cost (Huntington and Archiebeque, 1999; VandeHaar and St-Pierre, 2006). As a result of the emphasis on maximizing profitability, there was no incentive for dairy producers to feed diets that would reduce the environmental costs associated with dairy production. However, the turn of the 21st century has seen a paradigm shift towards sustainable animal agriculture, with environmental issues taking centre stage (Agle et al., 2010; Ryan et al., 2011). This shift is reflected by the emergence of policies and regulations geared towards reduction of environmental costs of dairy production, including excretion of N, which can be an air and water pollutant (VandeHaar and St-Pierre, 2006; Arriaga et al., 2009; Higgs et al., 2012).

Urea is the main form of N excreted by dairy cows. Under most dietary conditions, urea-N and NH_3-N make up 50 to 90% of total urinary N (Bristow et al., 1992). Urea-N in manure is broken down by microbial ureases found in faeces and soil, leading to the formation of volatile NH_3-N (VandeHaar and St-Pierre, 2006). In a study by Lee et al. (2011a) using ^{15}N-labeled urine or feces, 90% of NH_3-N emitted from mixed manure during the first 10 d of simulated storage was derived from urinary N. Consequently, animal agriculture is responsible for approximately 50% of atmospheric NH_3-N (VandeHaar and St-Pierre, 2006). Besides causing haze, atmospheric NH_3-N is also a key ingredient in the formation of acid rain and, thus, has a negative impact on ecosystem health (VandeHaar and St-Pierre, 2006). Atmospheric NH_3-N is also converted to small particle ammonium ions (NH_4^+) that are required for formation of particulate matter $\leq$ 2.5 µm in diameter ($PM_{2.5}$) that has been reported to compromise human health (Miller et al., 2007; Hristov, 2011b). Up to 20% of atmospheric $PM_{2.5}$ was estimated to be derived from NH_3-N released from livestock operations in parts of the United States (Hristov, 2011b). Some of the NH_3-N released under aerobic conditions common in topsoil undergoes nitrification, forming nitrates (NO_3^-; Tamminga, 1992). Leaching of NO_3^- into water bodies has a negative impact on ecological health, as it compromises drinking water quality and contributes to eutrophication. Microbial denitrification of nitrates, which occurs under anaerobic conditions, leads to emission

of volatile intermediates including nitrous oxide (N_2O) that contributes to global warming (Tamminga, 1992; VandeHaar and St-Pierre, 2006).

2.1.1 Nitrogen Utilization Efficiency in Dairy Cows

Efficiency of conversion of dietary N to milk protein by dairy cows is low, as over 70% of ingested N is excreted as faecal or urinary N (Castillo et al., 2000; Arriaga et al., 2009; Ryan et al., 2011). Capture of only less than 30% of dietary N for milk protein synthesis has huge economic implications, as protein is one of the most expensive components of dairy rations. Additionally, the irreversible loss of approximately 30 and 40% of dietary N in feces and urine, respectively, also has a detrimental effect on ecological health (VandeHaar and St-Pierre, 2006). The reasons for this low efficiency of N use are varied and range from the use of absorbed AA for hepatic and renal gluconeogenesis to AA transactions in the portal-drained viscera (PDV) and peripheral tissues that support protein turnover (Bergman and Heitmann, 1978). However, the indiscriminate breakdown of dietary protein and microbial AA to NH_3-N, which if not sequestered into microbial protein is lost from the rumen, is considered the major source of N utilization inefficiency in dairy cows (Tamminga, 1992).

The amount of dietary N that enters the ruminal NH_3-N pool varies depending on a number of factors, including the amount and fermentability of dietary CHO. According to Nolan (1975), 30 to 80% of dietary N is converted to NH_3-N. Besides dietary N, breakdown of microbial N also contributes to ruminal NH_3-N. Up to 50% of microbial protein synthesized in the rumen is degraded to NH_3-N, mainly due to predation of ruminal bacteria by protozoa (Leng and Nolan, 1984). Although this breakdown of bacterial N by protozoa provides AA-N and NH_3-N that could be recaptured for microbial growth, degradation of nucleic acids to various purine derivatives including xanthines represents an irreversible loss of N (McAllan, 1982). Endogenous N derived from the breakdown of BUN secreted across the ruminal wall, recycled salivary urea-N and sloughed off ruminal wall cells also add to the ruminal NH_3-N pool.

Ruminal NH_3-N concentration, which is the difference between production, and absorption and passage to the omasum, has been reported to range from 0.8 to 56 mg/dL (Satter and Roffler, 1974). Although a small proportion of ruminal NH_3-N is lost from the rumen due to

passage to post-ruminal sites in the fluid phase of digesta, ruminal disappearance of NH_3-N is mainly through sequestration into microbial protein or absorption across the ruminal wall into peripheral circulation (Hristov and Ropp, 2003). Therefore, the proportion of NH_3-N absorbed across the ruminal wall is dependent on the amount of NH_3-N in excess of requirements for microbial growth, as influenced by various factors including dietary N intake. Firkins and Reynolds (2005) estimated that as N intake increases, up to 42% of dietary N intake could be absorbed as NH_3-N across the PDV. As a result of its tissue toxicity, the absorbed NH_3-N is detoxified by hepatocytes to urea-N (Marini and Van Amburgh, 2006). Following synthesis, urea-N enters peripheral circulation (blood urea-N; BUN) and, given its small molecular size and solubility, it equilibrates in body tissues and fluids including milk (milk urea-N; MUN). A proportion of BUN is recycled to all parts of the GIT, including the rumen. Recycling of urea-N to the rumen is desirable because it is broken down by bacterial urease to NH_3-N that can potentially be sequestered into microbial protein (Marini and Van Amburgh, 2006). However, the BUN fraction not recycled to the GIT is excreted in urine (urine urea-N; UUN), and this represents an irreversible loss of dietary N and, thus, contributes to the low efficiency of N use.

2.2 Meeting Amino Acid Requirements of Dairy Cows

Dairy cows require amino acids (AA) for growth, maintenance and production functions. Traditionally, CP ($N \times 6.25$) has been the standard used for determination of protein quality of feeds, specification of animal AA requirements, and formulation of diets to meet those requirements (Satter and Roffler, 1975). However, better understanding of gastrointestinal (GIT) metabolism of dietary CP has led to its further classification as ruminally degradable- (RDP) and -undegradable protein (RUP; Bach et al., 2005). Ruminally degradable protein consists of true protein and nonprotein N (NPN).

The indiscriminate degradation of true protein by ruminal microbes yields peptides and AA, which can be directly sequestered into microbial protein (Bach et al., 2005). In addition, some of the AA derived from true protein are deaminated to produce NH_3-N that can be used for microbial growth. Dietary NPN in the form of AA, peptides and NH_3-N also contributes N and carbon (C) for MPS (Bach et al., 2005). However, a fraction of dietary CP is resistant to ruminal degradation (RUP), but can potentially be digestible in post-ruminal sites. As a result of constant

rumen turnover, the passage and subsequent breakdown of the microbial protein and digestible RUP in the abomasum and small intestine avails AA for absorption and, ultimately, utilization by the dairy cow. Endogenous protein mainly from the released digestive secretions including saliva, mucous and enzymes, and sloughed-off GIT cells also provides AA for absorption. Therefore, microbial protein, digestible RUP, and endogenous protein are the three constituents of metabolizable protein (MP) that supply AA to meet dairy cow requirements (Clark et al., 1992). Of these constituents, microbial protein is the most important, both quantitatively and qualitatively.

2.2.1 Breakdown of Dietary Protein in the Rumen

Ruminal bacteria (e.g., *Prevotella ruminicola* and *Butyrivibrio fibrisolvens*) and ciliated protozoa (e.g., *Entodinium caudatum*) are responsible for the breakdown of the bulk of dietary protein in the rumen. However, there is evidence indicating limited contribution of anaerobic fungi (e.g., *Neocallimastix frontalis*) to proteolysis (Wallace, 1996). Degradation is preceded by the attachment of ruminal bacteria to feed particles, and up to 75% of proteolytic activity has been shown to occur in the particulate compared to the fluid fraction of ruminal digesta (Brock et al., 1982). This attachment brings the ruminal bacteria in close proximity with the substrate, and is followed by release of different cell-associated and extra-cellular proteases including dipeptyl aminopeptidases and aminopeptidases which work in concert to degrade dietary protein to peptides and AA (Brock et al., 1982; Wallace, 1996). Peptides and AA are subsequently translocated into bacterial cells, where peptides are further broken down to AA by peptidases. The resultant intracellular AA are either incorporated into microbial protein if energy is available, or are deaminated, forming VFA, CO_2, CH_4 and NH_3-N if energy is limiting (Tamminga, 1979). Some proteolytic bacterial species including *Streptococcus bovis* can utilize peptides, AA and NH_3-N as N sources for protein synthesis, whereas fibrolytic species like *Fibrobacter succinogenes* have a preference for NH_3-N (Russell et al., 1992). Protozoa are also involved in protein degradation, as they engulf dietary insoluble protein molecules which they enzymatically degrade to peptides, AA and NH_3-N (Tamminga, 1979; Jouany, 1996; Belanche et al., 2012). A fraction of the resultant AA and NH_3-N is incorporated into protozoal protein,

whereas excess AA and NH_3-N are excreted, and are potentially available for bacterial protein synthesis in the rumen (Belanche et al., 2012).

2.2.2 Microbial Protein Production in the Rumen

Optimal N metabolism in the rumen is achieved when the bulk of dietary RDP is captured for, and maximizes the synthesis of microbial protein (NRC, 2001; Bach et al., 2005). Quantitatively, microbial protein is important as it is estimated to contribute on average 59% (range of 34 to 89%) of duodenal nonammonia nitrogen (NAN) flow (Clark et al., 1992). Microbial protein is also qualitatively superior compared to commonly-used high quality protein supplements like canola meal (CM) as its AA composition closely matches that of milk, especially for Lys and Met, which are considered the most limiting AA for milk production (O'Connor et al., 1993; NRC, 2001). Additionally, 80% of microbial protein is digestible such that the bulk of microbial AA is available for absorption in the small intestine (NRC, 2001). Therefore, optimizing ruminal N metabolism is key to maximizing the supply of AA to the small intestine and limiting N losses from the rumen as NH_3-N and, ultimately, as urea-N in urine.

2.2.3 Factors That Influence Microbial Protein Synthesis

Maximizing MPS is key to maximizing productivity, as duodenal microbial protein flow has been estimated to account for up to 65% of the variation in milk yield (Schwab and Ordway, 2004). Several factors including ruminal retention time and pH are known to have an impact on MPS (Stern et al., 1979; Bach et al., 2005). However, this literature review will focus on how ruminal fermentable protein and energy supply, and synchrony in RDP and fermentable energy availability affects MPS, as these factors are related to my thesis research.

2.2.3.1 Ruminally Degradable Protein Supply

The breakdown of RDP yields peptides, AA and NH_3-N, which are all N sources for MPS. Based on NRC (2001) recommendations, dietary RDP supply should be at least 9.5 to 10.5% of dietary DM to prevent a potential depression in MPS stemming from peptide, AA and/or NH_3-N deficiencies in the rumen (Broderick et al., 2007). Of these 3 N sources for MPS, NH_3-N has been studied the most, given the relative ease with which it can be quantified compared to peptides and AA (Firkins et al., 2007). Using $^{15}NH_3$, Leng and Nolan (1984)

estimated that 50 to 80% of bacterial N is derived from the ruminal NH_3-N pool, whose source is either dietary RDP or endogenous N, including recycled urea-N from blood or saliva. In a study to determine the effect of CHO source (dextrose, starch, NDF and CHO mix) on NH_3-N utilization in lactating dairy cows, 38 to 60% of bacterial N was derived from the ruminal NH_3-N pool (Hristov et al., 2005). As a result of this documented importance of ruminal NH_3-N for MPS, there has been a considerable amount of research to determine the ruminal NH_3-N concentration that would not only maximize its capture into microbial protein, but would also minimize its loss from the rumen.

It has been suggested that the optimum ruminal NH_3-N concentration for maximum MPS is 5 mg/dL (Satter and Slyter, 1974; Russell and Strobel, 1987). This was based on in vitro observations that when NH_3-N concentration was less than 5 mg/dL, MPS was suppressed, whereas NH_3-N in excess of 5 mg/dL did not lead to an increase in microbial N in mixed culture. In a study with pure cultures of ruminal bacteria, only 1.4 mg/dL of NH_3-N was sufficient to maximize MPS (Schaefer et al., 1980). This difference potentially reflects differences in NH_3-N requirements and utilization by different bacterial species that form the ruminal consortium (Russell et al., 1992; Reynal and Broderick, 2005). Discrepancies in the amount of NH_3-N required to support maximum MPS also exist between in vitro and vivo studies (Olde and Schaefer, 1987; Reynal and Broderick, 2005). Generally, the maximum amount required for MPS has been observed to be higher in in vivo compared to in vitro studies. In a study by Kang-Menarich and Broderick (1980), a ruminal NH_3-N concentration of 8.5 mg/dL was required to maximize bacterial growth in the rumen when nonlactating cows were fed a basal diet of corn and cottonseed hulls supplemented with an increasing amount of urea. After adjusting dietary RDP content (10.6, 11.7, 12.3 and 13.2% of DM) when feeding corn/alfalfa silage based diets by including varying amounts of solvent- and lignosulfonate-treated soybean meal and urea, Reynal and Broderick (2005) suggested that a minimum ruminal NH_3-N concentration of 11.8 mg/dL was required to maximize MPS. The differences between in vivo and in vitro studies have been attributed to the presence of particulate matter in the rumen that provides favourable nutrient or substrate milieu conditions for faster NH_3-N-fuelled bacterial growth rates, which is lacking in media used in vitro (Olde and Schaefer, 1987). The concentration of NH_3-N required for maximum MPS might also be positively correlated with diet fermentability, as it influences

bacterial attachment and nutrient milieu conditions (Olde and Schaefer, 1987; Erdman et al., 1986). In the study by Olde and Schaefer (1987), a higher NH_3-N concentration (12.5 vs. 6.1 mg/dL) was required to support a faster in situ fractional degradation rate of barley compared to corn grain (0.036 vs. 0.024/h) when feeding steers barley-based diets. Erdman et al. (1986) infused incremental amounts of urea into the rumen of cows fed ground corn and soybean hulls and measured DM digestion of various feedstuffs. Up to 50% of the variation in the NH_3-N required for maximum effective ruminal DM degradation of the feedstuffs resulted from differences in their fermentability (Erdman et al., 1986).

The use of ruminal NH_3-N as the sole indicator of RDP sufficiency is inaccurate due to its failure to fully account for amino-N contribution (Firkins et al., 2007). Based on estimates by Leng and Nolan (1984), amino-N can contribute 20 to 50% of bacterial N, and several studies (Maeng and Baldwin, 1976; Maeng et al., 1976; Van Kessel and Russell, 1996) have shown the stimulatory effect amino-N has on MPS. Addition of a mixture of 18 AA into the fermentation media containing NH_3-N as a N source and glucose or cellobiose as the energy substrates led to a 46 and 67% increase in microbial yield, respectively (Maeng et al., 1976). Isonitrogenous replacement of urea with an AA mixture also led to a 26% increase in microbial growth (19.3 vs. 24.4 mg of microbial cells per 100 mg of glucose; Maeng and Baldwin, 1976). This stimulatory effect can be partially explained by the presence of amylolytic bacteria in the rumen that are known to obtain up to 66% of their N needs for growth from amino-N (Russell et al., 1983). It could also result from the deamination of AA that would increase the availability of carbon skeletons for synthesis of microbial protein and/or use as an energy source (Bryant, 1973). Although it was widely believed that cellulolytic bacteria could not use peptides and AA for MPS (Russell et al., 1992), there is evidence to the contrary (Atasoglu et al., 2001). Using pure cultures of *Fibrobacter succinogenes*, *Ruminococcus flavifaciens* 17 and *Ruminococcus albus*SY3, Atosoglu et al. (2001) showed that both peptides and AA can stimulate, and are incorporated into microbial protein by cellulolytic bacteria. Therefore, amino-N is essential for optimal growth of both amylolytic and cellulolytic bacteria.

2.2.3.2 Ruminal Fermentable Energy Supply

Ruminal energy supply, determined by the amount and fermentability of dietary CHO, is a major factor that regulates MPS (Rohr, 1986; Agle et al., 2010), as it determines the fate of AA produced during ruminal proteolysis (Russell et al., 1983; Hristov et al., 2005). When fermentable CHO is deficient, rather than sequestration into microbial protein, the bulk of AA are deaminated to NH_3-N. However, an increase in fermentable CHO supply causes a decrease in NH_3-N production by ruminal microbes as a result of diversion and direct incorporation of amino-N into microbial protein (Russell et al., 1983; Hristov et al., 2005). Additionally, an adequate supply of fermentable CHO also enhances capture of NH_3-N for MPS given the positive correlation that exists between energy supply and microbial NH_3-N capture (Oba and Allen, 2003; Hristov et al., 2005). Increasing the amount of OM truly digested in the rumen from 7.7 to 11.3 kg/d (21 vs. 32% starch on a DM basis, respectively) when feeding isonitrogenous diets (18% CP on a DM basis) to lactating dairy cows led to a decrease in ruminal NH_3-N concentration and a 31% increase in microbial N flow at the duodenum (Oba and Allen, 2003). The type of CHO also has an impact on MPS in the rumen (Stern and Hoover, 1979). The more rapidly fermentable sugar and starch promote microbial growth to a greater extent compared to slowly-fermentable, ruminally-available fiber.

2.2.3.3 Synchrony in Ruminally Degradable Protein and Fermentable Energy Supply

An optimal rate of MPS can be achieved if the dietary supply of both protein and CHO is quantitatively adequate. However, besides amount, the rate and extent of ruminal degradation is a key determinant of nutrient availability for MPS (Nocek and Russell, 1988). Therefore, the rate and extent of ruminal protein and CHO also have to be synchronized to ensure maximum growth rates of ruminal microbes, and improved N use efficiency. A faster rate and/or greater extent of ruminal protein degradation relative to CHO degradation leads to an increase in ruminal NH_3-N concentration as a result of limited energy to drive the incorporation of RDP-derived AA into microbial protein. Conversely, limited amino- and NH_3-N supply due to a slower rate and/or limited extent of dietary protein degradation compared to CHO degradation in the rumen also compromises microbial growth (Nocek and Russell, 1988). Although it is reasonable to expect synchrony in ruminal protein and energy supply to maximize MPS and reduce the irreversible

loss of NH_3-N from the rumen, achieving that optimal balance has proven to be difficult due to a myriad of factors (Bach et al., 2005; Hall and Huntington, 2008; Cole and Todd, 2008). These include analytical challenges that hamper the precise and accurate determination or estimation of ruminal protein and CHO degradation of different feedstuffs that are influenced by various factors including ruminal pH and residence time. Additionally, physiological factors including the contribution of recycled urea-N to the ruminal NH_3-N pool, and the presence of ruminal microflora made up of different bacterial, protozoal and fungal species with different nutrient requirements, growth rates, rumen residence times and pH tolerance, result in synchrony being unattainable (Hall and Huntington, 2008; Cole and Todd, 2008).

2.2.4 Ruminally Undegradable Protein

Although microbial protein is of high quality, it cannot quantitatively meet the AA requirements of high producing dairy cows (Schwab et al., 1992; Santos et al., 1998). Therefore, there is need for RUP sources including dried distillers grains with solubles (DDGS) in diets for lactating cows. However, dietary RUP is only useful if its inclusion in the diet does not lead to a decrease in RDP supply that would compromise microbial growth in the rumen (Schwab et al., 1992; Santos et al., 1998; Ipharraguerre and Clark, 2005). Additionally, RUP should be digestible in the abomasum and small intestine. Its AA content should also complement microbial AA, especially for Lys and Met that are considered to be the most limiting AA for milk and milk protein synthesis under most feeding conditions in North America.

2.2.5 Endogenous Protein

Reentry of previously digested N into the GIT mainly as protein or urea-N is important in dairy cows as it contributes AA to the metabolizable pool (Tamminga et al., 1995). Endogenous N in the form of proteins contained in salivary, gastric, pancreatic and intestinal secretions, mucus and sloughed cells, and is available either as “free” endogenous protein or is incorporated into microbial protein in the rumen was shown to account for 24% of duodenal N flow (Ouellet et al., 2010). After addition of the fraction of recycled urea-N that was sequestered into microbial protein, endogenous N made up 34% of duodenal N flow. Although a number of dietary factors

including fermentability and abrasiveness determine duodenal endogenous N flow, it still makes a significant contribution to metabolizable AA supply for dairy cows (Ouellet et al., 2010).

2.3 Reducing the Environmental Cost of Dairy Production

Protein is overfed on most dairy farms in North America, with typical diets for high producing dairy cows containing an average of 18% CP on a DM basis (Huhtanen and Hristov, 2009; Pacheco et al., 2012). Huhtanen and Hristov (2009) attributed this to the belief that there is a strong linear relationship between dietary CP concentration and milk production. There is also widespread use of "safety margins" when formulating dairy rations as producers and nutritionists would rather feed high CP diets to ensure an adequate supply of metabolizable AA, as the consequences of underfeeding CP including its negative impact on milk and milk protein yields, are more economically costly (VandeHaar and St-Pierre, 2006; Pacheco et al., 2012).

There is increasing evidence (Broderick, 2003; Leonardi et al., 2003; Olmos Colmenero and Broderick, 2006b) that there is no economic merit in overfeeding protein, as diets containing 16.1 to 16. 7% CP have been shown to be adequate for early- to mid-lactation cows under specific dietary conditions. In studies by Leonardi et al. (2003), Broderick et al. (2003) and Broderick and Olmos Colmonero (2006b) increasing dietary CP from 16.1 to 18.8%, 16.7 to 18.4% and 16.5% to 17.9 and 19.4%, respectively, by replacing corn grain with soybean meal in corn/alfalfa silage based diets did not lead to a significant increase in both milk and milk protein yields. Additionally, results from meta-analyses by Ipharrauguerre and Clark (2005) and Huhtanen and Hristov (2009) clearly show diminishing returns in milk yield, and a decrease in efficiency of conversion of dietary N into milk protein at higher compared to lower dietary CP concentrations. Recently, using models to predict the impact of dietary CP (11.6, 15.5 and 19.4%) on lactation curves, Caccamo et al. (2012) showed that increasing dietary CP from 11.6 to 19.4% led to a significant increase mainly in peak milk production, possibly due to the inadequate energy supply during this period when DMI lags behind milk production. This supports results from earlier studies by Wu and Satter (2000) and Law et al. (2009), indicating that there is an opportunity to lower feed costs by feeding medium CP diets after early lactation without potentially compromising productivity. Additionally, the medium CP diets (16.1 to 16.7% CP) in studies by Leonardi et al. (2003), Broderick et al. (2003) and Olmos Colmonero

and Broderick (2006b) also caused a decrease in ruminal NH_3-N and MUN concentrations and, more importantly, a reduction in urinary N and urea-N excretion when compared to the high CP diets (18.4 to 19.4%). This decrease in urinary urea-N excretion is desirable, as it leads to a decrease in NH_3-N emission from manure (Agle et al., 2010; Powell et al., 2011).

Despite the documented improvement in N efficiency and reduction in N loss, feeding low to medium CP diets can also potentially compromise MP supply, and ultimately cow productivity. Decreasing CP content in diets fed to early and mid-lactation cows from 15.7 to 14.3% led to a decrease in milk, 4% FCM, and milk protein yields when feeding corn-silage based diets (Cabrita et al., 2011). Besides causing a decrease in ruminal NH_3-N, BUN and MUN concentration, feeding corn/alfalfa silage based diets containing 14.8% compared to 16.7% CP of dietary DM to mid-lactation cows also led to a 37 and 55% decrease in urinary excretion of N and urea-N, respectively (Lee et al., 2011b). However, the inadequate supply of MP on the 14.8% compared to 16.7% CP diet (-156 g/d vs. +44 g/d MP balance) also led to a 8% decrease in milk yield (Lee et al., 2011b). Therefore, finding the delicate balance between an improvement in dietary N conversion into milk, and a reduction in N loss to the environment when feeding low CP diets is a key goal in dairy research today.

Increasing metabolizable AA supply to maintain milk and milk protein production when feeding low CP diets by inclusion of RUP supplements and/or rumen-protected AA has been a strategy used to try and achieve this balance (Davidson et al., 2003; Lee et al., 2012). Compared to a high CP, moderate RUP diet (HPMU; 19.4% CP, 40% of CP as RUP), feeding a low CP, high RUP diet (LPHU; 16.8% CP and 46% of CP as RUP) to dairy cows during early lactation decreased ruminal NH_3-N, PUN and MUN concentrations without compromising milk and milk protein yields (Davidson et al., 2003). Moreover, the LPHU diet also led to a 34% decrease in urinary N excretion. In a study with early lactation cows, feeding a low CP diet (13.6%) that contained 87% of MP requirements (-317 g/d of MP balance; NRC, 2001) compared to a MP-adequate diet (15.7% CP and +9 g/d MP balance) led to a 36 and 47% reduction in urinary N and urea-N excretion, respectively (Lee et al., 2012). The MP-deficient diet also resulted in a 9 and 11% decrease in milk and milk protein yields, respectively. However, supplementing rumen protected Lys and Met to the MP-deficient diet improved milk and milk protein yields, whereas

addition of rumen-protected His together with Lys and Met led to a 100% recovery in milk and milk protein yields. In addition, the reduction in urinary N and urea-N excretion on the MP-deficient diet was maintained following supplementation with the rumen protected Lys, Met and His (Lee et al., 2012). Therefore, balancing for Lys, Met and His, which are the major AA limiting milk production in dairy cows in North America, in MP-deficient diets also offers an opportunity to increase efficiency of dietary N conversion into milk N, and reduce N loss.

Our ability to formulate diets that will increase the efficiency of N use in dairy cows, and ultimately reduce loss of N to the environment, is dependent on accuracy and precision of predicted flow of MP or individual AA to the duodenum. However, there is increasing concern about the shortcomings of commercially available dairy ration formulation programs including the National Research Council 2001 (NRC) models (Huhtanen and Hristov, 2009; Pacheco et al., 2012). For example, in a current study, Pacheco et al. (2012) evaluated AminoCow (AC), Agricultural Modeling and Training Systems (AMTS), Cornell-Penn-Miner (CPM) and NRC models, and was able to show the limited accuracy and precision of the 4 models when simultaneously estimating duodenal RUP and EAA flow. In the NRC (2001), which is one of the widely used models to formulate lactating cow diets in North America, MP supply is predicted based on dietary RDP and RUP. Depending on various factors including production level, the NRC (2001) recommends that 9.5 to 10.5% of dietary DM should be RDP so as to maximize MPS. However, there is evidence showing that the NRC (2001) potentially over-predicts RDP requirements and, thus, contributes to the over-feeding of protein to dairy cows (Cyriac et al., 2008; Agle et al., 2010). In the study by Agle et al. (2010), feeding a RDP-deficient diet (7.1% RDP as % of diet DM; 12.9% CP) compared to RDP-adequate diet (10.3% RDP as % of diet DM; 15.4% CP) to mid-lactation cows did not reduce milk and milk protein yields, and also increased N efficiency from 22.3 to 28.2%. Although ruminal NH_3-N was lower on the RDP-deficient diet, there were no differences in the amount of ruminal NH_3-N used for MPS (estimated using ^{15}N). However, there was a 26% decrease in the irreversible loss of ruminal NH_3-N that contributed to a 39% reduction in urinary N excretion on the 7.1% compared to the 10.3% RDP diet. Consequently, cumulative NH_3-N loss from manure under controlled laboratory conditions was shown to be lower on the 7.1% than the 10.3% diet (Agle et al., 2010). Similarly, feeding low CP diets (15.9 vs. 16.8%) did not compromise milk and milk protein yields,

increased N efficiency (33.5 vs. 27.7%) and reduced urinary N output by 24% (Cyriac et al., 2008).

One of the key reasons why the NRC (2001) over-predicts RDP requirements for optimal ruminal microbial growth is because the model does not account for the contribution of recycled urea-N to the ruminal NH_3-N pool available for MPS (Huhtanen and Hristov, 2009). Recycling of urea-N to the GIT in lactating dairy cows can potentially buffer the rumen from low NH_3-N concentration when diets are deficient in RDP (Huhtanen and Hristov, 2009). Additionally, sequestration of recycled urea-N into microbial protein adds to the MP pool available to meet cow requirements even when diets are deemed RDP-inadequate (Huhtanen and Hristov, 2009; Agle et al; 2010). Therefore, a key strategy that has received attention in recent years in an effort to improve ruminal N metabolism and reduce the irreversible loss of N to the environment is the harnessing of the urea-N recycling capacity of dairy cows (Reynolds and Kristen, 2008).

2.4 Urea-N Recycling

As ruminants, dairy cows have the ability to recycle urea-N synthesized in the liver to the GIT. In a recent study with lactating dairy cows, recycled urea-N accounted for 0.72 to 1.06 of digestible N intake, indicating its importance in maintenance of a positive N balance (Gozho et al., 2008). It is estimated that 40 to 80% of urea-N is recycled to all sections of the GIT (Lapierre and Lobley, 2001) depending on various factors including BUN and ruminal NH_3-N concentrations, and dietary fermentable CHO supply (Kennedy and Milligan, 1980; Cole and Todd, 2008). More importantly, recycling of urea-N to the rumen confers an evolutionary advantage to dairy cows (ruminants) as ruminal microbes can capture that N for protein synthesis, thus adding to metabolizable AA supply.

2.4.1 Transport of Urea-N Into The Rumen

Urea-N is recycled back to the rumen either through saliva or secretion across the ruminal epithelial cells, with the relative contribution of each of these 2 routes dependent on dietary characteristics (Lapierre and Lobley, 2001). An increase in dietary forage content that increases chewing and saliva production typically increases the amount of urea-N recycled in saliva (Lapierre and Lobley, 2001). Huntington (1989) observed that the relative salivary

contribution to total urea-N recycled to the rumen in steers was 46% higher on the forage than concentrate diet. Dietary CP content also has an impact on the relative contribution of each of the routes to urea-N entry into the rumen. Salivary urea-N flux did not differ between cows fed high or low CP diets (17.1 vs. 12.9% of diet DM; Kristensen et al., 2010). However, there was a dramatic decrease in ruminal extraction of arterial urea-N on the high CP diet, thereby increasing the contribution of salivary urea-N flux as a percentage of total urea-N recycled to the rumen. This indicates the increased importance of the contribution of salivary urea-N to total urea-N available in the rumen when dietary CP is high, as it counterbalances the down-regulation of urea-N secretion across the ruminal wall (Kristensen et al., 2010).

For a long time, it was thought that the passage of urea-N across the ruminal epithelial cells was solely by passive diffusion (Abdoun et al., 2006). This process was shown to be dependent on the presence of urease, an enzyme produced by ureolytic epimural bacteria (Houpt and Houpt, 1968). Urease breaks down urea-N during secretion to form NH_3-N and CO_2, and this is essential for maintenance of a concentration gradient that drives the passage of urea-N into the rumen. However, in recent years, several studies have also shown the presence of urea transport proteins in rumen epithelial cells, including urea transporter-B (UT-B) in dairy cows (Marini and Van Amburg, 2003; Marini et al., 2009). It has also been postulated that aquaporins, a family of water transporter proteins, could also be involved in the transport of urea-N into the rumen (Røjen et al., 2011a). However, the role of these proteins in facilitating the entry of urea-N across the ruminal epithelium is still equivocal.

2.4.2 Incorporation of Recycled Urea-N into Microbial Protein

Although the amount of urea-N recycled to the rumen is important, the proportion of that recycled urea-N that is incorporated into microbial protein is crucial, as it represents the AA-N available to meet animal requirements. Lapierre and Lobley (2001) estimated that up to 35 to 55% of recycled urea-N could potentially be used for anabolic purposes in cattle, offering a unique opportunity to fully harness this N salvage system. However, there have only been a few studies (Delgado-Elorduy et al., 2002; Al-Dehneh et al., 2007; Gozho et al., 2009) to date, which have focused on determining strategies to improve the sequestration of recycled urea-N into microbial protein in dairy cows. The amount of NH_3-N from recycled urea-N that is captured and

utilized by microbes is partially determined by the ruminal concentration of NH_3-N derived from dietary protein breakdown, which has a dilution effect (Reynolds and Kristensen, 2008). An example is the study by Marini and Van Amburg (2003) in which the absolute amount of urea-N that entered the GIT did not change when feeding Holstein heifers incremental amounts of dietary N (1.45, 1.89, 2.50, 2.97 and 3.40% of diet DM). However, there was a linear decrease in the percentage of total bacterial N derived from recycled urea-N (18.9 to 3.2%) as dietary N increased, possibly due to the increase in ruminal NH_3-N concentration (Marini and Van Amburg, 2003). Ruminally fermentable CHO drives microbial growth and, thus, its availability also determines the proportion of NH_3-N from recycled urea-N that is sequestered into microbial protein. Provision of a greater amount of ruminal-fermentable energy to lactating cows by feeding a high-grain compared to a high-forage diet led to a higher concentration of recycled urea-N in ruminal bacteria (37.5 vs. 12.7%) and duodenal digesta (19.1 vs. 7.4; Al-Dehneh et al., 1997). Therefore, dietary RDP and energy supply are key factors that influence sequestration of recycled urea-N into microbial protein in the rumen (Reynolds and Kristensen, 2008).

2.4.3 Factors That Regulate Urea Recycling

Urea-N recycling is regulated by a number of factors. However, this literature review will focus on dietary N and fermentable CHO supply, as these were part of my thesis research.

2.4.3.1 Dietary Nitrogen Supply

Considerable research has been conducted on regulation of urea-N recycling to the GIT, especially by dietary N status. However, this process is still not fully understood (Reynolds and Kristensen, 2008; Hristov et al., 2011a). It is now well established that an increase in dietary CP and RDP concentrations in diets fed to dairy cows causes an increase in net portal appearance of NH_3-N, urea-N synthesis in the liver and BUN concentration (Martineau et al., 2011). Despite this increase in the amount of urea-N synthesized, several studies have shown that the absolute amount of urea-N recycled to the GIT does not change with dietary CP concentration (Reynolds and Kristensen, 2008; Kristensen et al., 2010). However, the fractional transfer of urea-N increases with a decrease in dietary CP concentration as a result of an up-regulation of urea-N extraction across the PDV and ruminal wall, which also result in a decrease in urinary urea-N

excretion (Røjen et al., 2011b). This up-regulation of urea-N transport is an adaptive mechanism ensuring a supply of N for ruminal microbial growth when dietary N supply is inadequate. It is also desirable as it offers an opportunity to reduce the irreversible loss of urea-N in urine by dairy cows.

Ruminal NH_3-N and BUN concentrations are influenced by dietary N supply and, thus, play a role in regulating the flux of NH_3- and urea-N across the GIT and potential use of NH_3-N for anabolic purposes. An inverse relationship exists between ruminal NH_3-N concentration and transfer of urea-N to the rumen (Kennedy and Milligan, 1980), as permeability of ruminal epithelial cells to urea-N decreases as ruminal NH_3-N increases (Egan et al., 1986). In addition, an elevation in ruminal NH_3-N concentration also inhibits bacterial urease activity, which disrupts the concentration gradient that drives the diffusion of urea-N into the rumen (Cheng and Wallace, 1979). It had been reported that a positive correlation exists between BUN concentration and GIT entry of urea-N in cattle when BUN concentration is below 4 m*M* (Harmeyer and Martens, 1980). Lapierre and Lobley (2001) conducted regression analysis of a wide range of BUN and urea-N GIT entry data from numerous experiments and only observed a very weak correlation. However, in a recent study by Sunny et al. (2007), there was a high correlation between BUN concentration and GIT urea-N entry rate when BUN ranged from 2 to 5 m*M*. There has also been a suggestion that changes in dietary N status, and their effect on ruminal NH_3-N and BUN concentrations could potentially alter facilitated transport of urea-N across the ruminal wall. However, in recent studies, Røjen et al. (2011a, b) did not observe a correlation between dietary N concentration, and messenger RNA expression and protein abundance of UT-B and aquaporins in lactating dairy cows.

2.4.3.2 Dietary Fermentable Carbohydrate Supply

Increasing the dietary supply of ruminally fermentable CHO by either increasing fermentable starch intake (Huntington, 1989) or altering method and degree of grain processing (Alio et al., 2000; Delgado-Elorduy et al., 2002) has been shown to increase the movement of urea-N across the ruminal wall. Since an increase in energy availability for MPS needs to be matched by N supply, an up-regulation of urea-N recycling possibly occurs to avail additional NH_3-N for microbial growth. The potential reduction in ruminal NH_3-N concentration due to its

increased capture for MPS as energy supply increases is also favorable for optimal bacterial urease activity and influx of urea-N into the rumen (Kennedy and Milligan, 1980; Reynolds and Kristensen, 2008). In a study by Simmons et al. (2009), increasing the fermentable CHO content of diets fed to steers led to an increase in the expression of UT-B in ruminal tissue. Therefore, Simmons et al. (2009) suggested that this up-regulation in urea transporter expression could contribute to the observed increase in transepithelial movement of urea-N when diet energy intake increases. However, the functional significance of the observed changes in UT-B expression remains to be elucidated.

The movement of urea-N into the rumen is also modulated by dietary fermentable CHO supply through its effect on ruminal CO_2 and SCFA production, and pH (Abdoun et al., 2006). Urea-N influx into the rumen has been shown to increase when the ruminal concentration of CO_2 and butyrate increases (Thorlacius et al., 1971; Rémond et al., 1993). However, the exact mechanism behind the modulative effects of these fermentation products remains to be fully determined. Although sub-epithelial blood flow is stimulated by both CO_2 and butyrate, an increase in rate of blood flow to the rumen has been discounted as the reason behind the increase in urea-N transfer when energy intake increases (Rémond et al., 1993). Simmons et al. (2009) suggested that butyrate stimulates urea transporter expression including UT-B, leading to an increase in urea-N influx into the rumen when dietary energy supply increases. However, this remains to be substantiated. Increasing fermentable CHO intake also causes a decrease in ruminal pH due to an increase in SCFA production. In an in vitro study to elucidate the impact of mucosal pH (range of 5.4 to 7.4) on ruminal epithelial tissue permeability to urea-N, Abdoun et al. (2010) reported peak transfer of urea-N to occur within the physiological pH range of 6.0 to 6.4. After observing a decrease in urea-N transport as pH dropped below 6.0, Abdoun et al. (2010) suggested that ruminal pH < 6.0 decreases epithelial permeability by inducing conformational changes in proteins that facilitate the transfer of urea-N into the rumen (Abdoun et al., 2010). However, this theory also remains to be validated. Therefore, further studies are required to shed more light on regulation of urea-N recycling in dairy cows, which is central to improving N utilization efficiency.

2.4.4 The Role of the Kidney

Although it was not a major focus of my thesis research, it is worthwhile mentioning that the kidney plays a key role in urea-N recycling (Marini and Van Amburg, 2006). For example, feeding a low N diet (1.45% N) to heifers resulted in the reabsorption of 47% of urea-N filtered by the kidney compared to 8% for heifers fed a high N diet (3.40% N; Marini and Van Amburgh, 2003). The UT expressed in the kidney are involved in this important urea-N salvage mechanism that ensures a substantial amount of endogenous urea-N is recycled to the gut.

2.5 Distillers Grains

2.5.1 Ethanol Production in Saskatchewan

The need to address environmental concerns arising from the burning of fossil fuels was one of the major drivers of the creation of a legal framework promoting the development of alternative fuels, including bioethanol, by the Saskatchewan government. Initially, legislation mandating the blending of 1.0% of ethanol into all gasoline sold was implemented in November 2005, and this later increased to 7.5% in January 2007. As a result of these changes, ethanol production has increased dramatically in recent years, and this has also led to the production of millions of tonnes of different co-products including distillers grains (DGS). Therefore, the bioethanol industry has to dispose of the large quantities of these co-products in an environmentally-sustainable manner. Additionally, viability of the industry is heavily dependent on the sale of these co-products to complement revenue generated from ethanol (Bradford and Mullins, 2012).

Compared to eastern Canada and the United States where corn is the major feedstock, in western Canada wheat is the predominant feedstock for bioethanol production. However, fluctuations in wheat price have led to corn being increasingly used in combination with wheat in different ratios based on least cost and availability (Nuez-Ortín and Yu, 2011; Abdelqader and Oba, 2012). Consequently, there is a wide array of DGS products that are available, whose nutrient composition profiles are influenced mainly by the ratio of corn to wheat used. Currently, feeding nontraditional ingredients including co-products from the bioethanol (e.g., DGS) and agricultural processing industries (e.g., whey permeate) is of great interest to dairy producers, as

a result of rising feed costs (Martel et al., 2011; Bradford and Mullins, 2012). Therefore, the expansion of the bioethanol industry in western Canada offers a unique opportunity for the inclusion of the different co-products including wheat-based (W-DDGS) and blend dried distillers grains with solubles (B-DDGS) in dairy cow diets. However, besides increased availability and cost-effectiveness, use of W-DDGS and B-DDGS in dairy cow diets will largely depend on whether they are comparable to or are higher in nutritional quality than traditional feed ingredients (Vander Pol et al., 2008). Following years of substantial research mainly in the United States, corn-based dried distillers grains with solubles (C-DDGS) is extensively used in dairy rations, as its nutritional characteristics and feeding value are well documented (Schingoethe et al., 2009). However, there is a paucity of information on the potential use of W-DDGS and B-DDGS as feed ingredients for dairy cows in western Canada and the United States (Abdelqader and Oba, 2012). Ultimately, this information gap will curtail the ability of producers to control feed costs through the judicious use of these co-products.

2.5.2 Utilization of Wheat-based and Blend Distillers Grains in Dairy Cow Diets

The NDF content of DGS is high and it has been reported to range from 25.7 to 51.5 %, whereas its lignin content is typically less than 5% (Nuez Ortin and Yu, 2009; Abdelqader and Oba, 2012). As a result of its high fiber fermentability in the rumen, DGS has been considered for use as a nonforage fiber source (NFFS) that replaces either forages or concentrates in dairy diets (Bradford and Mullins, 2012). However, the small particle size of DGS is a major concern when feeding DGS as a NFFS, especially when partially replacing forages, as a result of significant changes in TMR particle size distribution. A reduction in dietary physically effective fiber (peNDF) content below the 22% threshold required for stimulation of chewing activity, saliva secretion and rumination causes a decrease in ruminal pH and contributes to milk fat depression (Mertens, 1997; Bradford and Mullins, 2012). Zhang et al. (2010a, b) documented these negative responses in ruminal pH and milk fat synthesis when partially replacing barley silage and grain with W-DDGS and B-DDGS in diets fed to lactating dairy.

There has been limited research on the utilization of W-DDGS and B-DDGS as protein supplements in diets fed to dairy cows. Their use depends on whether they can replace traditional protein supplements without compromising cow health and productivity. In western Canada and

parts of the United States, canola meal (CM) is the major protein supplement used in dairy diets (Hickling, 2008; Mulrooney et al., 2009). Although it is widely available, CM is usually a more expensive supplement compared to W-DDGS and B-DDGS (Hickling, 2008). Therefore, from an economic standpoint, it would be prudent to partially or completely replace CM with W-DDGS and B-DDGS in dairy cow diets, provided MP supply is not compromised. However, to our knowledge, there has been no studies to date on the impact of replacing traditional protein supplements such as CM with W-DDGS and B-DDGS on N utilization, microbial protein synthesis and metabolizable protein flow to post-ruminal sites. Therefore, this was a major focus of my thesis research.

Canola meal has a high CP content [mean 37.5% of DM; Canadian International Grains Institute (CIGI), 2011], which is comparable to W-DDGS. The CP content of W-DDGS is typically higher than C-DDGS (40 vs. 30%, on average), which is a reflection of the higher CP content of wheat compared to corn (Abdelqader and Oba, 2012). Therefore, as the proportion of corn used with wheat as feedstock increases, the CP content of the resultant B-DDGS decreases. Canola meal is considered a high quality protein source partly because it is highly digestible in the rumen. Over 60% of protein in CM is potentially degradable in the rumen and could provide an adequate amount of N to support MPS (Boila and Ingalls, 1993; Piepenbrink and Schingoethe, 1998). Piepenbrink and Schingoethe (1998) estimated that over 50% of microbial protein flow at the duodenum could be derived from ruminal degradation of CM. Compared to CM, W-DDGS and B-DDGS have a lower RDP content, ranging from 34.0 to 45.6% of CP (Nuez-Ortín and Yu, 2010a). As a result of these differences, replacing dietary CM with W-DDGS and B-DDGS reduces dietary RDP supply, which could potentially limit MPS in the rumen if amino- and NH_3-N deficiencies occur. Consequently, milk and milk protein yields could be compromised, thereby erasing potential financial gains from replacing the expensive CM with the cheaper DGS.

Besides microbial protein, high producing dairy cows have a requirement for RUP to maximize production. However, the value of RUP is dependent on the amount reaching the duodenum, and its AA composition and individual AA digestibility in the small intestine. Traditionally, DGS have been used as a source of RUP for dairy cows due to their high RUP content. The RUP content of W-DDGS and B-DDGS (wheat:corn = 70:30) was 54.4 and 63.8%

of CP, respectively (Nuez-Ortín and Yu, 2010b), which is higher than reported in CM (39.5% of CP; Piepenbrink and Schingoethe, 1998). Therefore, replacing CM with W- and B-DDGS would quantitatively improve duodenal RUP flow. The RUP from W-DDGS and C-DDGS is highly digestible in the small intestine (> 86%; Li et al., 2012). Additionally, over 80% of Lys and Met in RUP from W-DDGS and C-DDGS is also digestible. As a result of the high digestibilities, AA in W-DDGS and C-DDGS are available for absorption in the small intestine. However, replacing CM with W-DDGS and B-DDGS could qualitatively compromise RUP supply. The RUP fraction of both W-DDGS and C-DDGS contains ≤ 3.8% lysine (Li et al., 2012) compared to 5.1% lysine for CM (Piepenbrink and Schingoethe, 1998). Therefore, addition of W-DDGS and B-DDGS at the expense of CM could result in a failure to meet the recommended target of 7.2% lysine in MP required to maximize milk and milk protein production (NRC, 2001). This inadequacy in lysine content of DGS is reflected by positive production responses when diets containing C-DDGS were supplemented with ruminally protected Lys and Met (Nichols et al., 1998). Therefore, there is need for research to determine whether dietary RUP and RDP supply meets metabolizable AA requirements when W-DDGS and B-DDGS replace CM in diets for high producing dairy cows.

Although formulating W-DDGS and B-DDGS into dairy cow diets offers an opportunity to ensure the viability of both the ethanol and dairy industries, there are environmental concerns that need to be taken into consideration. Distillers grains have an atypically high N content relative to the N content of grain used as feedstock. Therefore, due to the low efficiency of N use in dairy cows, the bulk of N supplied by DGS is lost as excreted N. Additionally, inclusion of DGS to diets could potentially lead to overfeeding of N and increase its loss to the environment, especially when used to replace high energy, low CP ingredients including barley grain and silage (Schmit et al., 2009; Gehman and Kononoff, 2010). Therefore, there is need for research to determine feeding strategies that optimize nutrient utilization efficiency and, thus, ensure the judicious use of W-DDGS and B-DDGS in dairy cow diets. These include the use of medium CP diets and increasing diet CHO fermentability to reduce the loss of NH_3-N from the rumen, and UUN excretion. In addition, these strategies could also increase the transfer of urea-N to the rumen and its subsequent capture for MPS.

2.6 Conclusions

Dairy cows are inefficient in capturing dietary N for productive purposes. Loss of approximately 70% of dietary N in urine and feces is detrimental to environmental health. In addition, conversion of less than 30% of dietary N to milk protein also has huge economic implications for farmers, especially as feed costs continue to rise. Therefore, there has been considerable interest in feeding cheaper alternative feed ingredients including co-products from ethanol production as a strategy to address shrinking profit margins. The dramatic expansion of the bioethanol industry in Saskatchewan in recent years has led to large quantities of W-DDGS and B-DDGS being available for potential use as ruminant feed. However, there is a paucity of information on their utility as protein ingredients in lactating cow diets. Although it might be economically sustainable to feed W-DDGS and B-DDGS to dairy cows, most of the N they provide is excreted in urine and feces. However, research aimed at reducing N excretion by increasing the capture of dietary N into milk protein and, thus, ensuring the judicious use of W-DDGS and B-DDGS in dairy diets is lacking. Therefore, the overall hypothesis of my thesis research was that the efficiency of nutrient utilization can be optimized when co-products are included in lactating cow diets as partial or complete replacements for traditional feed ingredients. The overall objective was to determine nutritional strategies that optimize the efficiency of nutrient utilization when co-products partially or completely substitute for traditional feed ingredients in lactating cow diets.

3A. REPLACING CANOLA MEAL AS THE MAJOR PROTEIN SOURCE WITH WHEAT DRIED DISTILLERS GRAINS WITH SOLUBLES IN DAIRY COW DIETS. I. EFFECTS ON RUMINAL FUNCTION, MICROBIAL PROTEIN SYNTHESIS, OMASAL NUTRIENT FLOW, AND MILK PRODUCTION[1]

3A.1 Abstract

A study was conducted to determine the effects of replacing canola meal (CM) as the major protein source with wheat-based dried distillers grains with solubles (W-DDGS) on ruminal fermentation, microbial protein production, omasal nutrient flow and animal performance. Eight lactating dairy cows were fed in a replicated 4 × 4 Latin square design with 28-d periods (20 d of dietary adaptation and 8 d of measurements). Four cows in one Latin square were ruminally cannulated for measurements of ruminal fermentation characteristics and flow of nutrients at the omasal canal. Cows were fed either a standard barley silage-based TMR containing CM as the major protein supplement (0% W-DDGS, control) or diets formulated to contain 10, 15 and 20% W-DDGS (DM basis), with W-DDGS replacing primarily CM. Diets were isonitrogenous (18.9% CP) and contained 3.0, 3.2, 3.5 and 3.7% ether extract for 0, 10, 15 and 20% W-DDGS, respectively. Diets contained 50% forage and 50% concentrate. Inclusion of W-DDGS linearly increased DM intake (29.5, 31.2, 30.2 and 31.9 kg/d for 0, 10, 15 and 20% W-DDGS). The addition of W-DDGS in place of CM resulted in a 1.2 to 1.8 kg increase in milk yield (42.9, 44.7, 44.1 and 44.5 kg/d for 0, 10, 15 and 20% W-DDGS); however, there was a quadratic decrease in feed efficiency (i.e., milk yield/DM intake) as the dietary level of W-DDGS increased. There were no differences among treatments for milk fat, protein, and lactose concentrations; however, there were quadratic changes in milk yields of fat (1.48, 1.56, 1.62 and 1.55 kg/d for 0, 10, 15 and 20% W-DDGS, respectively), protein (1.44, 1.46, 1.49 and 1.42 kg/d) and lactose (1.96, 2.02, 2.09 and 1.93 kg/d). Ruminal fermentation characteristics did not change except that the inclusion of 20% W-DDGS resulted in a decrease and a tendency for a decrease in molar concentrations of isobutyrate and total VFA, respectively. Omasal flow of total bacterial

[1]A version of this chapter has been published: Chibisa, G. E., Christensen, D. A., and Mutsvangwa, T. 2012. Effects of replacing canola meal as the major protein source with wheat dried distillers grains with solubles on ruminal function, microbial protein synthesis, omasal flow, and milk production in cows. J. Dairy Sci. 94 (8): 3913-3927.

nonammonia N (NAN) and bacterial efficiency (g of total bacterial NAN flow/kg of organic matter truly digested in the rumen) were not different among diets; however, feeding W-DDGS resulted in a quadratic increase in nonammonia nonbacterial N (NANBN) flow at the omasal canal (271, 318, 336 and 311 g/d for 0, 10, 15 and 20% W-DDGS, respectively). These data indicate that W-DDGS can substitute for CM as the major protein source in dairy cow diets without negatively affecting ruminal fermentation, microbial protein production, and omasal nutrient flow, and can potentially increase DM intake and milk yield.

3A.2 Introduction

The use of wheat as the principal feedstock for ethanol production in western Canada has led to an increase in the availability of wheat-based dried distillers grains with solubles (W-DDGS). Numerous studies conducted mostly in the USA have demonstrated that corn-based dried distillers grains with solubles (C-DDGS) can be used as a suitable replacement for traditional protein supplements while maintaining or increasing levels of milk production (reviewed by Schingoethe et al., 2009). However, when compared with C-DDGS, W-DDGS contains less fat (49.8 vs. 165.3 g/kg) and more CP (393.2 g/kg vs. 320.1 g/kg) (Nuez-Ortín and Yu, 2009). Therefore, it is plausible that because of these differences in nutrient composition, the feeding value of W-DDGS for dairy cows could be different than that of C-DDGS. To date, there has been limited research on the utilization of W-DDGS in barley-based dairy diets that are commonly fed in western Canada. Because W-DDGS is high in NDF content, ranging from 25.9 to 54.1% (Dong et al., 1987; McKinnon and Walker, 2008), and this NDF is highly digestible (Nuez-Ortín and Yu, 2009), a limited number of studies have examined the inclusion of W-DDGS in dairy diets as a partial replacement for forage (Penner et al., 2009c; Zhang et al., 2010a,b) or barley grain (Zhang et al., 2010a). The major focus of these previous studies was to determine if feeding W-DDGS could potentially attenuate ruminal acidosis and, to our knowledge, there are no reported studies that have examined the impacts of replacing traditional protein supplements like canola meal (CM) with W-DDGS on N utilization, microbial protein production, and omasal flow of nitrogenous compounds.

In western Canada and parts of the USA, CM is the principal source of protein that is included in dairy diets because it is readily available and is a high quality protein supplement

(Hickling, 2008; Mulrooney et al., 2009). Recently, however, focus has turned to the use of W-DDGS in dairy diets as a strategy to control rising feed costs because W-DDGS is usually cheaper than CM (Hickling, 2008). When compared to CM, W-DDGS is greater in CP content (43.6 vs. 39.5%), but is lower in methionine (1.75 vs. 2.68 g/16 g N) and, particularly, lysine (2.04 vs. 5.30 g/16 g N) contents (Boila and Ingalls, 1994). In an in situ study, ruminal N degradability of W-DDGS was much lower (36.5 vs. 63.4%), whereas small intestinal digestibility (calculated as ileal disappearance minus ruminal disappearance) was much greater (57.5 vs. 26.6%) when compared with CM (Boila and Ingalls, 1994). Because of the differences in RDP content of these two protein supplements, replacement of CM with W-DDGS may negatively affect ruminal microbial protein production. Maximizing the supply of microbial protein is important as it can contribute more than 60% of metabolizable protein (MP) reaching the small intestine and, qualitatively, it has an AA profile that closely matches amino acid requirements for milk protein synthesis (NRC, 2001). Therefore, if substituting CM with W-DDGS compromises microbial protein synthesis due to deficiencies in RDP, this would be undesirable as it could negatively affect cow performance. Besides a requirement for microbial protein, high-producing dairy cows also require digestible RUP to augment the amino acid supply from microbial protein (Stern et al., 2006). Although W-DDGS is a good source of digestible RUP, its content of lysine is low (Boila and Ingalls, 1994). Therefore, another concern when replacing CM with W-DDGS in lactating cow diets is whether metabolizable lysine supply will be adequate to support milk and milk protein production (Boila and Ingalls, 1994). Despite these potential challenges in meeting MP requirements when including W-DDGS in dairy cow diets, limited research has been conducted to justify either partial or complete replacement of CM with W-DDGS. Therefore, this study was conducted to determine whether completely replacing CM (as the major protein source) in barley-based rations with increasing amounts of W-DDGS would have any impacts on ruminal fermentation characteristics, microbial protein production, omasal flow of nitrogenous fractions, and productivity of lactating dairy cows.

3A.3 Materials and Methods

3A.3.1 Animals and Experimental Design

Eight multiparous Holstein cows (718.5 ± 53.8 kg BW; 101.1 ± 39.2 days-in-milk) housed at the Greenbrae Dairy Research Facility (University of Saskatchewan) were used in this study. The experimental design was a replicated 4 × 4 Latin square with 28-d periods (20 d of dietary adaptation and 8 d of measurements). Four cows in one Latin square were ruminally-cannulated. All experimental cows were housed in individual tie-stalls and the experimental procedures used were all approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048), and were in accordance with the Canadian Council of Animal Care guidelines (Ottawa, ON).

3A.3.2 Experimental Treatments and Animal Management

The 4 dietary treatments consisted of a standard barley silage-based diet containing CM as the principal protein supplement (control, 0% W-DDGS) or diets formulated to contain 10, 15 and 20% W-DDGS (DM basis). Single batches of CM and W-DDGS were used for the entire study. The batch of W-DDGS was produced from 100% wheat (Terra Grain Fuels, Belle Plaine, SK). Because W-DDGS has a higher CP content than CM, W-DGGS replaced all CM and varying proportions of soybean meal in the concentrate portion of diets containing W-DDGS in order to make diets isonitrogenous. The ingredient and chemical composition of the 4 diets is given in Table 3A.1. Cows were fed experimental diets as total mixed rations (TMR) at 0830 and 1600 h for ad libitum intake. The forage:concentrate ratio of the TMR was 50:50. The forage component of the TMR was a mixture of barley silage (65% on a DM basis) and chopped alfalfa hay (35%).

3A.3.3 Data Collection and Sampling

Rumen fermentation characteristics, microbial protein production, omasal nutrient flow, total tract nutrient digestion, and N balance were determined using the 4 ruminally-cannulated cows in one Latin square. To quantify omasal digesta flow, indigestible NDF (iNDF; Reynal et al., 2005), $YbCl_3$ (Siddons et al., 1985), and CrEDTA (Udén et al., 1980) were used as digesta markers for the large particle (LP), small particle (SP), and fluid (FP) phases, respectively.

Table 3A.1 Ingredient and chemical composition of experimental diets fed to lactating dairy cows

	Diet			
	Control	10% DDGS	15% DDGS	20% DDGS
Ingredient Composition, % of diet DM				
Barley silage	32.3	32.3	32.3	32.3
Alfalfa hay	17.4	17.4	17.4	17.4
Barley	30.0	30.3	29.6	28.4
Wheat-based DDGS	_	10.0	15.0	20.0
Canola meal	8.8	_	_	_
Soybean meal	6.8	6.4	2.6	_
Corn gluten meal	0.96	0.96	0.96	0.34
Canola oil	0.55	0.55	0.55	0.55
Molasses	0.22	0.22	0.22	0.22
Mineral-vitamin mix[1]	1.6	1.6	1.6	1.6
Bicarbonate	0.88	0.88	0.88	0.88
Salt (Cobalt-Iodine)	0.33	0.33	0.33	0.33
Limestone	0.05	0.22	0.22	0.27
Dynamate[2]	0.16	0.16	0.16	0.16
Chemical composition				
DM, %	53.2	53.5	53.7	54.0
OM, % of DM	91.5	91.4	91.6	91.1
CP, % of DM	18.9	18.8	19.0	18.9
NDF, % of DM	32.6	32.3	32.8	33.6
ADF, % of DM	18.0	17.8	18.5	19.0
RDP,[3] % of DM	12.7	13.0	11.0	11.6
RUP,[4] % of DM	6.64	7.28	7.55	7.52
Ether extract, % of DM	3.0	3.2	3.5	3.7
NE_L,[5] Mcal/kg of DM	1.5	1.5	1.5	1.5

[1]Contained (/kg of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16%, Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

[2]Dynamate (Eastern Minerals Inc., Henderson, NC) contained 18% K, 11% Mg, and 22% S.

[3]RDP was calculated from in vivo measurements as: RDP, % of DM = (Total CP intake, kg/d – omasal RUP flow, kg/d) x 100/DM intake, kg/d.

[4]RUP was calculated from in vivo measurements as: RUP, % of DM = (Total omasal CP flow, kg/d – omasal microbial CP flow, kg/d) x 100/DM intake, kg/d.
[5]Calculated from NRC (2001).

To quantify ruminal microbial protein production, $(^{15}NH_4)_2SO_4$ (10 atom percent excess [APE] ^{15}N; Cambridge Isotope Laboratories, Andover, MA) was used as a microbial marker (Reynal et al., 2005). Preceding infusion of the marker solutions (d 13), a 500-mL rumen digesta sample was collected and stored at -20ºC for the determination of background ^{15}N (^{15}NB) abundance. A priming dose of the three marker solutions ($YbCl_3$, $[^{15}NH_4]_2SO_4$, and Cr-EDTA) equivalent to half the daily dose was then administered into the rumen via the ruminal cannula. Subsequently, marker solutions were continuously infused into the rumen using a peristaltic pump (Model: 205U, Watson and Marlow, Cornwall, UK) for the next 10 d (d 13 to d 23) at a constant rate of 1 L/d, providing 2.77 g of Cr (Binnerts et al., 1968), 3.35 g of Yb, and 0.22 g of ^{15}N (Brito et al., 2006) per day. The amount of marker solution infused each day (~1 L) was recorded. During each period, a 50 mL sub-sample of the marker solutions infused for each cow was collected and stored at room temperature for Cr, Yb and ^{15}N analysis.

The omasal sampling technique as described by Huhtanen et al. (1997) was used to collect omasal digesta. Briefly, this technique involved locating the omasal canal by hand and then inserting a sampling tube via the ruminal cannula. Sampling tubes were inserted at each sampling time in order to ensure correct positioning in the omasal canal and to minimize the potential negative impact on digesta passage and normal digestive function if the sampling tubes were left in place between samplings. Once the sampling tube had been inserted in the omasal canal, a 425-mL omasal digesta sample was collected from each cow at 0600, 1200 and 1800 h on d 20, 0000, 0800, 1400 and 2000 h on d 21, 0200, 1000, 1600 and 2200 h on d 22, and 0400 h on d 23, such that the collected samples were representative of a 24-h feeding cycle. After careful mixing to ensure representative sampling, the collected 425-mL sample was divided into 100, 125 and 200 mL subsamples. The 100 and 200 mL subsamples were immediately stored at -20ºC, and were pooled by cow over the 24-h cycle to yield 1.2 and 2.4 L composite samples, respectively. The 125-mL sub-samples were placed in an ice-bath following collection, and pooled over 2 sampling times to yield a 250-mL composite sample which was used for the isolation of particle-associated (PAB) and fluid-associated (FAB) bacteria using filtration and differential centrifugation as described by Brito et al. (2009). Briefly, the 250-mL composite samples were squeezed through two layers of cheesecloth. The resultant filtrand (solids) was washed with 250 mL of 0.85% saline solution, squeezed again through two layers of cheesecloth,

and then transferred to a 500-mL PAB container that contained 175 mL of a cold (5°C) 0.85% saline solution with 0.1% (wt/vol) Tween-80. The contents of the PAB container were then mixed thoroughly and held on ice. The filtrate from these initial filtration steps was collected, pooled, and held on ice in preparation for the isolation of fluid-associated bacteria (FAB). To isolate FAB, the filtrate was initially centrifuged (1,000 × *g*, 5°C, 5 min). The resultant pellet was transferred to the PAB container, whose contents were then blended on low speed for 30 sec before storage at 5°C until PAB isolation 24 h later. The supernatant was decanted and centrifuged (11,300 × *g*, 5°C, 30 min). Following centrifugation, the resultant supernatant was decanted and discarded, whereas the pellet was re-suspended in 50 mL of McDougall's buffer (McDougall, 1948). The suspension was then re-centrifuged (11,300 × *g*, 5°C, 30 min) and the resultant FAB pellet was stored at -20ºC. Following storage at 5°C for 24 h, contents of the PAB container were filtered through 2 layers of cheesecloth. The filtrate was then processed as previously described for FAB isolation, with the exception that the pellet obtained after the initial low speed centrifugation (1,000 × *g*, 5°C, 5 min) step was discarded. The resultant PAB pellet was then stored at -20ºC.

Apparent total tract nutrient digestibility and N balance were determined using 4-d total urine and feces collection as described by Plaizier et al. (2000), starting on d 24 of each experimental period. Total urine output was collected using indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA). Catheters were inserted at 0900 h on d 23, and were then connected to urine collection tubing when urine collections were initiated at 0900 h on d 24. Urine was collected into 20-L Carboy polyethylene containers into which 200 ml of H_2SO_4 had been added in order to achieve urine pH of less than 3. The acidification of urine was necessary to prevent microbial degradation and the loss of volatile NH_3-N. Daily urinary output was weighed, mixed thoroughly and a 5% sub-sample of the daily output was drawn, pooled for each cow during each collection period and stored at -20˚C until analyzed for total N. In addition, a 2-mL aliquot of urine was diluted with 8 mL of distilled water and stored at -20°C for later determination of urea-N and purine derivatives. Feces were collected into large steel trays which were positioned over the gutter behind each stall. Daily fecal output for each cow was determined by weighing and feces were then mixed thoroughly before 2.5% of daily output was sampled and stored at -20°C for later

chemical analysis. During each 4-d total collections period, TMR and orts samples were collected daily and stored at -20°C for later analysis.

To determine ruminal fermentation characteristics, approximately 1,000 mL of ruminal contents were collected from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula at 0800, 0900, 1000, 1100, 1200, 1500, 1800, and 2100 h on d 27, and 0000, 0300 and 0600 h on d 28. The ruminal contents were strained through 4 layers of cheesecloth. Ruminal fluid pH was then measured immediately using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA). Two 10-mL sub-samples of ruminal fluid were collected and mixed with chilled 25% (wt/vol.) meta-phosphoric acid (H_2PO_4) or 1% H_2SO_4 and stored at -20°C for later determination of VFA and NH_3, respectively. Ruminal pH was also continuously measured every 30 sec for 3 consecutive days starting on d 25 using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA) as described by Penner et al. (2006).

During each measurement period, individual cow feed intake was recorded daily. Samples of TMR and orts were collected daily and stored at -20°C for later analysis. Experimental cows were milked three times daily at 0430, 1230 and 1900 h and milk weights were recorded. At each milking on d 25 to 28, milk was collected into plastic vials containing 2-bromo-2-nitropropane-1-2-diol as a preservative. Daily milk samples from the 0430, 1230 and 1900 h milkings were then pooled proportionally based on milk yield from each milking and pooled milk samples were stored at 4°C before being sent to the Provincial Milk Testing Laboratory (Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK) for compositional analysis. On d 28, blood samples were collected at 1030 h from the coccygeal vein of each cow into 10-mL vacutainer tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ). Blood samples were centrifuged at 2,500 × *g* for 15 min at 4°C and the plasma obtained was stored at – 20°C until later analysis for urea-nitrogen (PUN).

3A.3.4 Sample Analyses

Pooled milk samples were analyzed for CP, lactose, and fat using an infrared analyzer (Foss System 4000, Foss Electric, Hillerød, Denmark) (AOAC, 1990; method 972.16). After the experiment, frozen TMR and fecal samples were thawed overnight at room temperature, pooled

per collection period for each cow, and subsequently dried in an oven at 60°C for 48 h (AOAC, 1990; method 930.15). Dried TMR and fecal samples were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Samples were analyzed for DM (AOAC, 1990; method 930.15), OM (AOAC, 1990; method 942.05), CP using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05), ether extract (AOAC, 1990; method 920.39), and ADF and NDF (Van Soest et al., 1991). Amylase and sodium sulfite were used for NDF determination. Frozen urine samples were thawed overnight at room temperature and then analyzed for N using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05). The plasma and dilute urine samples were analyzed for urea-N by the diacetyl monoxime-based method of Marsh et al. (1957) using a colorimetric urea-N kit (Stanbio Urea Nitrogen Kit, Procedure No. 0580; Stanbio Laboratory, Boerne, TX, USA).

The ^{15}NB samples were freeze-dried and ground through a 1-mm screen (Christy-Norris Ltd.) before being pulverized with a ball mill for later non-ammonia nitrogen (NAN) and ^{15}N analyses. The FAB and PAB pellets were freeze-dried and then ground with a mortar and pestle, after which composite samples of FAB and PAB were prepared for each cow per period by combining equal amounts (DM basis) from each sampling time. The 2.4-L omasal digesta composite samples were thawed at room temperature and fractionated into the three phases i.e., LP, SP and FP as described by Brito et al. (2009). Briefly, omasal digesta was squeezed through a single layer of cheesecloth. Solids retained on the cheesecloth were the LP. The filtrate was then centrifuged at 1000 × *g* for 5 min at 5°C, and the resultant pellet was the SP, whereas the supernatant was the FP. The LP, SP and FP samples were freeze-dried and then ground through a 1-mm screen (Christy-Norris mill) before Yb, Cr and iNDF analysis. To determine Cr and Yb concentrations, a 1-g sample of each of the 3 phases (LP, SP and FP) was combusted at 550°C for 8 h in a muffle furnace (AOAC, 1990) before nitric acid digestion as described by Vicente et al. (2004). Subsequently, Cr concentration was measured by atomic absorption spectrophotometry (Perkin Elmer 2300, Perkin-Elmer Corp, Norwalk, CT), whereas Yb concentration was quantified by atomic emission spectroscopy (Varian Spectra 220, Varian, Mulgrave, Australia). Indigestible NDF concentration was determined in duplicate TMR and LP samples, and in triplicate SP samples according to Ahvenjärvi et al. (2000). Briefly, 1.5, 3.0 and 3.5 g samples of LP, TMR and SP, respectively, were weighed into 5 × 10 cm nylon mesh bags

(6 µm pore size; part no. 03-6/5, Sefar America Inc., Depew, NY) before incubation for 12 d in the rumen of a ruminally-cannulated cow. Following incubation, bags were rinsed in water for 30 min before NDF analysis. The mean concentration of Cr in FP was 7.7 and 15.4 times greater than in SP and LP, respectively, whereas the Yb concentration in SP was 2.5 and 2.9 times greater than in FP and LP, respectively. The mean iNDF concentration was 6.3 times greater in LP than in SP. As a result of the concentrations of the 3 principal markers being distinctly greater in their respective digesta phases, the Cr, Yb and iNDF concentrations in LP and SP, and Cr and Yb concentrations in FP were used to physically reconstitute the omasal true digesta (OTD) flowing out of the rumen using the triple-marker method of France and Siddons (1986). Marker concentrations were also used to physically reconstitute a 2-g particle phase (PF) sample consisting of SP and LP.

To determine the flow of nutrients to the omasum, the OTD samples were analyzed for OM by combustion in a muffle furnace at 550°C for at 8 h, total N (Leco 2000; Leco Instruments, Inc., St. Joseph, MI), ADF and NDF. To quantify NH_3-N in OTD, 10 mL of 0.07 *M* sodium citrate (pH 2.2) was added to 0.5 g of OTD sample and the mixture vortexed and subsequently held at 39°C for 30 min in a forced-air oven. The extracts were then centrifuged at 18,000 × *g* for 15 min at 4°C, and the resultant supernatant was analyzed for NH_3-N using the phenol-hypochlorite method as described by Broderick and Kang (1980). Samples of ^{15}NB, FAB, PAB, FP, PF and OTD were prepared for ^{15}N analysis as described by Brito et al. (2009). Briefly, to volatize NH_3-N prior to ^{15}N analysis, ^{15}NB, FAB, PAB, FP, PF and OTD samples containing approximately 100 µg of N were weighed into 5 × 9-mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK). Subsequently, 50 µL of 72 m*M* K_2CO_3 was added to each capsule before incubation in a forced air oven at 60°C for 24 h. Enrichment of ^{15}N in NAN of the samples was then measured by combustion to N_2 gas in an elemental analyzer and continuous flow isotope ratio-mass spectrometry.

For amino acid analysis, TMR and OTD samples were prepared by acid hydrolysis (AOAC, 1995; method 994.12) to liberate individual amino acids from proteins. Briefly, approximately 100 mg TMR or OTD sample was weighed and then hydrolyzed under reflux in 6 *M* HCl at 110ºC for 24 h. Hydrolyzed samples were then neutralized with 25% (wt/vol) NaOH, cooled to room temperature, and then the neutralized samples were then diluted to 50 mL total

volume using sodium citrate buffer (pH 2.2). The separation and quantification of individual amino acids in hydrolyzed samples were performed using a high-performance liquid chromatograph (Shimadzu, Columbia, MD). For the determination of cysteine and methionine in TMR and OTD samples, performic acid oxidation was performed prior to acid hydrolysis to oxidize cysteine and methionine to cysteic acid and methionine sulfone, respectively (AOAC, 1995; method 994.12). Tryptophan was determined after hydrolysis of 100 mg of sample with 5 *M* NaOH at 120°C for 16 h using the method of Hugli and Moore (1972).

Frozen ruminal fluid samples were thawed at room temperature, centrifuged at 20,000 x *g* for 15 min at 4°C to obtain a clear supernatant. For ruminal fluid samples that were preserved with 25% H_2PO_4, supernatants were filtered through a 0.45-µm membrane and a 0.9-mL portion of the filtered supernatant was mixed with 0.1 mL of 10 mg/mL crotonic acid as an internal standard. Ruminal VFA were separated and quantified by gas chromatography (Agilent 6890, Mississauga, ON) as described by Erwin et al. (1961). For ruminal fluid samples that were preserved with H_2SO_4, supernatants were analyzed for ruminal NH_3-N using a phenol-hypochlorite assay (Broderick and Kang, 1980).

3A.3.5 Calculations and Statistical Analysis

The ruminal pH data that was recorded daily was averaged for each minute and summarized daily as minimum pH, mean pH and maximum pH. The degree and incidence of ruminal acidosis was determined from the pH data using three pH thresholds i.e., 5.8, 5.5 and 5.2. Ruminal acidosis was considered to occur when ruminal pH was < 5.8 (total ruminal acidosis). The pH profiles were further classified as mild RA when 5.8 > ruminal pH > 5.5, severe RA when 5.5 > ruminal pH > 5.2, and acute RA when ruminal pH < 5.2 (Penner et al., 2006). The duration (min/d) and total area (pH x min) that ruminal pH was below each threshold was calculated.

The flow of nutrients at the omasal canal was calculated as described by Brito et al. (2009) with minor modifications. Omasal flow of NAN, assumed to consist of PAB NAN, LAB NAN and non-NH_3 non-bacterial N (NANBN), was calculated as the difference between total omasal N and NH_3-N flow. The natural abundance of the 16 background ^{15}N digesta samples

averaged 0.36809 ± 0.00012 (S.D.). In a study by Ahvenjärvi et al. (2002), the background ^{15}N enrichment in bacteria was similar to that of the digesta. Based on those observations, ^{15}N enrichment in bacterial and omasal fractions in the current study was computed using ^{15}NB as ^{15}N enrichment (^{15}N APE) = ^{15}N atom % - 0.36809 (background ^{15}N atom %). Following the assumptions that FAB was representative of bacteria flowing with FP whereas PAB was representative of bacteria flowing with PF, omasal flows of FAB NAN, PAB NAN, total bacterial NAN, NANBN, RDP supply, and OM truly digested in the rumen (OMTDR) were computed as follows: FAB NAN flow = FP NAN flow × (FP ^{15}N APE ÷ FAB ^{15}N APE); PAB NAN flow = PF NAN flow × (PF ^{15}N APE ÷ PAB ^{15}N APE); total bacterial NAN flow = FAB NAN flow + PAB NAN flow; NANMN flow = total NAN flow – total bacterial NAN flow; RDP supply = total CP intake – (NANBN flow × 6.25); FAB DM flow = FAB NAN flow ÷ (% FAB NAN ÷ 100); PAB DM flow = PAB NAN flow ÷ (% PAB NAN ÷ 100); FAB OM flow = (FAB DM flow × % FAB OM) ÷ 100; PAB OM flow = (PAB DM flow × % PAB OM) ÷ 100; and total bacterial OM flow = FAB OM flow + PAB OM flow. Flows and intakes are in grams or kilograms per day and NAN concentrations are in g per g of OM.

All data on ruminal fermentation parameters, nutrient digestibilities, omasal flow and excretion were analyzed as a 4 x 4 Latin square using the MIXED procedure of SAS (SAS Institute, 2002) according to the following model: $Y_{ijk} = \mu + P_i + C_j + T_k + E_{ijk}$ where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the fixed effect of period i, C_j is the random effect of cow j, T_k is the fixed effect of dietary treatment k, and E_{ijk} is the residual error. Ruminal pH, VFA and NH_3-N data were analyzed accounting for repeated measures through the inclusion of the variable time in the repeated statement, as well as terms for time (hour), and interaction (diet × time) in the model previously described. Production data for the eight cows were analyzed using the MIXED procedure of SAS (SAS Institute, 2002) for a replicated 4 × 4 Latin square design according to the following model: $Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl}$ where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square i, P_j is the fixed effect of period j, $C_{k(i)}$ is the random effect of cow k (within square i), T_l is the fixed effect of dietary treatment l, ST_{il} is the interaction between square i and treatment l, and E_{ijkl} l is the residual error. The interaction term was removed from the model when $P > 0.25$. Orthogonal contrasts were used to test for linear and quadratic effects of level of W-DDGS in the diet.

Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. All reported values are least squares means, which were separated using the PDIFF test in SAS.

3A.4 Results

3A.4.1 Diet Composition

Dietary ingredient and chemical composition are presented in Table 3A.1. Because W-DDGS had a higher CP content than CM, W-DGGS replaced all CM and varying proportions of soybean meal in the concentrate portion of diets containing W-DDGS in order to make all diets isonitrogenous. Diets were also formulated to be isoenergetic. Dietary contents of RDP and RUP that were calculated from observed omasal flows of NAN fractions and DM intakes ranged from 11 to 12.7%, and 6.64 to 7.52% (% of DM), respectively. The AA composition of TMR is presented in Table 3A.2. Notably, lysine content decreased with increasing amounts of W-DDGS in TMR, reflecting the low concentration of lysine in W-DDGS.

3A.4.2 Feed Intake and Milk Production

Results on feed intake and milk production that are presented in Table 3A.3 are from all 8 cows that were used in the study. The inclusion of W-DDGS resulted in a linear increase in DM ($P < 0.01$) and CP ($P = 0.02$) intake (Table 3A.3). There was a tendency ($P = 0.05$) for a linear increase in milk production with W-DDGS; however, there was no diet effect ($P = 0.35$) on energy-corrected milk (ECM). Quadratic changes were observed in apparent DM efficiency (ECM/DMI; $P < 0.01$) and N efficiency (milk N/N intake; $P < 0.01$) with increasing W-DDGS in the diet. Milk fat, protein and lactose contents were not different ($P > 0.05$) among diets; however, the inclusion of W-DDGS resulted in quadratic changes ($P \leq 0.03$) in milk fat, protein and lactose yields. Feeding increasing amounts of W-DDGS tended to increase ($P = 0.10$) PUN concentration, which averaged 21.4 mg/dL for the diets containing W-DDGS compared to 20.3 mg/dL for the control diet (Table 3A.3).

Table 3A.2 Amino acid (AA) composition of total mixed rations (expressed as % of DM)

	Diet			
Item	Control	10% DDGS	15% DDGS	20% DDGS
Essential AA				
Arginine	0.853	0.686	0.636	0.628
Histidine	0.397	0.349	0.315	0.333
Isoleucine	0.780	0.709	0.658	0.733
Leucine	1.350	1.265	1.191	1.223
Lysine	0.926	0.702	0.642	0.593
Methionine	0.293	0.290	0.285	0.294
Phenylalanine	0.793	0.746	0.780	0.760
Threonine	0.671	0.609	0.608	0.602
Tryptophan	0.195	0.201	0.192	0.174
Valine	0.898	0.779	0.762	0.817
Nonessential AA				
Alanine	0.995	0.947	0.906	0.943
Asparagine	1.491	1.332	1.193	1.148
Cysteine	0.283	0.275	0.274	0.292
Glutamine	3.284	3.489	3.505	4.014
Glycine	0.798	0.703	0.704	0.742
Proline	1.431	1.392	1.510	1.605
Serine	0.689	0.686	0.668	0.686
Tyrosine	0.434	0.422	0.388	0.429

Table 3A.3 Dry matter (DM) and crude protein (CP) intakes, milk yield and composition, and plasma urea-N (PUN) of dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
DM intake, kg/d	29.7[c]	30.7[b]	30.0[bc]	31.8[a]	0.54	<0.01	<0.01	0.08
CP intake, kg/d	5.62	5.73	5.86	5.87	0.405	0.09	0.02	0.48
Milk yield, kg/d	42.9[b]	44.7[a]	44.1[ab]	44.5[a]	2.31	0.04	0.05	0.14
ECM,[3] kg/d	45.0	45.0	44.5	45.4	1.83	0.35	0.79	0.11
Feed efficiency[4]	1.47[ab]	1.49[a]	1.50[a]	1.44[b]	0.033	<0.01	0.15	<0.01
N efficiency[5]	24.1[bd]	25.2[ab]	25.1[ac]	24.5[cd]	0.69	<0.01	0.28	<0.01
Milk fat, %	3.60	3.57	3.43	3.56	0.109	0.44	0.46	0.34
Milk fat yield, kg/d	1.48[b]	1.56[ab]	1.62[a]	1.55[ab]	0.061	0.03	0.05	0.03
Milk protein, %	3.32	3.29	3.30	3.30	0.065	0.54	0.33	0.30
Milk protein yield, kg/d	1.44[bc]	1.46[ab]	1.49[a]	1.42[c]	0.043	0.03	0.64	<0.01
Milk Lactose, %	4.54	4.52	4.57	4.55	0.034	0.28	0.50	0.93
Milk lactose yield, kg/d	1.96[c]	2.02[b]	2.09[a]	1.93[d]	0.035	<0.01	0.14	<0.01
PUN, mg/dL	20.3	21.7	21.0	21.4	0.82	0.10	0.12	0.05

[a, b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Values are least squares means obtained from 8 cows.

[2]*P* values indicate overall diet, linear and quadratic effects.

[3]Energy-corrected milk (ECM), which was calculated as [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)] (Orth, 1992).

[4]Feed efficiency = ECM/DMI.

[5]Nitrogen efficiency = milk N (kg/d)/N intake (kg/d).

3A.4.3 Ruminal Fermentation Characteristics

The daily mean, minimum and maximum ruminal pH, and degree of SARA (duration and area below the pH thresholds of 5.5 and 5.8) were similar across diets (Table 3A.4). Ruminal VFA concentrations were unaffected by diet, except that the inclusion of 20% W-DDGS resulted in a decrease ($P < 0.01$) and a tendency ($P = 0.09$) for a decrease in the molar concentrations of isobutyrate and total VFA, respectively (Table 4). Ruminal NH_3-N concentrations were unaffected ($P = 0.89$) by diet. Because ruminal fermentation characteristics were measured at various time points relative to feeding, a time × treatment interaction for any of the ruminal variables that were measured would be indicative of differences in fermentation patterns; however, there was no treatment × time interaction for ruminal ammonia and VFA concentrations which indicates that fermentation patterns were essentially similar across diets (Table 3A.4).

3A.4.4 Nutrient Intakes, Ruminal Digestibilities, and Omasal Nutrient Flows

Intake, omasal flow, amount digested in the rumen, and apparent ruminal digestibility of DM were not affected ($P > 0.05$) by dietary treatment (Table 3A.5). Organic matter intake was similar ($P = 0.76$) across diets and averaged 29.2 kg/d. Dietary inclusion of W-DDGS resulted in a linear decrease ($P = 0.01$) in the amount of OM apparently digested in the rumen and a tendency for a linear increase ($P = 0.09$) in the amount of OM flowing at the omasal canal. However, the percent of OM apparently digested in the rumen was not affected by diet. Although the amount of OM truly digested in the rumen was not different, there was a tendency for a decrease ($P = 0.08$) in the percent of OM truly digested in the rumen with the inclusion of W-DDGS. There were quadratic changes ($P < 0.01$) in NDF and ADF intakes when W-DDGS was added to the diets; however, omasal flows, amounts and percents of NDF and ADF apparently digested in the rumen were not different across diets (Table 3A.5).

Table 3A.4 Ruminal fermentation characteristics of dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
Ruminal pH								
Mean	6.04	6.08	6.02	6.13	0.15	0.14	0.18	0.28
Minimum	5.53	5.55	5.50	5.60	0.18	0.23	0.29	0.24
Maximum	6.68	6.71	6.67	6.77	0.12	0.25	0.22	0.33
Duration, min/d								
pH < 5.8	379	312	405	319	179	0.43	0.67	0.83
pH < 5.5	110	115	141	113	78.5	0.92	0.83	0.66
Area, pH × min/d								
pH < 5.8	82.1	73.8	98.8	84.6	47.43	0.87	0.74	0.89
pH < 5.5	10.2	13.5	16.5	21.0	10.42	0.79	0.33	0.94
Ruminal VFA[3], m*M*								
Acetate	100.9	100.8	101.5	94.5	4.08	0.61	0.36	0.43
Propionate	26.0	25.8	28.2	26.4	2.36	0.62	0.56	0.59
Butyrate	14.8	15.6	15.0	13.9	1.16	0.49	0.38	0.24
Isobutyrate	1.07[a]	1.03[a]	0.98[a]	0.88[b]	0.07	<0.01	<0.01	0.23
Valerate	2.01	2.12	2.22	2.24	0.21	0.36	0.10	0.62
Isovalerate	1.48	1.41	1.32	1.12	0.23	0.39	0.12	0.64
Total VFA	147	147	150	140	6.05	0.09	0.14	0.05
Acetate:Propionate Ratio[3]	4.00	4.03	3.73	3.87	0.37	0.91	0.65	0.88
Ruminal NH_3-N[3], mg/dL	16.7	16.5	17.0	16.3	1.31	0.75	0.69	0.61

[a, b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Values are least squares means obtained from 4 ruminally-cannulated cows.
[2]*P* values indicate overall diet, time and diet × time effects.
[3]There were significant effects of sampling time on ruminal concentrations of NH_3-N, and total and individual VFA, and acetate:propionate ratio ($P < 0.01$). Diet x time of sampling interactions were not significant.

Table 3A.5 Nutrient flow from and digestion in the rumen of dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
DM								
Intake, kg/d	30.2	30.0	30.4	30.1	0.8	0.90	0.92	0.92
Omasal flow, kg/d	23.4	22.3	24.0	24.2	0.6	0.21	0.19	0.32
Apparent digestion, kg/d	6.67	7.36	6.61	6.24	0.70	0.48	0.38	0.32
Apparent digestion, % of DM intake	22.0	24.8	21.5	20.4	2.1	0.51	0.40	0.37
OM								
Intake, kg/d	29.2	29.0	29.5	29.1	0.7	0.76	0.97	0.84
Omasal flow, kg/d	18.7	18.0	19.3	19.5	0.4	0.12	0.09	0.28
Apparent digestion, kg/d	10.7^{a}	10.9^{ab}	9.9^{ab}	9.8^{b}	0.5	0.04	0.01	0.47
Apparent digestion, % of OM intake	35.6	37.3	34.9	33.4	1.3	0.30	0.17	0.27
True digestion, kg/d	18.5	19.2	16.9	17.7	0.58	0.19	0.19	0.81
True digestion, % of OM intake	62.7	63.8	61.4	59.6	1.33	0.22	0.08	0.32
NDF								
Intake, kg/d	10.2^{ab}	9.9^{b}	10.1^{b}	10.6^{a}	0.3	0.01	0.02	<0.01
Omasal flow, kg/d	6.03	6.14	6.14	6.51	0.40	0.75	0.42	0.72
Apparent digestion, kg/d	4.02	3.84	4.00	4.07	0.37	0.97	0.86	0.75
Apparent digestion, % of NDF intake	40.1	38.7	39.2	38.2	3.1	0.97	0.71	0.96
ADF								
Intake, kg/d	5.88^{a}	5.38^{c}	5.64^{b}	5.76^{ab}	0.18	<0.01	0.59	<0.01
Omasal flow, kg/d	3.56	3.31	3.43	3.64	0.18	0.46	0.57	0.26

Apparent digestion, kg/d	2.40	2.06	2.18	2.09	0.20	0.62	0.37	0.55
Apparent digestion, % of ADF intake	40.7	38.3	38.4	36.6	3.1	0.83	0.40	0.91

[a, b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Values are least square means obtained from 4 ruminally-cannulated cows.

[2]*P* values indicate overall diet, linear and quadratic effects.

3A.4.5 Omasal Flow of Nitrogen Fractions and Amino Acids, and Microbial Protein Synthesis

Intake of N was unaffected by diet ($P = 0.24$) and averaged 934 g/d (Table 3A.6). Similarly, N apparently digested in the rumen, when expressed as grams per day ($P = 0.62$) or as a proportion of N intake ($P = 0.22$), did not differ across diets. Although N truly digested in the rumen expressed as grams per day was similar ($P = 0.21$) across treatments, N truly digested in the rumen expressed as a proportion of N intake decreased linearly ($P = 0.02$) with increasing amounts of W-DDGS in the diet. Increasing the level of W-DDGS in the diet resulted in a tendency for a linear decrease ($P = 0.09$) in RDP supply when expressed as grams per day, and a linear decrease ($P = 0.02$) in RDP supply when expressed as a proportion of DM intake. The omasal flow of total N expressed as grams per day or as a proportion of N intake did not differ ($P = 0.14$) with dietary treatment. Ammonia N flowing at the omasum was similar ($P = 0.24$) across diets. Omasal flow of NAN expressed as grams per day tended to increase linearly ($P = 0.09$) with dietary inclusion of W-DDGS. There was a quadratic increase ($P < 0.01$) in the flow of NANBN at the omasum, expressed as grams per day and as a proportion of omasal NAN flow, whereas omasal NANBN flow expressed as a proportion of DM ($P = 0.05$) or N ($P = 0.03$) intakes increased linearly with the inclusion of W-DDGS in the diet. The supply of RUP when expressed as grams per day increased linearly ($P = 0.01$) with the addition of W-DDGS to the diet; however, there was no diet effect ($P = 0.28$) on RUP supply when expressed as a proportion of DM intake. Omasal flows of FAB NAN and PAB NAN when expressed as grams per day were similar ($P > 0.05$) across diets and, thus, the omasal flow of total bacterial NAN (i.e., FAB NAN plus PAB NAN) was not different ($P = 0.31$). However, there was a quadratic response ($P < 0.01$) in the omasal flow of total bacterial NAN when expressed as a proportion of total NAN flow. Efficiency of microbial protein synthesis did not differ ($P = 0.12$) among diets.

Omasal flow of all essential amino acids (EAA) did not differ ($P > 0.05$) among diets (Table 3A.7). However, there was a linear decrease ($P = 0.02$) from 17.0 to 15.9% in the omasal flow of lysine, when expressed as a proportion of total EAA, with increasing levels of W-DGGS in the diet,

Table 3A.6 Intake, digestibility, and omasal flow of N constituents in dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
N intake, g/d	927	947	918	942	34	0.24	0.78	0.88
N apparently digested in the rumen								
g/d	-133	-84	-148	-145	39	0.62	0.56	0.57
% of N intake	-16.2	-5.9	-22.2	-14.3	3.8	0.22	0.46	0.66
N truly digested in the rumen								
g/d	613	634	509	577	32.0	0.21	0.19	0.38
% of N intake	66.3	66.0	58.2	59.5	2.66	0.08	0.02	0.72
RDP supply								
g/d	3,805	3,889	3,413	3.472	216	0.23	0.09	0.94
% of DMI	12.7[a]	13.0[a]	11.0[b]	11.6[ab]	0.49	0.02	0.02	0.55
Flow at omasal canal								
N								
g/d	1,055	1,012	1,110	1,085	28	0.14	0.12	0.72
% of N intake	116	106	123	114	3.41	0.14	0.33	0.88
NH_3-N, g/d	42.4	32.9	45.8	43.0	4.32	0.24	0.46	0.46
NAN								
g/d	1,012	977	1,078	1,042	25	0.06	0.09	0.98
% of N intake	111	103	118	109	3.32	0.14	0.40	0.84
NANBN[5]								
g/d	271[c]	318[b]	336[a]	311[b]	24	<0.01	<0.01	<0.01
% of NAN flow	27.1[c]	32.2[a]	31.7[a]	29.3[b]	2.09	<0.01	0.02	<0.01

% of N intake	29.4	31.5	35.5	36.0	2.70	0.13	0.03	0.70
% of DM intake	0.91	1.03	1.07	1.08	0.080	0.13	0.05	0.29
RUP								
g/d	1,946[bd]	1,998[cd]	2,430[ab]	2,378[ac]	156	0.04	0.01	0.65
% of DM intake	6.64	7.28	7.55	7.52	0.493	0.28	0.12	0.35
FAB[3] NAN								
g/d	401	366	408	360	22.72	0.13	0.37	0.73
% of total bacterial NAN	54.1	53.4	55.6	51.3	2.51	0.49	0.56	0.47
PAB[4] NAN								
g/d	341	330	320	347	22.83	0.84	0.92	0.42
% of total bacterial NAN	45.9	46.6	44.4	48.7	2.51	0.49	0.56	0.47
Total bacterial NAN								
g/d	743	688	733	708	28.31	0.31	0.61	0.59
% of NAN	72.9[a]	67.8[c]	68.3[c]	70.7[b]	2.09	<0.01	0.02	<0.01
Microbial efficiency								
g of microbial N/kg OMTDR[6]	40.8	36.8	40.4	41.2	1.42	0.12	0.37	0.10

[1]Values are least square means obtained from 4 ruminally-cannulated cows.

[2]*P* values indicate overall diet, linear and quadratic effects.

[3]FAB = fluid-associated bacteria.

[4]PAB = particle-associated bacteria.

[5]NANBN = non-NH_3 nonbacterial N.

[6]OMTDR = OM truly digested in the rumen.

Table 3A.7 Omasal amino acid flow (g/d) in dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
Essential AA								
Arginine	222	195	222	207	13.8	0.49	0.78	0.67
Histidine	106	117	119	104	9.94	0.62	0.96	0.21
Isoleucine	257	239	261	248	10.4	0.46	0.94	0.79
Leucine	402	377	439	388	23.7	0.33	0.87	0.59
Lysine	353	322	355	319	14.8	0.35	0.40	0.88
Methionine	108	100	105	110	2.53	0.25	0.38	0.15
Phenylalanine	237	226	260	232	13.6	0.38	0.75	0.56
Threonine	241	242	270	221	17.5	0.25	0.77	0.38
Valine	259	237	279	251	15.5	0.34	0.82	0.85
BCAA[3]	919	853	979	887	47.7	0.45	0.90	0.81
TEAA[4]	2,287	2,170	2,395	2,057	106	0.37	0.47	0.51
Lysine, % EAA	17.0	16.6	16.3	15.9	0.28	0.12	0.02	0.98
Methionine, % EAA	5.34	5.15	4.69	5.58	0.234	0.18	0.86	0.23
Lysine:methionine	3.56^{ab}	3.53^{ab}	3.67^{a}	3.12^{b}	0.108	0.03	0.04	0.04
Non-essential AA								
Alanine	329	305	351	312	16.0	0.24	0.97	0.65
Asparagine	531	516	572	513	29.7	0.62	0.98	0.55
Cysteine	68.1^{b}	68.8^{ab}	78.8^{ab}	80.8^{a}	2.96	0.03	<0.01	0.84
Glutamate	782^{b}	846^{ab}	980^{a}	947^{ab}	41.3	0.03	<0.01	0.27
Glycine	252	266	276	215	18.4	0.15	0.50	0.38

Proline	238	242	288	273	12.5	0.07	0.02	0.43
Serine	214	205	235	215	12.7	0.44	0.56	0.65
Tryptophan	66.9	63.5	75.9	70.4	5.24	0.42	0.35	0.84
Tyrosine	192	175	192	176	6.37	0.35	0.45	0.97
TNEAA[5]	2,609	2,597	2,960	2,769	125	0.21	0.17	0.49
Total AA	4,855	5,004	5,435	4,549	285	0.28	0.78	0.30

[1]Values are least squares means obtained from 4 ruminally-cannulated cows.

[2]*P* values indicate overall diet, linear and quadratic effects.

[3]BCAA = branched-chain AA (isoleucine + leucine + valine).

[4]TEAA = total essential AA.

[5]TNEAA = total nonessential AA.

whereas omasal flow of methionine, when expressed as a proportion of total EAA, did not change, averaging 5.19%. Consequently, the inclusion of W-DDGS in the diet had a quadratic effect ($P = 0.04$) on the lysine:methionine ratio in omasal digesta. The addition of W-DDGS to the diet resulted in a linear increase ($P \leq 0.02$) in the omasal flow of cysteine, glutamate, and proline, whereas the flow of the other NEAA did not differ ($P > 0.05$).

3A.4.6 Excretion and Total Tract Digestibility of Nutrients

There were no differences in total urinary N excretion when expressed as grams per day ($P = 0.78$) or percent of N intake ($P = 0.36$; Table 3A.8). However, the dietary inclusion of W-DDGS had a quadratic ($P = 0.01$) effect on urinary urea-N excretion when expressed as grams per day, whereas urinary urea-N excretion when expressed as a percent of urinary N was unaffected by diet. When expressed as grams per day, there was a tendency ($P = 0.06$) for a linear increase in fecal N excretion with increasing levels of W-DDGS, but fecal N excretion when expressed as a percent of N intake was similar ($P = 0.59$) across diets. Total N excretion when expressed as grams per day was not different across diets ($P = 0.14$); however, there was a quadratic change ($P = 0.03$) in total N excretion when expressed as a percent of N intake. There were no differences ($P > 0.05$) among diets for apparent total-tract digestibilities of DM, OM, CP, ADF and NDF (Table 3A.8).

3A.5 Discussion

It is well-established that the type, amount and quality (e.g., true protein vs. non-protein nitrogen) of protein supplements that are included in a diet are key factors which can influence ruminal fermentation, and the flow of microbial NAN and NANBN to the small intestine in dairy cows (Clark et al., 1992; Ipharraguerre and Clark, 2005). In western Canada and parts of the USA, dairy cow diets typically contain CM as the principal source of protein because it is readily available and is a high quality protein supplement (Hickling, 2008; Mulrooney et al., 2009). However, a rapid expansion of the ethanol industry in western Canada has resulted in large quantities of W-DDGS becoming available for feeding dairy cows. Major differences in the chemical compositions and ruminal degradabilities of CM and W-DDGS have been reported (Boila and Ingalls, 1994), so the major objective of the current study was to determine the impact

Table 3A.8 Urinary N excretion, fecal excretion and apparent total tract digestibilities in dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
N intake, g/d	908	954	906	947	32.1	0.19	0.39	0.89
Urinary excretion								
Total, kg/d	40.7	42.2	42.0	44.4	4.26	0.40	0.20	0.78
Total N, g/d	374	350	342	356	22.5	0.78	0.56	0.42
Total N, % of N intake	40.3	38.0	36.6	38.2	1.36	0.36	0.20	0.22
Urea-N, g/d	270[a]	264[a]	240[b]	265[a]	23.1	0.01	0.07	0.01
Urea-N, % of urinary N	72.5	73.0	74.4	71.1	5.07	0.97	0.91	0.71
Fecal excretion								
DM, kg/d	9.04	9.34	9.23	9.47	0.19	0.14	0.05	0.78
OM, kg/d	8.11	8.53	8.55	8.70	0.16	0.18	0.09	0.30
N, g/d	231	241	247	249	6.5	0.21	0.06	0.53
N, % of N intake	25.5	25.5	26.7	26.9	0.92	0.59	0.22	0.95
Total N excretion								
g/d	628	610	555	599	18.3	0.14	0.12	0.13
% of N intake	66.7	62.7	63.4	64.9	1.1	0.10	0.33	0.03
Total tract apparent digestibility, %								
DM	69.2	69.1	69.6	68.7	0.41	0.52	0.60	0.37
OM	70.6	70.6	71.9	70.2	0.33	0.15	0.96	0.12
CP	74.5	74.5	73.3	73.2	0.92	0.60	0.24	0.96
ADF	45.0	42.6	47.0	45.3	1.73	0.28	0.48	0.85
NDF	46.5	45.0	48.1	47.0	1.35	0.24	0.33	0.82

[1]Values are least squares means obtained from 4 ruminally-cannulated cows.
[2]*P* values indicate overall diet, linear and quadratic effects.

of replacing CM as the major protein source with W-DDGS on microbial protein synthesis and omasal flow of N fractions. In the current study, CM, which is primarily a RDP source with an excellent AA profile, was completely replaced with W-DDGS, which provides a greater proportion of RUP, in dairy diets that were formulated to be isonitrogenous. Therefore, differences between CM and W-DDGS in ruminal protein degradability and AA composition were expected to have an impact on ruminal digestion and omasal flow of N fractions.

In the present study, we used the omasal sampling technique (Huhtanen et al., 1997) to collect omasal digesta. This procedure involved the insertion into the omasal canal via the ruminal cannula of a sampling tube that was connected to a compressor/vacuum pump for aspirating omasal digesta. Once inserted into the omasal canal, 12 spot samples of omasal digesta were then collected at 6-h intervals over a 3-d collection period such that the sampling times were equally distributed over a 24-h feeding cycle to ensure representative sampling. Our observations of animal behavior indicated that omasal sampling had very minimal effects on the cows, and this is supported by the high DM intakes and high levels of milk production. Omasal digesta flow was determined using the triple-marker method (France and Siddons, 1986). Estimates of digesta flow at the omasum that are obtained using omasal sampling can be erroneous if marker dysfunction occurs (Huhtanen et al., 2010). In the present study, the mean concentration of Cr in FP was 7.7 and 15.4 times greater than in SP and LP, respectively, whereas the Yb concentration in SP was 2.5 and 2.9 times greater than in FP and LP, respectively. The mean iNDF concentration was 6.3 times greater in LP than in SP. These results clearly indicate that the concentrations of the 3 principal markers were distinctly greater in their respective digesta phases, so marker dysfunction was likely not a problem. Titgemeyer (1997) suggested that omasal sampling data can be validated by determining how well it compares with reliable estimates published in the literature and whether it falls within biological limits. Our estimates of omasal flows of DM and OM ranged from 22.3 to 24.2 kg/d and 18.0 to 19.5 kg/d, respectively. These estimates are greater than those from published studies using similar marker techniques and omasal sampling (see Brito et al., 2006; Brito et al., 2007a, b; Reynal and Broderick, 2005), possibly reflecting the much greater DM intakes (30 kg/d) that were observed in the present study compared to those previous studies in which DM intakes were usually ≤24

kg/d. Greater DM intakes result in high passage rates and high omasal flows of DM and OM (Brito et al., 2006). Apparent digestion of DM in the rumen, when expressed as absolute amounts or as a proportion of intake, were unaffected by diet and ranged from 6.24 to 7.36 kg/d and 20.4 to 24.8%, respectively. The proportion of DM intake that was apparently digested in the rumen in the present study was lower than data obtained by other workers in dairy cows (33 to 36%, Brito et al., 2006; 36 to 38%, Olmos Colmenero and Broderick, 2006a; 35 to 42%, Brito et al., 2007b). Again, these discrepant results likely reflect the large differences in DM intakes among studies; the much greater DM intakes in our study resulted in high passage rates and, consequently, lower extents of ruminal digestion (Brito et al., 2006). When expressed as a proportion of OM intake, the apparent and true digestion of OM in the rumen ranged from 33.4 to 37.3% and 59.6 to 63.8%, respectively, and these data fall within the ranges of 30 to 60% and 40 to 70% for ruminal apparent and true digestion, respectively, which have been suggested to be typical (Titgemeyer, 1997). There were no dietary effects on apparent ruminal or total tract NDF and ADF digestion; however, it should be noted that apparent ruminal NDF digestion (mean = 39.1%) represented 83% of total tract NDF digestion (mean = 46.7%). Titgemeyer (1997) suggested that at least 80% of total tract NDF digestion should occur in the rumen when ruminants are fed by-products and good quality forage. Because our estimates of the proportion of dietary NDF that was digested in the rumen compare favorably with estimates that were derived using other experimental techniques (e.g., duodenal sampling) and modeling approaches (see Titgemeyer, 1997; Huhtanen et al., 2010), this suggests that problems with unrepresentative omasal digesta sampling or marker dysfunction were not encountered in the present study; thus, we can surmise that our measurements of omasal flow are reliable.

The dietary inclusion of W-DDGS in the current study had no effect on N intake, reflecting the similar dietary CP contents and DM intakes for the 4 ruminally-cannulated cows that were used in the metabolism study. As a consequence of similar N intakes, the flow of N at the omasal canal was similar across diets. Others (Brito et al., 2007a, b; Brito et al., 2009) have also reported omasal N flow to parallel N intake. The amount of dietary N that was apparently digested in the rumen did not change and was negative for all diets, indicating that omasal N flow was greater than N intake. In a meta-analytical study (Broderick et al., 2010) involving 32 experiments in which cattle were fed diets containing (% of DM) 9.9 to 23.7% CP, 7.5 to 17.7%

RDP and 2.3 to 9.8% RUP, it was estimated that zero ruminal N balance (i.e., when omasal N flow = N intake) occurred when dietary CP and RDP contents were 14.7 and 10.6%, respectively, and ruminal N balance became more positive as dietary CP and RDP contents increased. In the present study, mean dietary CP and RDP contents were 18.9 and 12.1%, respectively, so ruminal N balance would be expected to be positive based on the observations of Broderick et al. (2010). Others (Reynal et al., 2003; Olmos Colmenero and Broderick, 2006a) have also reported negative apparent N digestion in the rumen when dairy cows were fed diets containing >18% CP and this can be attributed to the N contribution of recycled urea-N in the rumen (Broderick et al., 2008). Even when dietary CP content exceeds 17%, our research group has demonstrated that substantial amounts of urea-N can be recycled to the rumen (Gozho et al., 2008). When ruminal N digestion was corrected for omasal outflow of microbial NAN, the amount of dietary N that was truly digested in the rumen was also unchanged by diet; however, when expressed as a proportion of N intake, there was a linear decrease in true N digestion in the rumen as CM was replaced as the major protein source with increasing amounts of W-DDGS. As a result, the supply of RDP decreased linearly from 3,805 g/d to 3,472 g/d for cows fed the control (0% W-DDGS) and 20% W-DDGS diets, respectively, representing a decrease from 12.7 to 11.6% when RDP supply is expressed as a proportion of DM intake. This response in RDP supply largely reflects the higher RUP content of W-DDGS (54% of CP; Nuez-Ortín and Yu, 2009) compared to CM (40% of CP; Piepenbrink and Schingoethe, 2009). A decrease in dietary RDP supply below the recommended 9.5 to 10.5% of DM (NRC, 2001) can depress microbial protein synthesis due to a deficiency in ruminal NH_3-N, and total free AA and peptides (Broderick et al., 2007). A shortcoming of the present study is that diets contained nearly 19% CP, primarily because of the high CP content of W-DDGS which made it difficult to formulate diets containing lower CP contents. Because of the high dietary CP levels, RDP supply in diets containing increasing levels of W-DDGS was still above the current recommended levels (NRC, 2001) which would mask any potential responses to changes in RDP supply. With lower CP diets, Reynal and Broderick (2005) reported greater microbial protein yields when RDP supply was increased. Further evidence that RDP supply was not limited by the dietary inclusion of W-DDGS is provided by the fact that ruminal NH_3-N concentrations did not change with diet and, at all sampling times (data not shown) during a 24-h feeding cycle, were always greater than 5

mg/dL which has been suggested as the minimum ruminal NH_3-N concentration that is required for maximum microbial protein synthesis (Satter and Slyter, 1974). Although other studies (Balcells et al., 1993; Reynal and Broderick, 2005) have reported higher concentrations of ruminal NH_3-N (11 mg/dL) as being needed to optimize microbial protein synthesis, it is noteworthy that ruminal NH_3-N concentrations on all diets were always above 10 mg/dL at all sampling times during a 24-h feeding cycle and it is unlikely that replacing CM with W-DDGS would have caused a ruminal NH_3-N deficiency. Although ruminal NH_3-N is the major source of N for microbial growth (Brito et al., 2007a), there is clear evidence that the ruminal availability of preformed AA and peptides can stimulate microbial growth in vivo (Chikunya et al., 1996) and in in vitro cultures (Argyle and Baldwin, 1989; Carro and Miller, 1999). In the current study, we did not measure ruminal AA and peptide concentrations, but it is unlikely that the ruminal availability of AA and peptides could have limited microbial growth because RDP supply was well within the recommended levels (NRC, 2001). Clearly, more research is warranted in which dairy cows are fed diets containing W-DDGS and formulated to contain 16-17% CP.

Omasal flow of NAN, when expressed as grams per day, tended to increase linearly when increasing amounts of W-DDGS were added to the diet. Because N intakes and omasal flow of microbial NAN were similar across diets, this response can be attributed to greater amounts of dietary protein escaping ruminal degradation with increasing amounts of W-DDGS being included in the diet. Also, we observed a quadratic increase in the omasal flow of NANBN, which is collectively composed of RUP and endogenous protein. The omasal flow of NANBN accounted for 24 to 28.6% of total NAN flow, which falls within the range that has been reported by others (Brito et al., 2006; Brito et al., 2007a, b). Because RUP is the major contributor to NANBN flow, the increased supply of RUP when increasing amounts of W-DDGS were added to the diet accounts for the greater omasal flows of NANBN with W-DDGS.

Maximizing the intestinal supply of microbial protein is important for high-producing dairy cows, so a major objective of the current study was to determine the effects of replacing CM with W-DDGS on microbial NAN supply. Omasal flows of FAB NAN and PAB NAN, when expressed as grams per day, were similar across diets and, thus, the omasal flow of total bacterial NAN (i.e., FAB NAN plus PAB NAN) was not different. The lack of significant effects of replacing CM with W-DDGS on bacterial NAN supply likely reflects that 11 to 12.7% RDP in

diets containing CM or W-DDGS was adequate to support ruminal microbial growth. Not surprisingly, across all diets, 71 to 76% of total NAN in omasal digesta was of bacterial origin. Although the relative contribution of bacterial NAN to total NAN flow can vary considerably depending primarily on dietary and ruminal conditions that influence microbial growth, this range is in close agreement with data obtained by other workers in dairy cows (69 to 74%, Olmos Colmenero and Broderick, 2006a; 67 to 79%, Brito et al., 2007a). When expressed as a proportion of total bacterial NAN flow, omasal flows of PAB NAN and FAB NAN contributed 44 to 49% and 46 to 51%, respectively, of total bacterial NAN flow and did not differ with diet. In other studies, PAB NAN and FAB NAN contributed, respectively, 55 and 45% (Brito et al., 2006) and 57 and 43% (Reynal and Broderick, 2005) of total bacterial NAN flow, which is in close agreement with our data. To our knowledge, this is the first study reporting the effects of replacing CM with W-DDGS on omasal flows of total NAN and bacterial NAN, so direct comparisons of our data with other published data with W-DDGS are not possible. Furthermore, only a few studies (Janicek et al., 2008 and Kelzer et al., 2009) have reported effects on microbial N flow to the small intestine when C-DDGS is included in diets fed to dairy cows. Although the inclusion of 30% C-DDGS reduced RDP supply, duodenal microbial N flow was not suppressed (Janicek et al., 2008). Similarly, Kelzer et al. (2009) did not observe any detrimental effects of feeding 15% C-DDGS on duodenal microbial N supply. In the present study, our estimates of omasal flows of bacterial NAN ranged from 688 to 743 g/d and were greater than microbial N yields that were reported previously in dairy cows fed C-DDGS (158 to 200 g/d, Kelzer et al., 2009; 256 to 270 g/d, Janicek et al., 2008). This discrepancy in results could be accounted for, among other numerous factors, by methodological differences in markers that were used to measure microbial NAN yields (Brito et al., 2006). Janicek et al. (2008) and Kelzer et al. (2009) estimated microbial N supply indirectly by using urinary excretion of purine derivatives, whereas ^{15}N was used in the current study. It is clearly established that using urinary purine derivative excretion grossly underestimates microbial yields when compared to ^{15}N as a microbial marker (Reynal et al., 2005; Brito et al., 2006). Additionally, there is a positive correlation between post-ruminal microbial N flow and DM intake (Moorby et al., 2006; Broderick et al., 2010). Cows in the present study consumed 4.0 to 6.7 kg/d more DM compared to those in the studies of Janicek et al. (2008) and Kelzer et al. (2009), and these large

differences in DM intakes could partly account for the disparity in estimates of microbial N supply. In numerous studies conducted with lactating dairy cows under a wide range of dietary conditions, estimates of microbial NAN supply obtained using ^{15}N as a microbial marker and omasal sampling ranged from 375 to 480 g/d (Olmos Colmenero and Broderick, 2006a; Brito et al., 2006; Brito et al., 2007b), which are still lower relative to estimates reported in the current study. Again, large differences in DM intake and other factors (e.g., source and amount of both dietary N and carbohydrate, ruminal dilution rate, ruminal pH) that influence ruminal microbial growth account for discrepant estimates of microbial NAN flow. Microbial NAN flows determined with omasal sampling and using an HPLC or spectrophotometric assay for purine analysis ranged from 239 to 717 g/d and 302 to 740 g/d, respectively (Reynal et al., 2003), thus indicating that our estimates are not unrealistic.

Microbial efficiency averaged 39.8 g/kg of OMTDR across diets in the current study and was higher than the range of 13.5 to 30.8 g/kg of OMTDR that was reported in a meta-analytical review of ruminal N metabolism data from studies using the omasal sampling technique under varied dietary conditions (Broderick et al., 2010). A major determinant of microbial efficiency is DM intake. An increase in DM intake leads to a decrease in the rumen retention time of microbes and, thus, a decrease in microbial maintenance energy requirements which would then result in an increase in microbial efficiency (Russell et al., 1992). In the present study, DM intake was greater than the range of 6.0 to 27.4 kg/d in DM intake that was reported in those studies used in the meta-analysis by Broderick et al. (2010), thus possibly accounting for our greater estimates of microbial efficiency. Microbial efficiency was not suppressed when W-DDGS was added to diets. Besides a requirement for N, rumen microbes also require energy primarily supplied by the fermentation of OM (Clark et al., 1992; Ipharraguere et al., 2005). Feeding W-DDGS had no effect on OM truly digested in the rumen, which averaged 62% across dietary treatments, so we can surmise that energy availability did not limit microbial growth in diets containing W-DDGS.

Although W-DDGS is a good source of digestible RUP, its content of lysine is especially lower when compared to CM (Boila and Ingalls, 1994). Together, lysine and methionine are the two most limiting amino acids for milk and milk protein production on a wide variety of diets that are typically fed to dairy cows in North America (Schwab et al., 1992), so the major

challenge when feeding W-DDGS as a substitute for CM is providing a sufficient amount of lysine (Boila and Ingalls, 1994; Mulroney et al., 2009; Schingoethe et al., 2009). Because dietary lysine content decreased from 0.926 to 0.593% of DM when dietary W-DDGS was increased from 0 to 20% W-DDGS (reflecting the lower lysine content of W-DDGS compared to CM), and because DM intakes were similar for cows that were used in the metabolism study, lysine intake (data not shown) decreased when W-DDGS replaced CM. We had anticipated that this decrease in lysine intake coupled with a higher RUP supply primarily from lysine-deficient W-DDGS would result in a decrease in lysine supply at the omasal canal; however, this was not the case as omasal lysine flows were unaffected by diet and the reasons for these observations are not clear. Amino acids in omasal digesta are primarily contributed by microbial NAN and feed protein that escapes ruminal degradation (i.e., RUP), plus small amounts of endogenous protein (NRC, 2001). As microbial NAN supply was not altered by diet, we can surmise that omasal lysine supply from that source was roughly equal across diets; consequently, it is plausible that a greater RUP supply as more W-DDGS was fed could have increased omasal lysine supply. Both methionine intake and omasal flow were unaffected when W-DDGS replaced CM. Based on NRC (2001), the ideal lysine:methionine ratio for optimum use of MP for both maintenance and milk protein production is 3:1. Across dietary treatments, the lysine:methionine ratio in omasal digesta ranged from 3.12:1 to 3.67:1, well above the NRC (2001) recommendation and suggesting that both lysine and methionine were not limiting in the present study. However, it should be noted that these lysine:methionine ratios were calculated from measured omasal flows of lysine and methionine, and do not account for intestinal digestibility.

When DM intakes for all 8 cows that were used in the study were included in the statistical analysis, feeding W-DDGS resulted in a linear increase in DM intake. This response in DM intake is somewhat different from that observed in the metabolism study (with only 4 cows) where no differences in DM intake were observed, possibly reflecting the differences in sample size. In a limited number of studies, the inclusion of W-DDGS (Zhang et al., 2010b) or a corn/wheat-based DDGS (produced from 70% corn and 30% wheat; Zhang et al., 2010a) at 20% of diet DM increased DM intake by 3.0 to 3.6 kg/d; however, it should be noted that these studies did not involve feeding graded levels of DDGS. In the current study, the inclusion of up to 20% W-DDGS linearly increased DM intake by 0.3 to 2.1 kg/d, a response that was lower

than that reported in the studies of Zhang et al. (2010a,b). This difference in the magnitude of responses in DM intakes when W-DDGS is included in the diet could possibly be attributed to the much lower DM intakes for control cows in those previous studies (~21.3 kg/d) compared to the current study (29.7 kg/d), which could have limited responses in DM intake as it was already near maximal. In a study by Janicek et al. (2008), the partial replacement of both corn silage and concentrates with C-DDGS at 10, 20, and 30% of diet DM linearly increased DM intake, thus supporting our observations in the current study. It is not clear why DDGS increases DM intake when it partially replaces forage or concentrate in dairy diets. When compared to forages, DDGS has a smaller particle size (Zhang et al., 2010b). Because ration particle size is inversely related to DM intake (Kononoff and Heinrichs, 2003), the inclusion of DDGS as a partial replacement for forage could increase DM intake due to faster rates of passage (Allen and Grant, 2000). However, in the study by Zhang et al. (2010b), cows fed DDGS as a partial replacement for forage exhibited a slower rate of passage yet their DM intake was greater than in control animals. In the current study, greater DM intakes for cows fed W-DDGS were not accompanied by greater omasal flows or decreased total-tract nutrient digestibility, thus suggesting that faster rates of passage were not responsible for the greater DM intakes that were observed. It is possible that the effects of including DDGS on DM intake will depend on the type of feed ingredient that is replaced (Janicek et al., 2008). It should be noted that other studies have observed that partially replacing concentrate with DDGS had no effect on DM intake (Sasikala-Appukuttan et al., 2008; Mulrooney et al., 2009; Mjoun et al., 2010; Christen et al., 2010) or tended to reduce DM intake (Anderson et al., 2006).

In the current study, actual milk production increased by 1.2 to 1.8 kg/d when W-DDGS replaced CM. These observations support previous studies in which the inclusion of W-DDGS (Zhang et al., 2010b) or a corn/wheat-based DDGS as a partial replacement for barley silage or concentrate increased milk yield by 2.8 to 3.4 kg/d, although the magnitude of response was different. Others (Anderson et al., 2006; Janicek et al., 2008) also reported that feeding increasing amounts of C-DDGS resulted in more milk production, a response that was attributed to corresponding increases in DM intake. In the current study, the 0.3 to 2.1 kg/d increase in DM intake when W-DDGS was fed contributed an estimated 0.5 to 3.2 Mcal of additional NE_L intake. Assuming caloric values of 9.29, 5.71 and 3.95 Mcal/kg for fat, protein, and lactose

(NRC, 2001) and using mean milk composition data for cows fed W-DDGS (i.e., fat, protein, and lactose contents of 3.52, 3.30, and 4.55%, respectively; Table 3), the additional NE_L intake would correspond to a 0.3 to 2.2 kg/d increase in milk yield. Therefore, it is likely that the 1.2 to 1.8 kg/d increase in milk yield when cows were fed W-DDGS can partly be attributable to a greater DM intake when compared to control cows. Milk protein content did not differ with diet, possibly reflecting that the dietary inclusion of W-DDGS had no detrimental effects on post-ruminal MP and EAA supply; however, milk protein yield changed quadratically with the inclusion of W-DDGS. Any diet-induced changes in omasal NAN flow as a result of differences in the contributions of either NANBN or microbial NAN flow can result in changes in milk protein yield (Broderick et al., 2010); therefore, the increase in NANBN and NAN flowing at the omasum when feeding W-DDGS could have contributed to the change in milk protein yield. Also, the greater milk protein yield with feeding W-DDGS could be partly attributed to the increase in milk yield. Milk N efficiency ranged from 24.1 to 25.2%, which is typical for cows fed high CP diets (NRC, 2001). Milk fat percentage, which is an indirect indicator of rumen health, did not change when CM replaced W-DDGS. All diets fed in this study were formulated to contain 50% forage and provided an adequate amount of physically-effective fiber to maintain rumen function and prevent milk fat depression. Penner et al. (2009c) did not observe changes in either chewing activity or milk fat concentration when feeding 10% W-DDGS as a partial replacement for concentrate in diets that contained 50% forage. According to Schingoethe et al. (2009), up to 40% DDGS can be included in dairy rations without causing milk fat depression, provided the diets contain an adequate amount of physically-effective fiber. Feeding DDGS-containing diets with forage and forage NDF contents below 50 and 22%, respectively, can result in milk fat depression (Kalscheur, 2005). Zhang et al. (2010b) reported a decrease in ruminal pH and milk fat concentration as a result of a decrease in dietary forage NDF and physically-effective fiber content when W-DDGS replaced barley silage.

Wheat-based DDGS is high in NDF that is highly-digestible, so it can be considered as a non-forage fiber source (Zhang et al., 2010b). In conjunction with its very low starch content, these characteristics of W-DDGS have led to recent studies (Zhang et al., 2010a,b) that have examined if feeding W-DDGS as a partial replacement for forage or concentrate could potentially attenuate ruminal acidosis in dairy cows. Results from these studies indicate that

when DDGS replaced barley silage or barley grain, ruminal acidosis was unaffected (Zhang et al., 2010a); however, partially replacing barley silage with DDGS worsened ruminal acidosis in a separate study (Zhang et al., 2010b). There is a general consensus that feeding DDGS as a partial replacement for barley silage or high starch concentrates has limited capacity in attenuating ruminal acidosis (Zhang et al., 2010a,b; Beliveau and McKinnon, 2009) because the small particle size of DDGS decreases chewing activity and, in turn, salivary secretion. In the present study, W-DDGS replaced portions of protein ingredients and, not surprisingly, had no effect on ruminal pH. Ruminal fermentation patterns were largely unaffected by the addition of W-DDGS. The lack of diet effects on ruminal acetate, butyrate, propionate, and total VFA concentrations following the inclusion of W-DDGS is in agreement with observations made in other studies (Anderson et al., 2006; Christen et al., 2010; Zhang et al., 2010a). In the present study, isobutyrate concentrations were lower in cows fed W-DDGS compared to CM, thus supporting results from other studies (Zhang et al., 2010b). The higher BCAA intake in cows fed CM compared to those fed W-DDGS (data not shown) could have provided more precursors for branched chain fatty acid synthesis (Johnson et al., 1994).

Although isonitrogenous diets were fed in the present study, changes in ruminal protein degradability were expected to have an impact on N excretion. Increasing the amount of W-DDGS in the diet had no effect on total urinary N excretion, possibly reflecting the similar N intake across diets as a positive correlation exists between N intake and urine N loss (Castillo et al., 2001). Fecal excretion of N tended to increase linearly with the inclusion of W-DDGS. Cows fed W-DDGS also excreted 64% of consumed N in feces and urine, on average, which was lower than the 67% that was observed for cows receiving 0% W-DDGS, possibly reflecting the decrease in RDP supply with W-DDGS. Overall, the impact of feeding W-DDGS as a substitute for CM on N loss to the environment was minimal.

3A.6 Conclusions

Based on results from this study, W-DDGS can completely substitute for CM as the major protein source and be used as the main protein supplement in lactating cow diets. Inclusion of W-DDGS had no detrimental effects on ruminal fermentation, microbial protein production, and flow of nutrients at the omasal canal. Cows fed diets containing W-DDGS exhibited greater

DM intakes and milk yields when compared to those fed CM as the major protein source. In conclusion, up to 20% W-DDGS can be added to lactating cow rations without negatively affecting ruminal function and can potentially increase DM intake and milk yield.

3B. REPLACING CANOLA MEAL AS THE MAJOR PROTEIN SOURCE WITH WHEAT DRIED DISTILLERS GRAINS WITH SOLUBLES IN DAIRY COW DIETS. II. EFFECTS ON OMASAL FATTY ACID FLOW, AND MILK FATTY ACID COMPOSITION[1]

3B.1 Abstract

Wheat dried distillers grains with solubles (W-DDGS) has a high fat content and is now commonly fed to dairy cows in western Canada. The objective was to determine the effects of replacing canola meal (CM) with W-DDGS on omasal fatty acid (FA) flow and milk FA composition. Four ruminally-cannulated lactating dairy cows were used in a 4 × 4 Latin square design with 28-d periods. Cows were fed either a standard barley silage-based TMR (0% W-DDGS, control) or TMR containing 10, 15 and 20% W-DDGS, with W-DDGS replacing CM as the major protein source. The omasal flow of C18:1 *trans*-10, C18:1 *trans*-11, C18:2n-6, C18:3n-3, conjugated linoleic acid (CLA) *cis*-9, *trans*-11, and total CLA increased linearly with increasing levels of W-DDGS. There were quadratic changes in milk concentrations of total C18:2, C18:3n-3, total n-3 polyunsaturated fatty acid (PUFA), and total n-6 PUFA as the level of W-DDGS increased. Feeding increasing amounts of W-DDGS resulted in a linear increase in milk concentrations of CLA *cis*-9, *trans*-11, CLA *trans*-11, *trans*-13, and total CLA, whereas milk concentrations of C18:1 *trans*-11 tended to increase linearly when level of W-DDGS increased. Feeding increasing amounts of W-DDGS resulted in quadratic changes in milk concentrations of C18:2n-6 and C18:3n-3. In summary, feeding increasing amounts of W-DDGS increased milk concentrations of key FA like C18:2n-6, C18:3n-3, CLA *cis*-9, *trans*-11, and total CLA, which can potentially improve human health.

[1]A version of this chapter has been published: Chibisa, G. E., Christensen, D. A., and Mutsvangwa, T. 2013. Replacing canola meal as the major protein source with wheat dried distillers grains alters omasal fatty acid flow and milk fat composition in dairy cows. Can. J. Anim. Sci. 93:137-147.

3B.2 Introduction

In western Canada, wheat is a major feedstock that is being used for ethanol production because it is readily available at a relatively low cost when compared to corn [Canadian International Grains Institute (CIGI), 2011]. As a by-product of ethanol production, large quantities of wheat-based dried distillers grains with solubles (W-DDGS) are now available as livestock feed. Although W-DDGS is high in neutral detergent fibre [NDF; mean 38.8% of dry matter (DM); CIGI, 2011], the NDF is highly-digestible (Nuez-Ortín and Yu, 2009) and this would increase its energy value for dairy cows. Additionally, W-DDGS is relatively high in crude fat (CF; mean 5.4% of DM; CIGI, 2011). Thus, W-DDGS is an attractive energy source that can increase the energy density of dairy cow diets, and feeding it to dairy cows has been reported to increase milk production (Zhang et al., 2010; Chibisa et al., 2012).

In western Canada and parts of the USA, solvent-extracted canola meal (CM) is the principal source of protein that is included in dairy diets; therefore, the increased utilization of W-DDGS in dairy diets will be displacing CM. When compared to CM, W-DDGS is greater in CF content (5.4 vs. 3.5%; CIGI, 2009, 2011). Therefore, the replacement of CM with increasing amounts of W-DDGS can increase dietary content of CF, which might negatively affect DM intake, milk production, and milk fat concentration (NRC, 2001). Additionally, wheat oil is a rich source of polyunsaturated fatty acids (PUFA), particularly linoleic acid (C18:2n-6; Becker, 2008). Because of the high PUFA content, feeding increasing amounts of W-DDGS can disrupt ruminal biohydrogenation (BH) of PUFA and result in the ruminal accumulation of various BH intermediates (Shingfield et al., 2010). Of the BH intermediates that have been demonstrated to play a role in milk fatty acids (FA) synthesis, C18:1 *trans*-10 (Bauman and Griinari, 2001) and conjugated linoleic acid (CLA) *trans*-10, *cis*-12 (Baumgard et al., 2001; Peterson et al., 2003) have been shown to be potent inhibitors of milk FA synthesis in dairy cows, thus resulting in milk fat depression (MFD). Feeding increasing levels of corn-based DDGS has been reported to linearly decrease milk fat content in cows (Leornadi et al., 2005). Under the Canadian milk supply management system, milk quota is measured in kg of milk fat and the multiple-component pricing system also puts a price premium in milk fat. Therefore, MFD in dairy herds fed W-DDGS would be undesirable for economic reasons; however, there is very limited information on how feeding increasing amounts of W-DDGS can alter milk fat content.

On the other hand, the high PUFA content of W-DDGS and its impact on ruminal BH can positively influence milk FA composition. Among the PUFA, oleic (C18:1), C18:2n-6, and linolenic (C18:3n-3) acids have been demonstrated to improve human health (see review by Wang et al., 2011). Of the ruminal BH intermediates that are of biological importance, some specific isomers of CLA (particularly *cis*-9, *trans*-11) and vaccenic acid (C18:1 *trans*-11) can also have potential benefits on human health (Wang et al., 2011) when their content in milk fat is increased. It is well-established that milk *cis*-9, *trans*-11 CLA can be derived from two main sources: 1) *cis*-9, *trans*-11 CLA produced from incomplete ruminal BH of dietary C18:2n-6 (Harfoot and Hazlewood, 1988); and 2) *de novo* mammary synthesis of *cis*-9, *trans*-11 CLA by desaturation of C18:1 *trans*-11 (Griinari et al., 2000). Therefore, feeding increasing amounts of W-DDGS can potentially increase milk content of CLA. To our knowledge, only one study (Zhang et al., 2010) has examined the effects of the inclusion of W-DDGS on milk FA composition in dairy cows. In that study, the dietary inclusion of 20% W-DDGS as a partial replacement for barley silage reduced milk fat concentration and increased milk concentrations of C18:2n-6; however, there were no changes in milk concentrations of any of the key ruminal BH intermediates (i.e., C18:1 *trans*-10, C18:1 *trans*-11, CLA *cis*-9, *trans*-11 or *trans*-10, *cis*-12). To our knowledge, there are no studies that have examined the effects of replacing CM with increasing amounts of W-DDGS on changes in ruminal BH and milk FA composition. Therefore, the objective of this study was to evaluate the effects of replacing CM with increasing amounts of W-DDGS on omasal flow of FA and milk FA composition in dairy cows.

3B.3 Materials and Methods

All experimental procedures that were used in this study were approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048), and were in accordance with the Canadian Council of Animal Care (1993) guidelines.

3B.3.1 Animals, Experimental Design and Treatments

Four ruminally-cannulated Holstein cows (719 ± 71 kg BW; 111 ± 49 DIM) housed at the Greenbrae Dairy Research Facility (University of Saskatchewan) were used in this study, which was part of a larger study that was designed to determine the effects of replacing CM with

W-DDGS on milk production and N metabolism (Chibisa et al., 2012). The experiment was designed as a 4 × 4 Latin square consisting of 28-d experimental periods with the last 8 d used for data and sample collection. The 4 dietary treatments consisted of a standard barley silage-based diet containing CM as the principal protein supplement (control, 0% W-DDGS) or diets formulated to contain 10, 15 and 20% W-DDGS (DM basis). Detailed descriptions of cow management, feeding, and diet ingredient and chemical composition were reported previously (Chibisa et al., 2012).

3B.3.2 Omasal Digesta, Feed, and Milk Sample Collection

Omasal digesta flow was determined using the triple marker method and the omasal sampling technique as described by Huhtanen et al. (1997), and all protocols for infusion of digesta markers, omasal sample collection, marker analyses, and reconstitution of omasal true digesta (OTD) were reported previously (Chibisa et al., 2012). Samples of TMR were collected daily from d 24 to d 28 and were stored at -20°C for later FA analysis. Experimental cows were milked three times daily at 0430, 1230 and 1900 h and milk weights were recorded. At each milking on d 25 to 28, milk was collected into plastic vials without preservative. Daily milk samples from the 0430, 1230 and 1900 h milkings were then pooled proportionally based on milk yield from each milking and pooled milk samples were stored at -20°C until FA analysis.

3B.3.3 Omasal Digesta, Feed, and Milk Fatty Acid Analysis

A detailed description of TMR and omasal digesta processing prior to analysis was reported previously (Chibisa et al., 2012). In preparation for FA analysis, pooled milk samples were thawed at 38°C in a water bath (AOAC 1990). Milk, TMR, and reconstituted OTD samples were analyzed for FA using procedures described by AlZahal et al. (2009). Briefly, lipids for FA analysis were extracted according to Bligh and Dyer (1959) with minor modifications as described by AlZahal et al. (2009). Extracted FA were methylated using 100 μL of 0.5 *M* sodium methoxide ($NaOCH_3$; Sigma-Aldrich, St. Louis, MO) as a catalyst, and quantification of FA methyl esters was performed using gas-liquid chromatography (GLC; Agilent 6890N, Agilent Technologies, Palo Alto, CA) equipped with a split/splitless injector, a flame ionization detector at 250°C (Agilent Technologies), and a capillary column (CP-Sil 88 column; 100 m, 0.25 mm,

0.2 µm of film thickness, Varian Inc., Mississauga, Ontario, Canada), with GLC conditions as described by AlZahal et al. (2009). Hydrogen was used as a carrier gas at a column flow rate of 1 mL/min. Identification of FA peaks was by comparison of retention times with known FA methyl ester standards (GLC #463, #UC-59-M, 21:0, 23:0, and 26:0) that were obtained from Nu-Chek Prep Inc. (Elysian, MN). The individual isomers of C18:1 and C18:2 FA were identified as described by AlZahal et al. (2009).

3B.3.4 Statistical Analysis

Data on FA intake, omasal FA flow, and milk FA composition are reported as least square means ± SEM. Data were analyzed as a 4 x 4 Latin square using the PROC MIXED procedure of SAS (SAS User's Guide 2004) according to the following model: $Y_{ijk} = \mu + P_i + C_j + T_k + E_{ijk}$ where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the random effect of period i, C_j is the random effect of cow j, T_k is the fixed effect of dietary treatment k, and E_{ijk} is the residual error. Orthogonal contrasts were used to test for linear and quadratic effects of level of W-DDGS in the diet. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

3B.4 Results

3B.4.1 Ingredient and Diet Composition

When compared to CM, W-DDGS contained more CF, C16:0, C18:2n-6, C18:3n-3 and total PUFA, but less C18:1 (Table 3B.1). Substituting W-DDGS for CM increased the dietary contents of CF, C16:0, C18:2n-6, C18:3n-3 and total PUFA, but dietary content of C18:1 decreased (Table 3B.2). As expected, these differences reflected the differences in CF and FA compositions of CM and W-DDGS.

3B.4.2 Intake and Omasal Flow of FA

Feeding W-DDGS resulted in a linear increase ($P < 0.05$) in CF intake (Table 3B.3). Intakes of C16:0, C18:2n-6, C18:3n-3 and total PUFA increased linearly ($P < 0.05$) as the dietary level of W-DDGS increased.

Table 3B.1 Fatty acid compositions of **c**anola meal and wheat-based dried distillers grains with solubles that were used in formulating experimental diets

	Ingredient	
Composition	Canola meal	W-DDGS[1]
Total fatty acids, g/100 g of DM	2.81	4.68
Fatty acids, mg/g of DM		
C4:0	0.191	0.040
C6:0	0.027	0.027
C8:0	0.023	0.032
C12:0	0.045	0.003
C14:0	0.067	0.106
C14:1 *cis*-9	0.000	0.008
C15:0	0.070	0.114
C16:0	2.57	9.59
C16:1 *cis*-9	0.277	0.080
C17:0	0.020	0.080
C18:0	0.633	0.954
C18:1	14.51	7.04
C18:2n-6	8.32	26.68
C18:3n-3	1.07	1.75
C20:0	0.072	0.081
C20:1 *cis*-11	0.059	0.209
C24:0	0.114	0.000
Total PUFA[2]	9.38	28.43

[1]Wheat-based dried distillers grains with solubles.

[2]Total PUFA = C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-3.

Table 3B.2 Crude fat and fatty acid composition of experimental diets fed to lactating dairy cows

	Diet			
Diet Composition	Control	10% DDGS	15% DDGS	20% DDGS
Crude fat, % of DM	3.0	3.2	3.5	3.7
Fatty acid, mg/g of DM				
C10:0	0.022	0.020	0.021	0.020
C12:0	0.042	0.039	0.040	0.038
C14:0	0.251	0.226	0.253	0.231
C14:1 *cis*-9	0.019	0.017	0.019	0.016
C15:0	0.107	0.060	0.074	0.076
C16:0	6.0	6.5	7.0	7.3
C16:1 *cis*-9	0.114	0.064	0.083	0.065
C18:0	1.3	1.0	1.0	1.0
C18:1 *cis*-9	8.6	7.8	7.9	8.5
C18:1 *cis*-11	1.0	0.5	0.5	0.5
C18:2n-6	8.7	11.6	13.6	14.8
C18:3n-3	3.1	3.5	3.7	3.8
C20:0	0.175	0.154	0.152	0.151
C20:1 *cis*-11	0.017	0.011	0.013	0.013
C20:2n-6	0.151	0.119	0.115	0.126
C20:3n-3	0.037	0.043	0.044	0.045
C22:0	0.165	0.154	0.155	0.152
C24:0	0.193	0.184	0.193	0.188
C26:0	0.070	0.068	0.073	0.078
Total PUFA[1]	12.0	15.3	17.5	18.6

[1]Total PUFA = C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-3.

Table 3B.3 Total crude fat and individual fatty acid intakes (g per d) of dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
Total crude fat intake	941	915	1,058	1,165	43	0.08	0.04	0.11
Fatty acid intake								
C10:0	0.670	0.568	0.643	0.620	0.025	0.13	0.28	0.12
C12:0	1.30	1.11	1.21	1.21	0.05	0.14	0.26	0.09
C14:0	7.83	6.48	7.61	7.25	0.30	0.11	0.37	0.11
C14:1 *cis*-9	0.589	0.480	0.583	0.506	0.022	0.09	0.14	0.27
C15:0	3.33*a*	1.71*b*	2.23*b*	2.38*b*	0.10	0.03	0.03	0.02
C16:0	187	185	212	228	8.6	0.09	0.04	0.16
C16:1 *cis*-9	3.53*a*	1.84*b*	2.47*b*	2.02*b*	0.10	0.03	0.02	0.03
C18:0	38.8	28.4	29.2	30.0	1.2	0.06	0.04	0.04
C18:1 *cis*-9	268	223	240	266	10	0.10	0.53	0.05
C18:1 *cis*-11	31.1*a*	14.6*b*	14.9*b*	16.6*b*	0.7	<0.01	<0.01	<0.01
C18:2n-6	274	335	411	466	17	0.06	0.03	0.75
C18:3n-3	97	99	113	120	4.6	0.09	0.04	0.33
C20:0	5.44	4.43	4.56	4.75	0.19	0.09	0.09	0.06
C20:1 *cis*-11	0.536*a*	0.317*b*	0.375*b*	0.397*ab*	0.016	0.04	0.04	0.02
C20:2n-6	4.68	3.42	3.45	3.97	0.15	0.05	0.06	0.03
C20:3n-3	1.16	1.22	1.32	1.42	0.05	0.10	0.05	0.41
C22:0	5.12	4.43	4.66	4.79	0.19	0.15	0.21	0.09
C24:0	6.02	5.26	5.80	5.91	0.23	0.15	0.60	0.10
C26:0	2.19	1.96	2.21	2.45	0.09	0.09	0.07	0.07
Total PUFA[3]	379*b*	436*ab*	529*ab*	591*a*	23	0.04	0.02	0.75

a,*b* Means within a row with different superscripts differ ($P < 0.05$).
[1]Values are least square means obtained from 4 cows.
[2]*P* values indicate overall diet, linear and quadratic effects.
[3]Total PUFA = C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-3.

The dietary inclusion of W-DDGS resulted in a linear increase ($P < 0.01$) in omasal flow of CF (Table 3B.4). The dietary inclusion of W-DDGS resulted in a linear increase ($P \leq 0.05$) in the omasal flows of C16:0, C18:0, total C18:1 *cis*, total C18:1, total C18:2, C18:3n-3, total CLA, total saturated FA, and total monounsaturated FA (MUFA), whereas the omasal flows of total C18:1 *trans*, total n-3 PUFA, total n-6 PUFA, and total PUFA tended to increase linearly ($P \leq 0.09$). Of the C18:1 FA, omasal flows of *cis*-9, *cis*-12, *cis*-13, *trans*-4, *trans*-6+7+8, *trans*-9, *trans*-10, *trans*-11, *trans*-12, *trans*-13+14, and *trans*-16 increased linearly ($P \leq 0.04$) as the dietary inclusion of W-DDGS increased, whereas there was a tendency ($P = 0.07$) for a quadratic change in omasal flow of *cis*-11. Omasal flows of C18:2n-6, C18:2 *trans*-9, *cis*-12, C18:2 *trans*-9, *trans*-12, CLA *cis*-9, *trans*-11, and CLA *trans*, *trans* increased linearly ($P \leq 0.05$) as the level of dietary W-DDGS increased.

3B.4.3 Milk Fatty Acid Composition

Feeding increasing amounts of W-DDGS resulted in a linear decrease ($P \leq 0.05$) in milk concentrations of C13:0 *iso*, C15:0 *iso*, C16:1 *cis*-9, C17:0, C18:0 *iso*, total <C16, and total branched-chain fatty acids (BCFA), and a tendency for a linear decrease ($P \leq 0.07$) in milk concentrations of C17:0 *iso* and C18:0 (Table 3B.5). There were quadratic changes ($P \leq 0.04$) in milk concentrations of C4:0, C8:0, C10:0, C12:0, C15:0 *anteiso*, C16:0 *iso*, total C18:2, C18:3n-3, C20:1 *cis*-11, C22:0, C24:0, total n-3 PUFA, total n-6 PUFA and total PUFA, and a tendency for quadratic changes ($P \leq 0.10$) in milk concentrations of C13:0, and C14:0 *iso* as the level of dietary W-DDGS increased. Milk concentrations of C18:3n-6, C20:4n-3, C22:4n-3, C22:4n-6 and C22:5n-3 did not differ among diets ($P > 0.05$), but feeding increasing amounts of W-DDGS resulted in a linear increase ($P \leq 0.01$) in milk concentrations of total C18:1 *trans*, total CLA, C20:2n-6 and C20:3n-3, and a tendency for a linear increase ($P \leq 0.09$) in milk concentrations of C20:3n-6 and C20:4n-6. Of the C18:1 isomers, milk concentrations of *cis*-11 decreased linearly ($P = 0.03$), whereas milk concentrations of *trans*-6+7+8, *trans*-12, *trans*-13+14, and *trans*-16 increased linearly, and those of *cis*-12 and *trans*-11 tended to increase linearly ($P \leq 0.07$) when dietary level of W-DDGS increased. There was a quadratic change ($P < 0.05$) in milk concentration of C18:1 *trans*-9 with increasing amounts of W-DDGS in the diet. Of the non-conjugated C18:2 isomers, the addition of W-DDGS to the diet resulted in a quadratic ($P < 0.01$)

Table 3B.4 Omasal crude fat and individual fatty acid flows (g per d) in dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
Crude fat omasal flow	829*b*	974*ab*	1,074*ab*	1,158*a*	65	0.01	<0.01	0.56
Fatty acid omasal flow								
C10:0	0.426	0.428	0.448	0.486	0.046	0.59	0.23	0.61
C12:0	1.95	1.83	2.03	2.21	0.19	0.34	0.22	0.31
C13:0 *iso*	0.635*b*	0.566*c*	0.715*a*	0.653*b*	0.059	<0.01	<0.01	0.67
C13:0 *anteiso*	2.40*ab*	2.25*b*	2.66*ab*	2.86*a*	0.22	0.04	0.02	0.07
C13:0	0.633	0.486	0.676	0.611	0.061	0.24	0.49	0.36
C14:0 *iso*	2.10*a*	1.75*b*	2.08*a*	2.22*a*	0.35	<0.01	0.02	<0.01
C14:0	10.81	10.11	9.08	10.40	1.91	0.83	0.72	0.50
C14:1 *cis*-9	0.255	0.217	0.298	0.277	0.055	0.80	0.66	0.90
C15:0 *iso*	4.33	4.20	4.46	4.51	0.36	0.93	0.63	0.81
C15:0 *anteiso*	11.0	11.4	12.6	12.2	0.6	0.13	0.05	0.40
C15:0	10.40	9.89	12.99	10.63	0.87	0.13	0.36	0.32
C16:0 *iso*	3.36	2.74	3.72	4.24	1.04	0.88	0.43	0.61
C16:0	141*b*	180*ab*	202*a*	218*a*	13	<0.01	<0.01	0.33
C16:1 *cis*-9	4.29	4.64	5.95	4.21	0.52	0.13	0.65	0.08
C17:0 *iso*	2.31	2.46	2.53	2.30	0.27	0.91	0.98	0.50
C17:0 *anteiso*	0.950	1.026	1.174	1.205	0.109	0.35	0.09	0.84
C17:0	4.78	5.03	5.54	5.63	0.36	0.07	0.01	0.69
C18:0	402*b*	479*ab*	513*ab*	554*a*	40	0.02	<0.01	0.51
C18:1 *cis*								

Total	73.8*b*	74.6*ab*	80.5*ab*	88.9*a*	4.3	0.05	0.01	0.30
Cis-9	57.5	56.8	63.2	67.4	3.3	0.14	0.04	0.48
Cis-11	11.60	9.16	7.77	8.59	1.22	0.10	0.07	0.10
Cis-12	5.08*c*	7.15*bc*	8.76*b*	12.39*a*	0.88	<0.01	<0.01	0.14
Cis-13	0.437*b*	0.518*b*	0.543*b*	0.881*a*	0.074	<0.01	<0.01	0.12
C18:1 *trans*								
Total	84.7	92.2	106.7	140.1	7.2	0.12	0.07	0.19
Trans-4	1.11	1.18	1.42	1.53	0.13	0.14	0.03	0.88
Trans-5	0.651	0.627	0.683	0.786	0.062	0.46	0.19	0.40
Trans-6+7+8	5.50*b*	5.94*ab*	7.07*ab*	7.60*a*	0.43	0.02	<0.01	0.91
Trans-9	3.34*b*	3.47*b*	4.12*ab*	4.82*a*	0.23	<0.01	<0.01	0.20
Trans-10	8.93*b*	9.47*ab*	11.88*ab*	13.07*a*	1.30	0.02	<0.01	0.69
Trans-11	34.0*b*	37.7*ab*	50.9*ab*	54.5*a*	4.3	0.02	<0.01	0.99
Trans-12	7.50*c*	9.09*b*	10.15*b*	13.24*a*	0.63	<0.01	<0.01	0.03
Trans-13+14	13.6*c*	16.2*c*	19.9*b*	25.1*a*	1.6	<0.01	<0.01	0.18
Trans-16	7.62*c*	9.31*b*	9.39*b*	12.39*a*	0.77	<0.01	<0.01	0.05
C18:1 total	164	173	181	224	10	0.09	0.05	0.12
C18:2 non-conjugated								
Cis-9, *cis*-12	25.3	33.1	35.7	41.4	1.99	0.10	0.05	0.46
Cis-9, *trans*-12	1.67	1.88	1.99	2.27	0.27	0.60	0.36	0.45
Trans-9, *trans*-12	0.266*b*	0.362*ab*	0.352*ab*	0.591*a*	0.066	0.04	<0.01	0.31
Trans-9, *cis*-12	1.11	1.17	1.14	1.72	0.14	0.07	0.05	0.07
Trans-11, *cis*-15	2.96	2.70	3.57	3.86	0.55	0.29	0.11	0.54
C18:2 conjugated								
Cis-9, *trans*-11	7.42	10.63	12.26	11.69	1.44	0.15	0.05	0.22
Trans-9, *cis*-11	0.129	0.116	0.186	0.145	0.018	0.15	0.17	0.34

Trans-10, *cis*-12	0.462	0.356	0.332	0.278	0.081	0.47	0.15	0.75
Trans-11, *trans*-13	0.657	0.636	0.695	0.580	0.063	0.44	0.46	0.40
Trans, trans[3]	1.17	1.44	1.61	1.71	0.16	0.14	0.03	0.59
C18:2 total	30.8*c*	38.5*bc*	45.0*ab*	48.7*a*	2.2	<0.01	<0.01	0.39
CLA[4]	9.80	13.16	15.03	14.50	1.56	0.15	0.05	0.24
C18:3n-3	7.08	7.48	8.23	8.64	0.52	0.21	0.05	0.99
C19:0	0.320	0.284	0.470	0.701	0.118	0.26	0.29	0.56
C20:0	6.93*b*	7.62*b*	8.25*ab*	9.39*a*	0.59	<0.01	<0.01	0.48
C20:1 *cis*-11	0.184	0.156	0.201	0.278	0.030	0.09	0.04	0.11
C20:2n-6	0.531	0.529	0.633	0.823	0.109	0.26	0.08	0.40
C20:3n-3	0.509	0.501	0.837	0.665	0.161	0.45	0.29	0.62
C20:3n-6	0.163	0.256	0.182	0.218	0.017	0.21	0.37	0.28
C20:4n-6	0.156	0.133	0.175	0.154	0.017	0.45	0.65	0.95
C22:0	3.96	4.48	4.84	5.11	0.33	0.08	0.02	0.97
C22:3n-3	0.340	0.350	0.413	0.349	0.086	0.93	0.82	0.68
C24:0	4.01	4.64	5.04	5.24	0.37	0.10	0.02	0.51
C26:0	5.21*b*	6.11*ab*	7.25*ab*	7.70*a*	0.75	0.03	<0.01	0.65
Total BCFA[5]	26.5	26.9	30.3	30.0	1.9	0.35	0.11	0.82
Total SFA[6]	619*b*	737*ab*	802*ab*	859*a*	56	0.02	<0.01	0.47
Total MUFA[7]	169	178	186	229	11	0.10	0.05	0.13
Total n-3 PUFA[8]	7.85	8.60	9.38	9.57	0.65	0.24	0.06	0.65
Total n-6 PUFA[9]	26.0	34.0	37.0	42.4	2.1	0.11	0.06	0.45
Total PUFA	34.7	41.9	45.6	52.6	2.5	0.12	0.06	0.93
n-6:n-3	3.81	3.74	3.82	4.34	0.27	0.54	0.27	0.38

a,b Means within a row with different superscripts differ ($P < 0.05$).

[1]Values are least square means obtained from 4 cows.

[2]*P* values indicate overall diet, linear and quadratic effects.
[3]Unresolved peak of CLA t*rans*-9, *trans*-11 + CLA t*rans*-10, *trans*-12.
[4]CLA = total conjugated linoleic acid (CLA *cis*-9, *trans*-11 + CLA *trans*-9, *cis*-11 + CLA *trans*-10, *cis*-12 + CLA *trans*-11, *trans*-13 + CLA *trans*-9, *trans*-11 + CLA *trans*-10, *trans*-12).
[5]Total BCFA = C13:0 *iso* + C13:0 *anteiso* + C14:0 *iso* + C15:0 *iso* + C15:0 *anteiso* + C16:0 *iso* + C17:0 *iso* + C17:0 *anteiso* + C18:0 iso.
[6]Total SFA = Total BCFA + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0 + C26:0.
[7]Total MUFA = C14:1 *cis*-9 + C16:1 *cis*-9 + C18:1 *trans*-4 + C18:1 *trans*-5 + C18:1 *trans*-6/*trans*-7/*trans*-8 + C18:1 *trans*-9 + C18:1 *trans*-10 + C18:1 *trans*-11 + C18:1 *trans*-12 + C18:1 *trans*-13/*trans*-14 + C18:1 *cis*-9 + C18:1 *cis*-11 + C18:1 *cis*-12 + C18:1 *cis*-13 + C18:1 *trans*-16 + C20:1 *cis*-11.
[8]Total n-3 PUFA = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:3n-3 + C22:5n-3.
[9]Total n-6 PUFA = C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6.

Table 3B.5 Milk fatty acid (FA) profiles (g/100 g of FA) in dairy cows fed increasing amounts of wheat-based dried distillers grains with solubles (W-DDGS)[1]

	Diet					*P* value[2]		
Item	Control	10% DDGS	15% DDGS	20% DDGS	SEM	Diet	Linear	Quadratic
C4:0	3.80	2.24	2.66	3.53	0.49	0.16	0.87	0.04
C6:0	1.61	1.61	1.49	1.67	0.07	0.22	0.60	0.22
C8:0	1.19*b*	1.27*a*	1.21*b*	1.19*b*	0.06	<0.01	0.16	<0.01
C10:0	3.18*ab*	3.43*a*	3.35*ab*	3.01*b*	0.19	0.05	0.16	0.01
C11:0	0.375	0.361	0.388	0.349	0.022	0.16	0.30	0.24
C12:0	3.97*ab*	4.25*a*	4.19*a*	3.64*b*	0.205	0.04	0.09	0.01
C13:0 *iso*	0.026*ab*	0.025*a*	0.024*ab*	0.020*b*	0.002	0.03	0.04	0.20
C13:0 *anteiso*	0.097*a*	0.090*ab*	0.099*a*	0.081*b*	0.006	0.03	0.03	0.10
C13:0	0.219	0.229	0.224	0.202	0.012	0.14	0.11	0.09
C14:0 *iso*	0.094*ab*	0.098*a*	0.091*ab*	0.087*b*	0.015	0.03	0.02	0.07
C14:0	12.5	12.8	12.5	11.7	0.5	0.19	0.12	0.14
C14:1 *cis*-9	1.001	0.897	1.007	0.841	0.071	0.11	0.07	0.32
C15:0 *iso*	0.254*a*	0.237*ab*	0.225*b*	0.217*b*	0.013	0.01	<0.01	0.40
C15:0 *anteiso*	0.462	0.486	0.500	0.459	0.021	0.06	0.89	0.02
C15:0	1.33	1.26	1.36	1.22	0.07	0.08	0.16	0.36
C16:0 *iso*	0.362*a*	0.302*b*	0.295*b*	0.298*b*	0.033	<0.01	<0.01	<0.01
C16:0	30.2	31.0	30.0	29.0	1.25	0.47	0.27	0.32
C16:1 *cis*-9	1.67*a*	1.51*ab*	1.53*ab*	1.43*b*	0.122	0.03	<0.01	0.56
C17:0 *iso*	0.417	0.409	0.397	0.354	0.011	0.10	0.06	0.14
C17:0 *anteiso*	0.158	0.162	0.186	0.167	0.010	0.29	0.29	0.29
C17:0	0.750	0.737	0.753	0.688	0.023	0.09	0.05	0.16

C18:0 *iso*	0.046	0.035	0.033	0.032	0.004	0.14	0.05	0.29
C18:0	10.5*c*	11.7*a*	10.7*bc*	11.4*ab*	0.7	<0.01	0.07	0.19
C18:1 *cis*								
Total	18.9	17.9	18.1	18.7	0.8	0.77	0.86	0.33
Cis-9	18.0	17.1	17.2	17.2	0.7	0.80	0.84	0.35
Cis-11	0.692	0.526	0.550	0.499	0.037	0.07	0.03	0.23
Cis-12	0.240	0.267	0.275	0.414	0.034	0.11	0.07	0.14
Cis-13	0.066	0.065	0.060	0.049	0.011	0.31	0.45	0.77
C18:1 *trans*								
Total	2.86	2.97	3.35	3.81	0.23	0.06	0.01	0.46
Trans-4	0.026	0.024	0.024	0.025	0.002	0.52	0.40	0.27
Trans-5	0.023	0.021	0.025	0.023	0.003	0.39	0.37	0.87
Trans-6+7+8	0.252	0.264	0.279	0.299	0.012	0.20	0.04	0.68
Trans-9	0.283	0.257	0.266	0.310	0.015	0.12	0.21	0.04
Trans-10	0.334	0.383	0.400	0.459	0.058	0.21	0.41	0.95
Trans-11	0.906	0.936	1.093	1.148	0.093	0.26	0.06	0.26
Trans-12	0.290*b*	0.331*b*	0.365*b*	0.452*a*	0.020	<0.01	<0.01	0.19
Trans-13+14	0.483	0.488	0.595	0.700	0.077	0.22	0.05	0.54
Trans-16	0.238*c*	0.308*b*	0.315*b*	0.371*a*	0.013	<0.01	<0.01	0.60
C18:1 total	21.8	20.9	21.5	22.5	0.8	0.54	0.46	0.24
C18:2 non-conjugated								
Cis-9, *cis*-12	1.83*c*	2.54*b*	2.79*ab*	2.93*a*	0.104	<0.01	<0.01	<0.01
Trans-11, *Cis*-15	0.060	0.041	0.052	0.065	0.010	0.26	0.50	0.09
C18:2 conjugated								
Cis-9, *trans*-11	0.364	0.373	0.432	0.469	0.029	0.09	0.02	0.63
Trans-9, *cis*-11	0.008	0.006	0.007	0.009	0.001	0.34	0.46	0.11

Trans-10, *cis*-12	0.007	0.006	0.007	0.007	0.001	0.94	0.98	0.60
Trans-11, *trans*-13	0.017	0.015	0.018	0.024	0.002	0.11	0.04	0.13
Trans, trans[3]	0.040	0.039	0.042	0.050	0.004	0.22	0.08	0.24
C18:2 total	1.89*c*	2.58*b*	2.83*ab*	3.00*a*	0.10	<0.01	<0.01	<0.01
CLA[4]	0.434	0.441	0.506	0.560	0.032	0.07	0.01	0.45
C18:3n-3	0.475*c*	0.531*b*	0.550*a*	0.524*b*	0.022	<0.01	<0.01	<0.01
C18:3n-6	0.043	0.041	0.038	0.038	0.004	0.78	0.35	0.84
C19:0	0.037	0.035	0.045	0.035	0.005	0.53	0.86	0.47
C20:0	0.188*b*	0.198*ab*	0.195*ab*	0.210*a*	0.009	0.02	<0.01	0.45
C20:1 *cis*-11	0.123*a*	0.118*b*	0.124*a*	0.126*a*	0.007	<0.01	<0.01	<0.01
C20:2n-6	0.031	0.036	0.039	0.042	0.003	0.08	0.01	0.57
C20:3n-3	0.018*b*	0.020*ab*	0.022*ab*	0.024*a*	0.001	0.03	<0.01	0.79
C20:3n-6	0.134*b*	0.150*a*	0.141*ab*	0.147*ab*	0.010	0.02	0.07	0.19
C20:4n-3	0.029	0.031	0.028	0.026	0.003	0.44	0.30	0.44
C20:4n-6	0.160	0.168	0.166	0.167	0.021	0.08	0.09	0.15
C20:5n-3	0.045*a*	0.044*a*	0.038*b*	0.037*b*	0.002	<0.01	<0.01	0.82
C22:0	0.056	0.060	0.057	0.056	0.003	0.07	0.46	0.04
C22:1 *cis*-13	0.005	0.005	0.008	0.007	0.002	0.70	0.47	0.95
C22:4n-3	0.009	0.009	0.010	0.008	0.001	0.90	0.86	0.63
C22:4n-6	0.029	0.029	0.029	0.030	0.005	0.91	0.76	0.65
C22:5n-3	0.070	0.075	0.065	0.064	0.010	0.17	0.11	0.41
C23:0	0.005	0.007	0.007	0.007	0.001	0.30	0.28	0.15
C24:0	0.023	0.027	0.025	0.024	0.002	0.09	0.88	0.04
C26:0	0.016	0.017	0.018	0.017	0.002	0.84	0.65	0.54
Summation by source[5]								
<C16	30.1*a*	29.1*ab*	29.4*ab*	28.3*b*	0.9	0.04	0.02	0.95

C16:0 and C16:1	32.2	32.8	31.8	30.8	1.3	0.42	0.19	0.36
>C16	38.0	38.1	38.7	40.7	1.2	0.38	0.13	0.47
Total BCFA[6]	2.14*a*	2.00*b*	1.95*b*	1.89*b*	0.07	<0.01	<0.01	0.16
Total SFA[7]	72.0	72.4	71.4	70.4	0.9	0.46	0.18	0.46
Total MUFA[8]	24.6	23.5	24.1	25.0	0.8	0.64	0.66	0.27
Total n-3 PUFA[9]	0.646*c*	0.710*ab*	0.715*a*	0.679*bc*	0.032	<0.01	0.02	<0.01
Total n-6 PUFA[10]	2.22*c*	2.96*b*	3.20*ab*	3.36*a*	0.13	<0.01	<0.01	<0.01
Total PUFA	2.85*b*	3.68*a*	3.90*a*	4.05*a*	0.16	<0.01	<0.01	<0.01

a,b Means within a row with different superscripts differ ($P < 0.05$).

[1]Values are least square means obtained from 4 cows.

[2]*P* values indicate overall diet, linear and quadratic effects.

[3]Unresolved peak of CLA t*rans*-9, *trans*-11 + CLA t*rans*-10, *trans*-12.

[4]CLA = total conjugated linoleic acid (CLA *cis*-9, *trans*-11 + CLA *trans*-9, *cis*-11 + CLA *trans*-10, *cis*-12 + CLA *trans*-11, *trans*-13 + CLA *trans*-9, *trans*-11 + CLA *trans*-10, *trans*-12).

[5]Fatty acids classified based on their source: <C16 are derived from *de novo* synthesis (C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 *iso* + C13:0 *anteiso* + C13:0 + C14:0 *iso* + C14:0 + C15:0 *iso* + C15:0 *anteiso* + C15:0); >16 are preformed fatty acids that are extracted from the blood by the mammary gland (C17:0 *iso* + C17:0 *anteiso* + C17:0 + C18:0 *iso* + C18:0 a*nteiso* + C18:0 + total C18:1 + total C18:2 + total CLA + C18:3n-3 + C18:3n-6 + C19:0 + C20:0 + C20:1 *cis*-11 + C20:2n-6 + C20:3n-3 + C20:3n-6 + C20:4n-3 + C20:4n-6 + C20:5n-3 + C22:0 + C22:1 *cis*-13 + C22:4n-3 + C22:4n-6 + C22:5n-3 + C23:0 + C24:0 + C26:0); and C16 + C16:1 are derived from both *de novo* synthesis and blood extraction (C16:0 *iso* + C16:0 + C16:1 *cis*-9).

[6]Total BCFA = C13:0 *iso* + C13:0 *anteiso* + C14:0 *iso* + C15:0 *iso* + C15:0 *anteiso* + C16:0 *iso* + C17:0 *iso* + C17:0 *anteiso* + C18:0 *iso*.

[7]Total SFA = Total BCFA + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0 + C26:0.

[8]Total MUFA = C14:1 *cis*-9 + C16:1 *cis*-9 + C18:1 *trans*-4 + C18:1 *trans*-5 + C18:1 *trans*-6/*trans*-7/*trans*-8 + C18:1 *trans*-9 + C18:1 *trans*-10 + C18:1 *trans*-11 + C18:1 *trans*-12 + C18:1 *trans*-13/*trans*-14 + C18:1 *cis*-9 + C18:1 *cis*-11 + C18:1 *cis*-12 + C18:1 *cis*-13 + C18:1 *trans*-16 + C20:1 *cis*-11.

[9]Total n-3 PUFA = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:3n-3 + C22:5n-3.

[10]Total n-6 PUFA = C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6.

change in milk concentration of *cis*-9, *cis*-12, and a tendency for a quadratic ($P = 0.09$) change in milk concentration of *trans*-11, *cis*-15. Of the conjugated 18:2 isomers, there was a linear increase ($P \leq 0.04$) in milk concentrations of CLA *cis*-9, *trans*-11 and CLA *trans*-11, *trans*-13, and a tendency for a linear increase ($P = 0.08$) in milk concentration of CLA *trans*, *trans* with increasing levels of dietary W-DDGS; however, milk concentrations of CLA *trans*-9, *cis*-11 and CLA *trans*-10, *cis*-12 were unaffected by diet ($P > 0.10$). When FA were grouped based on their origin, milk concentrations of *de novo* synthesized FA (i.e., <C16) linearly decreased ($P < 0.05$) with increasing levels of dietary W-DDGS, but milk concentrations of preformed FA (i.e., >C16) and FA derived from both *de novo* synthesis and the diet (i.e., C16 + C16:1) were unaffected ($P > 0.10$) by diet.

3B.5 Discussion

Because W-DDGS is higher in CF content when compared to CM, the inclusion of increasing amounts of W-DDGS resulted in greater levels of total dietary CF. It is well-documented that dietary fat levels exceeding 6 to 7% (DM basis) reduce fiber digestion and, consequently, lower DM intake in dairy cows (NRC, 2001). Additionally, the potential negative effects of supplemental fat on fiber digestion and DM intake are more pronounced when unsaturated rather than saturated fats are added to the diet (Firkins and Eastridge, 1994; Allen, 2000). In the present study, the addition of increasing amounts of W-DDGS made diets more unsaturated, primarily because the dietary content of C18:2n-6 increased by 33, 56, and 70% in diets containing 10, 15, and 20% W-DDGS, respectively, when compared to the control diet. However, even at the highest inclusion level of W-DDGS (i.e., 20%), dietary CF level was only 3.7%, which is well below the threshold range at which negative effects have been observed. In the larger study (Chibisa et al., 2012), we reported that the dietary inclusion of W-DDGS linearly increased DM intake and tended to linearly increase milk production without any negative effects on both ruminal and total tract digestion of fiber. This suggests that W-DDGS can safely be included in dairy cow diets up to 20% of DM without any negative effects on performance.

A major objective of this study was to evaluate the effects of replacing CM with increasing amounts of W-DDGS, which has greater contents of total fat and PUFA when compared to CM, on omasal flow of FA and milk FA composition. Our results indicate that

feeding increasing amounts of W-DDGS resulted in more substrate being made available for ruminal BH, thus increasing omasal outflow of some key PUFA and BH intermediates and, consequently, altering milk FA composition. Because of the FA composition of feed ingredients that are commonly used, dairy cow diets typically contain C18:2n-6 and C18:3n-3 as the major PUFA. These PUFA, together with other dietary unsaturated FA that are of minor importance, undergo extensive BH in the rumen due to the activities of ruminal microorganisms. When expressed as a proportion of their intakes, the daily amounts of C18:2n-6 and C18:3n-3 in omasal digesta ranged across diets from 9.5 to 11.0% and 7.9 to 8.2%, respectively, suggesting that ruminal BH of these FA was quite extensive in the present study. Complete ruminal BH of these FA yields C18:0 and, omasal digesta was significantly enriched in C18:0 as its outflow was approximately 10-, 17-, 18-, and 18-fold greater than its intake for the control, 10, 15, and 20% W-DDGS diets, respectively. Ruminal BH pathways result in the formation of various C18:1 *trans*, C18:1 *cis*, and non-conjugated and conjugated C18:2 isomers that can flow out of the rumen and be absorbed at the small intestine (Chilliard et al., 2007), thus influencing the FA composition of ruminant products like milk. Numerous factors are known to influence the extent of ruminal BH of dietary FA, among which the source and amount of supplemental PUFA appear to have the most profound effects (Chilliard et al., 2007). In the present study increasing the ruminal supply of PUFA, particularly C18:2n-6, by feeding increasing amounts of W-DDGS linearly increased the omasal flows of C18:2n-6 and C18:3n-3, reflecting the greater intakes of these PUFA in cows fed W-DDGS. More interestingly, feeding W-DDGS linearly increased or tended to linearly increase the omasal flows of various BH intermediates (C18:1 *trans*-10, C18:1 *trans*-11, and CLA *cis*-9, *trans*-11) that are known to have important biological effects. It is known that ruminal BH of C18:2n-6 yields primarily CLA *cis*-9, *trans*-11 and C18:1 *trans*-11 as the major BH intermediates, with CLA *trans*-10, *cis*-12 and C18:1 *trans*-10 also produced when ruminal pH is depressed (Bauman and Griinari, 2003; Shingfield et al., 2006). Ruminal BH of C18:3n-3, on the other hand, yields primarily C18:2 *trans*-11, *cis*-15 and C18:1 *trans*-11 as BH intermediates (Bauman and Griinari 2003; Shingfield et al. 2006). It is not surprising, therefore, that the omasal flows of these key BH intermediates increased when dietary intakes of C18:2n-6 and C18:3n-3 increased with greater levels of W-DDGS, and this response can be attributed to more substrate being available for BH. Others (Loor et al., 2005; Sterk et al., 2011) have

demonstrated increases in ruminal BH intermediates when diets that increased the intakes of C18:2n-6 and C18:3n-3 were fed to ruminants. Results from a recent study (Aldai et al., 2012) in which rolled barley was partially replaced with 20 or 40% W-DDGS failed to detect any differences in any of the major C18:1 (*cis* or *trans*), C18:2 or CLA BH intermediates in ruminal digesta (Aldai et al., 2012). This contradicts our observations from the present study, if we assume that the FA composition of omasal digesta is reflective of that of ruminal digesta. In the study of Aldai et al. (2012), C18:2n-6 intakes [calculated from dietary C18:2n-6 contents and DM intakes that were reported by Walter et al. (2010), a companion paper to Aldai et al. (2012)] of cows fed W-DDGS ranged from 159 to 205 g/d, whereas C18:2n-6 intakes in cows fed W-DDGS in the current study ranged from 335 to 466 g/d. A possible explanation for these discrepant results could, therefore, relate to these differences in C18:2n-6 intakes as more substrate was available for ruminal BH in the current study when compared to that of Aldai et al. (2012). Differences could also be attributed to the fact that we replaced CM with W-DDGS in the present study, whereas Aldai et al. (2012) replaced barley grain with W-DDGS which would have reduced dietary levels of ruminally-fermentable carbohydrate. It is well-established that diets that are low in ruminally-fermentable carbohydrate will result in a more extensive ruminal BH of dietary PUFA, thus reducing ruminal accumulation and outflow of key BH intermediates (Bauman and Griinari, 2001).

Perusal of the literature indicates that numerous studies have been conducted in the last two decades to examine how dietary factors alter bovine milk FA composition (Mansbridge and Blake, 1997). A major focus of these studies was to decrease the saturated FA content of milk, especially C14:0 and C16:0, which have been demonstrated to have undesirable hypercholesterolemic effects and to increase the risk of coronary heart disease (Berner, 1993). More importantly, focus has also been placed on enhancing the milk fat content of some PUFA, which have been demonstrated to have positive effects on human health (Wang et al., 2011). Among the PUFA, specific isomers of CLA (particularly *cis*-9, *trans*-11) and C18:3n-3 can potentially improve human health. Ruminant products like milk are the major source of CLA for human consumption and CLA *cis*-9, *trans*-11 has been associated with a wide range of positive health benefits in various experimental models (Wang et al., 2011). In the present study, milk fat contents of the major FA of dietary origin (i.e., C18:2n-6 and C18:3n-3) and the various ruminal

BH intermediates that are considered to be of biological importance (i.e., C18:1 *trans*-11, CLA *cis*-9, *trans*-11, and total CLA) increased with dietary levels of W-DDGS. Although we did not measure plasma FA concentrations in the present study, we can surmise that the increased omasal flows of these FA that we observed likely increased their plasma concentrations, thus increasing substrate availability to the mammary gland as these preformed FA (>C16) are extracted from the blood and directly incorporated into milk fat. Other studies have reported that milk concentrations of long-chain FA (i.e., >C16) generally mirror their omasal flows (Reveneau et al., 2012) or plasma concentrations (Abdelqader et al., 2009). The linear increase in milk concentrations of total CLA (as much as 29% at 20% W-DDGS when compared to the control diet) that we observed with increasing levels of W-DDGS was largely attributed to increases in CLA *cis*-9, *trans*-11, which represented about 85% of total CLA in milk fat. Although CLA *cis*-9, *trans*-11 that escapes complete ruminal BH can contribute to milk CLA (Harfoot and Hazlewood, 1988), the primary source of milk CLA *cis*-9, *trans*-11 is *de novo* mammary synthesis by desaturation of C18:1 *trans*-11 by Δ^9-desaturase (Griinari et al., 2000). In the present study, omasal outflow of C18:1 *trans*-11 linearly increased with dietary W-DDGS level and the greater substrate (C18:1 *trans*-11) availability to the mammary gland is the major reason for the observed increases in milk CLA *cis*-9, *trans*-11. Zhang et al. (2010) examined the inclusion of 20% W-DDGS as a partial replacement for barley silage in dairy cow diets and observed an increase in milk concentration of C18:2n-6; however, in contrast to the present study, they did not observe any changes in milk concentrations of any of the key ruminal BH intermediates (i.e., C18:1 *trans*-10, C18:1 *trans*-11, CLA *cis*-9, *trans*-11 or *trans*-10, *cis*-12). Because Zhang et al. (2010) did not measure ruminal outflow of FA, the reasons for the discrepancy in results are not clear, but could be attributed to the fact that W-DDGS replaced CM in the present study, whereas W-DDGS replaced barley silage in the study by Zhang et al. (2010).

Increasing the intake of PUFA in dairy cows has often been associated with a decrease in mammary fat secretion (NRC, 2001), a response that is now known to be mediated via increased formation of C18:1 *trans* FA and CLA isomers from ruminal BH (Bauman and Griinari, 2001). Of the BH intermediates that have been demonstrated to play a role in milk FA synthesis, C18:1 *trans*-10 (Bauman and Griinari 2001) and CLA *trans*-10, *cis*-12 (Baumgard et al. 2001; Peterson

et al. 2003) have been shown to be potent inhibitors of *de novo* synthesis of short-chain FA in the mammary gland. Milk fat contents (3.72, 3.85, 3.55, and 3.54% for cows fed the control, 10, 15 and 20% W-DDGS diets, respectively; $P = 0.09$) and yields (1.64, 1.67, 1.60, and 1.54 kg/d; $P = 0.06$) ($n = 4$ ruminally-cannulated cows) tended to decrease linearly as the dietary level of W-DDGS increased. In conjunction with the observed changes in milk fat, we also observed a linear decrease in the milk concentrations of FA <C16. This suggests some degree of inhibition of *de novo* synthesis of short-chain FA in the mammary gland, although this was quite small (only a 2.3 to 6% decrease when compared to control cows). There were no differences in the omasal flow of CLA *trans*-10, *cis*-12 and milk concentrations of this FA were very low, suggesting that it was likely not involved in modulating milk fat synthesis. However, the omasal flow of C18:1 *trans*-10 was increased by 6, 32, and 46% (compared to the control diet) when 10, 15, and 20% W-DDGS, respectively, was included in the diet. These increases in the omasal flow of C18:1 *trans*-10 could account for the decrease in milk fat secretion, although milk concentrations of C18:1 *trans*-10 were unaffected. In those studies that have reported negative effects of dietary PUFA on milk fat content, milk concentrations of C18:1 *trans*-10 have also been elevated (Abdelqader et al., 2009; Reveneau et al., 2012). However, in a recent study (Lock et al., 2007) the abomasal infusion of C18:1 *trans*-10 failed to inhibit milk fat synthesis in dairy cows, suggesting that other isomers might also be involved in the regulation of *de novo* milk fat synthesis.

3B.6 Conclusions

Results from the present study indicate that the inclusion of W-DDGS up to 20% of diet DM increases dietary PUFA content (particularly C18:2n-6); however, feeding W-DDGS tended to cause MFD and this is undesirable under the Canadian supply management system in which milk quota is based on kg of milk fat and the multiple-component pricing system puts a price premium on milk fat. Feeding greater amounts of W-DDGS increased the omasal flows of C18:2n-6, C18:3n-3, total C18:1 *trans*, total CLA and various BH intermediates (C18:1 *trans*-10, C18:1 *trans*-11, and CLA *cis*-9, *trans*-11) that are known to be of biological significance. These changes in omasal flows of these key FA are consistent with expected changes in ruminal BH pathways when dietary levels of PUFA are increased. Additionally, the observed changes in milk

concentrations of these key FA largely reflected their observed changes in omasal flow. From the standpoint of human health and making bovine milk more appealing to consumers, the observed increases in milk concentrations of C18:2n-6, C18:3n-3, total PUFA, total CLA, and CLA *cis*-9, *trans*-11 are of interest because of their potential to promote better human health.

In my first experiment (Chapter 3), feeding up to 20% W-DDGS (on a DM basis) as a replacement for CM did not compromise ruminal fermentation, microbial protein production and nutrient supply to post-ruminal sites. More importantly, dietary inclusion of W-DDGS resulted in a desirable increase in DMI and milk production. Therefore, based on my results, W-DDGS can be used as a complete substitute for CM in lactating cow diets. Although W-DDGS is the traditional co-product from bioethanol production in western Canada, B-DDGS is becoming increasingly available. However, compared to W-DDGS, information on the nutritional value of B-DDGS for dairy cows is scarce. Blend DDGS typically contains less CP and RDP than W-DDGS. To my knowledge, there are no reports in the literature comparing the feeding value of B-DDGS and W-DDGS for dairy cows. In recent years, there has been considerable interest in feeding low CP diets to lactating cows as a strategy to reduce feed costs, improve the efficienct of N utilization and minimize the irreversible loss of N to the environment. However, feeding diets containing as low as 15% CP has been reported to result in an undesirable decrease in milk production in some studies, whereas in others, lactation performance was not compromised. A possible explanation for these mixed results could be related to the magnitude of the decrease in dietary CP content and RDP supply. Given the differences in RDP content between W-DDGS and B-DDGS, it is plausible that the dietary inclusion of W-DDGS or B-DDGS as the major source of supplemental protein could influence animal responses. Therefore, the major goal of my second experiment (Chapter 4) was to determine how interactions between the type of distillers co-product (W-DDGS vs. B-DDGS) and diet CP content (15 vs. 17%) alter ruminal N utilization, urea-N recycling to the GIT, omasal nutrient flow, and production performance in lactating dairy cows.

4. EFFCTS OF FEEDING WHEAT OR CORN-WHEAT DRIED DISTILLERS GRAINS WITH SOLUBLES IN LOW OR HIGH CRUDE PROTEIN DIETS ON RUMINAL FUNCTION, OMASAL NUTRIENT FLOW, UREA-N RECYCLING, AND PERFORMANCE IN DAIRY COWS.

4.1 Abstract

A study was conducted to determine the effects of including either wheat-based (W-DDGS) or corn-wheat blend (B-DDGS) dried distillers grains with solubles as the major protein source in low or high crude protein (CP) diets fed to dairy cows on ruminal function, microbial protein synthesis, omasal nutrient flows, urea-N recycling, and milk production. Eight lactating Holstein cows (768.5 ± 57.7 kg body weight; 109.5 ± 40.0 days-in-milk) were used in a replicated 4 × 4 Latin square design with 28-d periods (18 d of dietary adaptation and 10 d of measurements) and a 2 × 2 factorial arrangement of dietary treatments. Four cows in one Latin square were ruminally-cannulated for the measurement of ruminal fermentation characteristics, microbial protein synthesis, urea-N recycling kinetics, and omasal nutrient flow. The treatment factors were type of distillers co-product (W-DDGS vs. B-DDGS) and dietary CP content (15.2 vs. 17.3%; DM basis). The B-DDGS was produced from a mixture of 15% wheat and 85% corn grain. All diets were formulated to contain 10% W-DDGS or B-DDGS on a DM basis. There was no diet effect on dry matter intake. Yields of milk, fat, protein and lactose, and plasma urea-N (PUN) and milk urea-N (MUN) concentrations decreased in cows fed the low CP compared to those fed the high CP diet. Although feeding B-DDGS tended to reduce ruminal ammonia-N (NH_3-N) concentration compared to feeding W-DDGS (9.3 vs. 10.5 mg/dL), there were no changes in PUN and MUN concentrations. Additionally, dietary inclusion of B-DDGS compared to W-DDGS did not affect ruminally-degradable protein (RDP) supply, omasal flows of total N, microbial non-ammonia N (NAN), RUP and total NAN, or urea-N recycling kinetics and milk production. However, feeding the low CP compared to the high CP diet decreased N intake, RDP supply, ruminal NH_3-N concentration, and omasal flows of N, microbial NAN, and total NAN. Feeding the low CP compared to the high CP diet also decreased endogenous urea-N production, urea-N recycled to the gastrointestinal tract, and urea-N excretion in urine. In summary, our results indicate that both W-DDGS and B-DDGS can be included as the major protein sources in dairy cow diets without compromising nutrient supply and production performance. However,

feeding the low CP compared to the high CP diet decreased omasal flows of microbial protein and metabolizable protein which, in turn, decreased milk production.

4.2 Introduction

Historically, wheat has been the major feedstock used for ethanol production in western Canada. However, fluctuations in the price of wheat in recent years have resulted in corn being increasingly used in combination with wheat in different ratios based on least cost and availability (Nuez-Ortín and Yu, 2011; Abdelqader and Oba, 2012). Therefore, both wheat-based (W-DDGS) and corn-wheat blend (B-DDGS) dried distillers grains with solubles are available for use in dairy cow diets. Results from a few recent studies showed that W-DDGS can be used to either partially or completely replace barley forage and grain (Zhang et al., 2010a, b) or canola meal (Chibisa et al., 2012) in lactating cow diets without negatively affecting animal performance. However, compared to W-DDGS, there is limited information on the feeding value of B-DDGS, whose nutrient composition is mainly influenced by the ratio of wheat to corn in the feedstock that is used for ethanol production (Canadian International Grains Institute [CIGI], 2011).

A major difference between B-DDGS and W-DDGS is in their content and ruminal degradability of CP. As the proportion of corn to wheat increases in the feedstock, there is a decrease in the CP content of B-DDGS compared to W-DDGS (Boila and Ingalls, 1994a, b; Nuez-Ortín and Yu, 2009; CIGI, 2011). Changes also occur in the CP degradation kinetics in the rumen. Boila and Ingalls (1994a) and Nuez-Ortín and Yu (2010a, b) observed a decrease in the effective degradability of B-DDGS CP as a result of the increase in the amount of zein, which is known to be resistant to ruminal degradation (Oran Little et al., 1968). Therefore, when compared to W-DDGS, the inclusion of B-DDGS in dairy cow diets could potentially reduce dietary RDP supply, thus causing a deficiency in ruminal NH_3-N which could suppress microbial protein synthesis in the rumen. In vitro estimates of RDP-derived microbial protein available for absorption in the small intestine were lower for B-DDGS compared to W-DDGS (138.8 vs. 160.6 g/kg of DM), suggesting limited ruminal NH_3-N supply (Nuez-Ortín and Yu, 2010a). In a recent study with lactating cows fed iso-nitrogenous diets, Abdelqader and Oba (2012) reported a lower ruminal NH_3-N concentration when diets contained B-DDGS (a mixture of 50% corn-based DDGS and 50% W-DDGS) compared to 100% W-DDGS (12.3 vs. 14.0 mg/dL), but

dietary effects on RDP supply, ruminal microbial protein production and post-ruminal flow of MP were not measured. Dietary CP content can also have major effects on RDP supply, as the amount of dietary CP that is degraded in the rumen increases with dietary CP content (Olmos Colmonero and Broderick, 2006a). When dietary CP content is reduced in diets containing either W-DDGS or B-DDGS as the major protein sources (which could potentially alter RDP supply), it is important to determine how changing dietary CP content and RDP supply interact to alter N utilization and production responses.

Urea-N recycling buffers the rumen from low NH_3-N concentrations and, thus, can maintain microbial growth when dietary RDP supply is limited (Huhtanen and Hristov, 2009; Agle et al; 2010). It is known that a greater proportion of endogenous urea-N is recycled to the rumen when dietary CP content is reduced (Lapierre and Lobley, 2001). Under such feeding conditions, the fractional contribution of NH_3-N from recycled urea-N to microbial protein synthesis increases, provided energy supply is not limiting (Marini and Van Amburgh, 2003). Therefore, the potential decrease in ruminal NH_3-N concentration when feeding B-DDGS compared to W-DDGS could increase the rate of urea-N transfer into the rumen and its subsequent sequestration into microbial protein. Based on the limited data that is available, the RUP content of W-DDGS and B-DDGS ranges from 45.0 to 63.8% of CP (Nuez-Ortín and Yu, 2010a; Li et al., 2012).

When corn-based DDGS (C-DDGS; a high RUP source) was compared with urea (no RUP value) as a N supplement in steers fed corn-based diets, both urea-N recycling to the gastrointestinal tract (GIT) and the proportion of microbial NAN arriving at the small intestine that was derived from recycled urea-N tended to be greater with C-DDGS (Brake et al., 2010). This suggests that recycled urea-N might be a more important source of N for ruminal microbial growth when high RUP diets are fed (Wickersham et al., 2009b; Brake et al., 2010).

Although several studies have been carried out to determine the nutritional characteristics and feeding value of W-DDGS, there is limited information on the use of B-DDGS as a protein supplement for dairy cows. We hypothesized that differences in the RDP content between W-DDGS and B-DDGS would result in differences in ruminal N utilization, urea-N recycling to the GIT, and omasal nutrient flow, and that these effects would be dependent on dietary CP content.

Therefore, the primary objective of this study was to determine how interactions between the type of distillers co-product (W-DDGS vs. B-DDGS) and diet CP content (15 vs. 17%) alter ruminal N utilization, urea-N recycling to the GIT, omasal nutrient flow, and production performance in lactating dairy cows.

4.3 Materials and Methods

4.3.1 Animals and Experimental Design

Eight lactating dairy cows (768.5 ± 57.7 kg BW; 109.5 ± 40.0 DIM) were used in a replicated 4 × 4 Latin square design with 28-d periods (20 d of dietary adaptation and 8 d of measurements) and a 2 × 2 factorial arrangement of dietary treatments. Four cows in one Latin square were ruminally-cannulated for the measurement of ruminal fermentation characteristics, microbial protein synthesis, urea-N recycling kinetics, and omasal nutrient flows. All cows were housed in individual tie-stalls at the Greenbrae Dairy Research Facility (University of Saskatchewan). The University of Saskatchewan Animal Care Committee approved the use of cows for this experiment (UCACS Protocol No. 20040048), and they were cared for in accordance with the Canadian Council of Animal Care (1993) regulations.

4.3.2 Experimental Treatments and Feeding Management

The treatment factors were type of distillers co-product (W-DDGS vs. B-DDGS) and dietary CP content (15.2 vs. 17.3%, DM basis). All diets were formulated to contain 10% (on a DM basis) W-DDGS or B-DDGS. Single batches of W-DDGS and B-DDGS were used for the entire study. The batch of W-DDGS was produced from 100% wheat (Terra Grain Fuels, Belle Plaine, SK, Canada), whereas the batch of B-DDGS was produced from 85% corn and 15% wheat (Husky Energy, Minnedosa, MB, Canada). Cows were fed twice daily at 0900 and 1600 h as TMR for ad libitum intake. The forage:concentrate ratio of the TMR was 50:50, with the forage component of the TMR being a mixture of barley silage (65% on a DM basis) and chopped alfalfa hay (35%; Table 4.1).

Table 4.1 Ingredient and chemical composition of diets

	Low CP		High CP	
	W- DDGS	B-DDGS	15% DDGS	20% DDGS
Ingredient Composition, % of diet DM				
Alfalfa hay	17.8	17.8	17.8	17.8
Barley silage	31.2	31.2	31.2	31.2
Soybean hulls	2.2	2.7	0.45	0.22
Cottonseed hulls	3.6	2.2	0.45	0.22
Barley grain	29.0	29.0	29.0	29.0
Wheat-based DDGS	10.0	_	10.0	_
Blend DDGS	_	10.0	_	10.0
Canola meal	0.89	1.1	3.3	2.2
Corn gluten meal	0.45	1.1	1.1	2.7
Soybean meal	0.45	0.45	2.2	2.2
Fat canola oil	0.60	0.60	0.60	0.60
Molasses	0.24	0.24	0.24	0.24
Mineral-vitamin mix[1]	1.8	1.8	1.8	1.8
Sodium bicarbonate	0.97	0.97	0.97	0.97
Salt	0.36	0.36	0.36	0.36
Limestone	0.24	0.24	0.24	0.24
Dynamate[2]	0.17	0.17	0.17	0.17
Chemical composition				
DM, %	54.6	54.4	54.9	54.5
OM, % of DM	94.0	94.1	93.9	94.1
CP, % of DM	15.0	15.3	17.2	17.4
Fat, % of DM	3.67	4.65	4.23	4.83
NDF, % of DM	35.2	34.9	32.6	32.5
ADF, % of DM	19.2	18.7	17.6	17.0
NE_L,[3] Mcal/kg	1.68	1.70	1.74	1.75

[1]Contained (/kg of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16%, Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

[2]Dynamate (Eastern Minerals Inc., Henderson, NC) contained 18% K, 11% Mg, and 22% S.

[3]Estimated using CPM-Dairy (v 3.0.8, Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; William H. Miner Agricultural Research Institute, Chazy, NY) using the chemical analysis of feed ingredients.

4.3.3 Data Collection and Sampling

Urea-N recycling kinetics, N balance, apparent total tract nutrient digestion, ruminal fermentation characteristics, microbial protein synthesis, and omasal nutrient flow were determined using the 4 ruminally-cannulated cows in one Latin square. On d 21 (0900 h) of each experimental period, the 4 ruminally-cannulated cows were fitted with temporary vinyl catheters (0.86 mm I.D. × 1.32 mm O.D.; Scientific Commodities Inc., Lake Havasu City, AZ) in the right and left jugular veins to facilitate continuous isotope infusion and blood sampling. Urea-N transfer to the GIT and whole body N balance were subsequently determined from d 22 to 26 as described by Lobley et al. (2000). Briefly, urine and fecal samples were collected on d 21 for analysis of ^{15}N natural abundance. Thereafter, continuous infusion of double-labeled urea ([$^{15}N^{15}N$]-urea, 99.8 atom % ^{15}N; Cambridge Isotope Laboratories, Andover, MA) prepared in 0.15 *M* sterile saline started at 0800 h on d 22 of each experimental period and ended at 0600 h on d 28. Individual cow N intake from d 13 to d 20 was used to determine the daily [$^{15}N^{15}N$]-urea dosage rate, based on a targeted plateau urinary [$^{15}N^{15}N$]-urea enrichment of 0.15 atom percent excess (APE). Total fecal and urine collections were conducted from 0800 h on d 22 to 0800 h on d 26 (96 h). Large steel trays placed behind each stall were used to collect feces. On each day, fecal output for each cow was thoroughly mixed and quantitatively transferred to pre-weighed plastic tubs for weighing. Subsequently, a 2.5% fecal subsample was collected and stored at -20°C for later chemical analysis. Indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) used for total urine collection were inserted at 0800 h on d 21 before connection to urine collection tubing at the start of [$^{15}N^{15}N$]-urea infusion (0800 h on d 22). Urine was collected into 20-L carboy polyethylene containers containing 150 mL of concentrated HCl, and this acidification (pH < 3) of urine during collection was necessary to prevent microbial growth and volatilization of NH_3-N. Daily urine output was recorded, before collection of a 5% subsample that was pooled by cow for each period and stored at -20°C for later N analysis. A daily 50-mL urine sample was also collected (d 23 to 26) and stored at -20°C for later analysis of urinary enrichments of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea. Additionally, a 2-mL urine sample was diluted with 8 mL of distilled water and stored at -20°C until analysis for urea-N.

To measure the flow of nutrients at the omasal canal, $YbCl_3$ (Siddons et al., 1985; Yang and Beauchemin, 2005) was used as a digesta marker. On d 21 of each experimental period, a 500-mL omasal digesta sample was collected for determination of background ^{15}N (^{15}NB) abundance. A priming dose of $YbCl_3$ that was equivalent to half the daily dose was then administered into the rumen via the ruminal cannula. Subsequently, a $YbCl_3$ solution was continuously infused into the rumen using a peristaltic pump (Model: 205U, Watson and Marlow, Cornwall, UK) for the next 7 d (d 21 to 28) at a constant rate of 1 L/d, providing 3.35 g of Yb per day. The amount of marker solution infused each day (~1 L) was recorded. During each period, a 50-mL sub-sample of the marker solution that was infused for each cow was collected and stored at room temperature for Yb analysis. The omasal sampling technique as described by Huhtanen et al. (1997) was used to collect omasal digesta. Briefly, this technique involved locating the omasal canal by hand and then inserting a sampling tube via the ruminal cannula. Sampling tubes were inserted at each sampling time in order to ensure correct positioning in the omasal canal and to minimize the potential negative impact on digesta passage and normal digestive function if the sampling tubes were left in place between samplings. Once the sampling tube had been inserted in the omasal canal, a 600-mL omasal digesta sample was collected from each cow at 0900, 1500 and 2100 on d 26; 0300, 1200 and 1800 h on d 27; and 0000, and 0600 h on d 28, such that the collected samples were representative of a 24-h feeding cycle. Omasal samples were stored at -20ºC and pooled by cow per period over the 24-h cycle.

To measure ruminal short-chain fatty acid (SCFA) and NH_3-N concentrations, and bacterial ^{15}N enrichment, approximately 400 mL of ruminal digesta was also collected at each omasal sampling time from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula. The ruminal contents were strained through 4 layers of cheesecloth. Two 10-mL sub-samples of ruminal fluid were then collected and mixed with chilled 25% (wt/vol.) meta-phosphoric acid (H_2PO_4) or 1% H_2SO_4 and stored at -20°C for later determination of SCFA and NH_3-N, respectively. After straining through cheesecloth, the remaining solid digesta was mixed with 500 mL of 0.15 *M* saline solution, and homogenized in a blender (NuBlend, Waring Commercial, Torrington, CT) for 60 s to dislodge particle-associated bacteria. The blended mixture was squeezed through four layers of cheesecloth to obtain a second filtrate. The two filtrates were mixed and 375 mL of the mixture were used to isolate mixed ruminal bacteria by

differential centrifugation. Briefly, filtrates were centrifuged at 500 × *g* for 20 min at 4°C to remove protozoa and residual feed particles. The supernatant was collected and centrifuged at 20,000 × *g* for 20 min at 4°C to obtain a ruminal bacterial pellet. Bacterial pellets were then pooled by cow per period and stored at -20°C.

To determine ruminal pH, approximately 1,000 mL of ruminal contents were collected from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula at 0900, 1000, 1100, 1200, 1400 and 1600 h on d 27. The ruminal contents were strained through 4 layers of cheesecloth. Ruminal fluid pH was then measured immediately using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA). At the same time points as for ruminal fluid sampling on d 27, blood samples were collected from the contra-lateral jugular vein into 10-mL vacutainer tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ). The collected samples were centrifuged at 1,500 × *g* for 15 min at 4°C, and the plasma obtained was stored at -20^{o}C until analyzed for urea-nitrogen (PUN).

Feed intake was recorded daily throughout the experiment. Samples of the TMR and orts were collected from d 23 to d 25 and were stored at -20°C for later analysis. Cows were milked three times daily at 0430, 1230, and 1900 h, and milk weights were recorded throughout the experiment. Milk samples were collected daily on 3 consecutive days (d 23, 24, and 25) from all three milkings into vials containing 2-bromo-2-nitropropane-1-2-diol as a preservative. Samples were submitted to the Alberta Central Milk Testing Laboratory (Edmonton, Alberta, Canada) for CP, fat, lactose, and MUN analyses.

4.3.4 Sample Analyses

After the experiment, frozen TMR and fecal samples were thawed overnight at room temperature, pooled per collection period for each cow, and subsequently dried in an oven at 60°C for 48 h (AOAC, 1990; method 930.15). Dried TMR and fecal samples were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Samples were analyzed for DM (AOAC, 1990; method 930.15), OM (AOAC, 1990; method 942.05), CP using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05), ether extract (AOAC, 1990; method 920.39), and ADF and NDF (Van Soest et al., 1991). Amylase

and sodium sulfite were used for NDF determination. Frozen urine samples were thawed overnight at room temperature and then analyzed for N using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05). The plasma and dilute urine samples were analyzed for urea-N by the diacetyl monoxime method (Marsh et al., 1957) using a colorimetric urea-N kit (Stanbio Urea Nitrogen Kit, Procedure No. 0580; Stanbio Laboratory, Boerne, TX, USA). Milk samples were analyzed for fat, CP, lactose and MUN using infrared spectroscopy (MilkoScan 605; Foss Electric, Hillerød, Denmark; AOAC, 1990; method 972.16).

To determine the enrichments of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea in daily urine samples (50-mL subsample), urinary urea-N was isolated by passing urine containing 1.5 mg of urea-N through a pre-packed cation exchange resin column (AG-50W-×8 Resin, 100-200 mesh, H^+ form; Biorad, Richmond, CA) as described by Archibeque et al. (2001). Following application of urine, 7 mL of N-free water was added to the column before elution of urea-N into test tubes using 20 mL of N-free water. Following air-drying (60°C) of the eluate, three 1-mL rinses of N-free water were used to quantitatively transfer urea-N from the test tubes into 17- × 60-mm borosilicate glass tubes. Subsequently, the urea-N samples were freeze-dried and 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). Analytical conditions used during IRMS should lead to the production of ions with mass/charge (m/z) values of 28, 29, and 30 for [$^{14}N^{14}N$]-, [$^{14}N^{15}N$]- and [$^{15}N^{15}N$]-urea, respectively. Standards prepared using [$^{15}N^{15}N$]-urea (99.8 atom % ^{15}N) and [$^{14}N^{14}N$]-urea (natural abundance urea; 0.364 atom% ^{15}N) were also analyzed, and results used to correct for [$^{14}N^{15}N$]-urea produced during non-monomolecular reactions (Lobley et al., 2000). Daily fecal samples were thawed overnight at room temperature before DM analysis (AOAC, 1990; method 930.15). Dried fecal samples were subsequently ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England) and were analyzed for ^{15}N enrichment by combustion to N_2 gas in an elemental analyzer and continuous flow isotope-ratio mass spectrometry (Lobley et al., 2000).

In preparation for non-ammonia nitrogen (NAN) and ^{15}N analyses, the omasal ^{15}NB and bacterial pellet samples were freeze-dried. Freeze-dried ^{15}NB samples were then ground through

a 1-mm screen (Christy-Norris Ltd.) before being pulverized with a ball mill. Freeze-dried bacterial pellet samples were ground using a mortar and pestle. The composited omasal digesta samples were freeze-dried and ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, UK). To determine Yb concentration, a 1-g sample was combusted at 550°C for 8 h in a muffle furnace (AOAC, 1990) before nitric acid digestion as described by Vicente et al. (2004). Subsequently, Yb concentration was measured by atomic absorption spectrophotometry (Perkin Elmer 2300, Perkin-Elmer Corp, Norwalk, CT). The Yb concentration in omasal digesta was then used to determine omasal DM flow (France and Siddons, 1986). To determine the flow of nutrients to the omasum, the freeze-dried omasal digesta samples were analyzed for OM by combustion in a muffle furnace at 550°C for at 8 h, total N (Leco 2000; Leco Instruments, Inc., St. Joseph, MI), ADF and NDF. To quantify NH_3-N, 10 mL of 0.07 *M* sodium citrate (pH 2.2) was added to 0.5 g of omasal digesta and the mixture vortexed and subsequently held at 39°C for 30 min in a forced-air oven. The extracts were then centrifuged at 18,000 × *g* for 15 min at 4°C, and the resultant supernatant was analyzed for NH_3-N using the phenol-hypochlorite method as described by Broderick and Kang (1980). Samples of ^{15}NB, bacterial pellets, and omasal digesta were prepared for ^{15}N analysis as described by Brito et al. (2009). Briefly, to volatize NH_3-N prior to ^{15}N analysis, ^{15}NB, bacterial pellets, and omasal digesta samples containing approximately 100 µg of N were weighed into 5 × 9-mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK). Subsequently, 50 µL of 72 m*M* K_2CO_3 was added to each capsule before incubation in a forced air oven at 60°C for 24 h. Enrichment of ^{15}N in NAN of the samples was then measured by combustion to N_2 gas in an elemental analyzer and continuous flow isotope ratio-mass spectrometry.

Frozen ruminal fluid samples were thawed at room temperature and pooled by cow for each period. This was followed by centrifugation at 20,000 x *g* for 15 min at 4°C to obtain a clear supernatant. For ruminal fluid samples that were preserved with 25% H_2PO_4, supernatants were filtered through a 0.45-µm membrane and a 0.9-mL portion of the filtered supernatant was mixed with 0.1 mL of 10 mg/mL crotonic acid as an internal standard. Ruminal SCFA were then separated and quantified by gas chromatography (Agilent 6890, Mississauga, ON) as described by Erwin et al. (1961). For ruminal fluid samples that were preserved with H_2SO_4, supernatants

were analyzed for ruminal NH_3-N using a phenol-hypochlorite assay (Broderick and Kang, 1980).

4.3.5 Calculations and Statistical Analyses

Urinary ^{15}N enrichment of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]-urea and total fecal ^{15}N excretion were used to calculate urea-N kinetics using the model described by Lobley et al. (2000). Based on this model, a fraction of urea-N synthesized in the liver (urea-N entry rate, UER) is irreversibly lost in urine (urinary urea-N elimination, UUE), whereas the remaining fraction is recycled to the GIT (GIT entry rate, GER). Urea-N recycled to the GIT is hydrolyzed by bacterial urease to NH_3-N, which has 3 fates: 1) excretion in feces (urea-N in feces, UFE); 2) reabsorption into portal blood and re-entry into the ornithine cycle (ROC); or 3) utilization for anabolic purposes (UUA), which is assumed to be primarily ruminal microbial protein synthesis (Lobley et al., 2000).

Omasal true digesta NAN content was calculated as total N – NH_3-N. Omasal digesta and bacterial pellet ^{15}N enrichment (APE) was calculated as: ^{15}N APE = sample ^{15}N percent – mean background sample ^{15}N percent. Bacterial NAN flow was then calculated by multiplying omasal NAN flow by the ratio of omasal digesta ^{15}N enrichment to bacterial pellet ^{15}N enrichment (Wickersham et al., 2009b). The omasal flow of bacterial NAN that was derived from recycled urea-N was calculated as bacterial NAN flow × (bacterial ^{15}N enrichment ÷ urinary ^{15}N enrichment) (Wickersham et al., 2009a). Apparent ruminal nutrient digestibilities were calculated as nutrient intake – omasal flow of nutrient, with flows expressed as kilograms per day (Reynal and Broderick, 2005). Organic matter truly digested in the rumen (OMTDR) was determined as OM intake – (omasal OM flow – microbial OM flow). Omasal flow of nonammonia nonmicrobial N (NANMN), RUP, and RDP were also calculated as described by Reynal and Broderick (2005) as follows: NANMN flow = total NAN flow – microbial NAN flow; RUP flow = total CP flow – microbial CP flow; and RDP supply = total CP intake – RUP flow.

All data on ruminal fermentation parameters, nutrient digestibilities, omasal flow, and excretion, and urea-N kinetics were analyzed as a 4 × 4 Latin square using the PROC MIXED

procedure of SAS (SAS Institute, 2004) according to the following model: $Y_{ijk} = \mu + P_i + C_j + T_k + E_{ijk}$ where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the fixed effect of period i, C_j is the random effect of cow j, T_k is the fixed effect of dietary treatment k, and E_{ijk} is the residual error. Ruminal pH and PUN concentration data were analyzed accounting for repeated measures through the inclusion of the variable sampling time in the repeated statement, as well as sampling time × dietary treatment in the model previously described. Production data for the 8 cows were analyzed using the MIXED procedure of SAS (SAS Institute, 2004) for a replicated 4 × 4 Latin square design according to the following model: $Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl}$ where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square i, P_j is the fixed effect of period j, $C_{k(i)}$ is the random effect of cow k (within square i), T_l is the fixed effect of dietary treatment l, ST_{il} is the interaction between square i and treatment l, and E_{ijkl} l is the residual error. Factorial contrasts were used to test the main effects of type of distillers co-product (W-DDGS vs. B-DDGS), dietary CP content (low [15.2%] vs. high [17.3%]), and their interaction (type of distillers co-product × dietary CP content). The interaction term was removed from the model when $P > 0.15$. Treatment differences were considered significant when $P \leq 0.05$ and tendencies when $0.05 < P \leq 0.10$.

4.4 Results

4.4.1 Dietary Characteristics

Dietary ingredient and chemical composition are presented in Table 4.1. Experimental diets were formulated to contain 15 or 17% CP (DM basis) and chemical analysis showed only marginal maximum deviations (+0.3 and +0.4 percentage units, respectively) from the intended dietary CP contents (Table 4.1).

4.4.2 Production Parameters

Results on feed intake and milk production that are presented in Table 4.2 are from all 8 cows that were used in the study. Dietary treatment had no effect ($P > 0.05$) on DMI. Cows fed the high CP diet had greater ($P < 0.01$) N intakes when compared to those fed the low CP diet (Table 4.2). Cows fed the high CP diet had greater ($P < 0.01$) milk yield, energy-corrected milk yield, and feed efficiency compared to those fed the low CP diet. However, milk N efficiency did

Table 4.2 Dry matter intake (DMI), and milk yield and composition of cows fed low or high CP diets containing either wheat-based dried distillers grains with solubles (DDGS) or blend DDGS as the major protein source (n = 8)

	CP		DDGS			*P* value	
Variable	Low	High	Wheat	Blend	SEM	CP	DDGS
DM intake, kg/d	28.7	29.1	28.7	29.1	0.79	0.28	0.39
N intake, kg/d	0.698	0.810	0.744	0.764	0.019	<0.01	0.17
Milk yield, kg/d	39.6	42.6	40.9	41.3	1.78	<0.01	0.73
ECM[1], kg/d	40.2	43.9	41.8	42.3	1.41	<0.01	0.52
Feed efficiency[2]	1.41	1.51	1.46	1.46	0.035	0.01	0.93
N efficiency[3]	27.4	26.4	27.0	26.9	0.84	0.29	0.86
Milk fat, %	3.63	3.68	3.65	3.67	0.108	0.52	0.78
Milk fat yield, kg/d	1.43	1.56	1.48	1.50	0.052	<0.01	0.45
Milk protein, %	3.10	3.22	3.14	3.18	0.096	0.02	0.43
Milk protein yield, kg/d	1.22	1.36	1.28	1.30	0.041	<0.01	0.56
Milk lactose, %	4.58	4.60	4.60	4.59	0.029	0.51	0.70
Milk lactose yield, kg/d	1.82	1.96	1.88	1.89	0.085	<0.01	0.78
MUN[4], mg/dL	12.6	14.9	13.9	13.6	0.64	<0.01	0.73
PUN[5], mg/dL	15.8	19.0	17.4	17.4	1.06	<0.01	0.96

[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/DMI

[3]Nitrogen efficiency = milk N (kg/d)/N intake (kg/d)

[4]Milk urea-N

[5]Plasma urea-N

not differ ($P > 0.05$) with diet. There was no diet effect ($P > 0.05$) on milk fat and lactose contents, but milk protein content was higher ($P = 0.02$) in cows fed the high compared to those fed the low CP diet. Feeding the high CP diet also resulted in greater ($P < 0.01$) milk fat, protein and lactose yields, and MUN and PUN concentrations compared to feeding the low CP diet.

4.4.3 Ruminal Fermentation Characteristics

Feeding the high CP diet resulted in a higher ($P < 0.01$) ruminal NH_3-N concentration compared to feeding the low CP diet, whereas feeding W-DDGS tended ($P = 0.06$) to increase ruminal NH_3-N concentration compared to feeding B-DDGS (Table 4.3). There were no diet effects ($P > 0.05$) on ruminal acetic, propionic, valeric and total SCFA concentrations, acetate:propionate ratio and ruminal pH. However, ruminal butyric, isobutyric, isovaleric and total branched-chain fatty acid (BCFA) concentrations were higher ($P < 0.05$) in cows fed the high compared to those fed the low CP diet.

4.4.4 Ruminal Digestion and Nutrient Flow

Although there was no diet effect ($P > 0.05$) on DMI, cows fed the high CP diet tended ($P = 0.10$) to have a higher OM intake and, consequently, a higher omasal OM flow ($P = 0.10$), compared to those fed the low CP diet (Table 4.4). The amount and percentage of DM and OM apparently digested in the rumen did not differ ($P > 0.14$) among diets. Additionally, there was no diet effect ($P > 0.12$) on the amount and percentage of OM truly digested in the rumen. Intake of NDF and the amount of NDF apparently digested in the rumen tended to be lower ($P = 0.09$) for cows fed the high CP compared to those fed the low CP diet. Intake of ADF ($P < 0.02$) and the amount of ADF apparently digested in the rumen ($P < 0.03$) were lower in cows fed the high compared to those fed the low CP diet.

4.4.5 Intake, Digestibility, and Omasal Flow of Nitrogen Fractions

Cows fed the low CP diet consumed less N ($P < 0.01$) compared to those fed the high CP diet (Table 4.5); however, N apparently digested in the rumen, when expressed as g per day or as a proportion of N intake, did not differ ($P \geq 0.17$) among diets. Feeding cows the high CP compared to the low CP diet increased ($P = 0.03$) the absolute amount of N truly digested in the

Table 4.3 Ruminal fermentation characteristics of cows fed low or high CP diets containing either wheat-based dried distillers grains with solubles (DDGS) or blend DDGS as the major protein source (n = 4)

	CP		DDGS			*P* value	
Variable	Low	High	Wheat	Blend	SEM	CP	DDGS
Ammonia-N, mg/dL	8.7	11.2	10.5	9.3	1.06	<0.01	0.06
Acetic acid, m*M*	76.7	77.6	77.9	76.3	2.81	0.61	0.35
Propionic acid, m*M*	23.2	23.7	23.9	22.9	1.11	0.60	0.29
Butyric acid, m*M*	13.2	14.4	13.7	13.9	0.83	<0.01	0.51
Valeric acid, m*M*	1.76	1.85	1.88	1.74	0.088	0.32	0.15
Isobutyric acid, m*M*	1.01	1.11	1.07	1.06	0.039	<0.01	0.61
Isovaleric acid, m*M*	1.48	1.67	1.53	1.62	0.14	0.02	0.19
Total BCFA[1], m*M*	2.49	2.78	2.60	2.68	0.18	<0.01	0.28
Total SCFA[2], m*M*	117	120	120	118	4.5	0.26	0.33
Acetate:Propionate	3.31	3.30	3.27	3.35	0.072	0.92	0.44
Mean pH	6.26	6.22	6.21	6.27	0.102	0.28	0.16

[1]BCFA = branched-chain fatty acids.

[2]SCFA = short-chain fatty acids.

Table 4.4 Nutrient flow from and digestion in the rumen of cows fed low or high CP diets containing either wheat-based dried distillers grains with solubles (DDGS) or blend DDGS as the major protein source (n = 4)

	CP		DDGS			*P* value	
Variable	Low	High	Wheat	Blend	SEM	CP	DDGS
DM							
Intake, kg/d	27.7	28.5	28.2	28.0	0.77	0.12	0.65
Omasal flow, kg/d	20.9	22.2	21.9	21.2	0.73	0.14	0.43
Apparent digestion, kg/d	6.78	6.35	6.36	6.78	0.36	0.34	0.36
Apparent digestion, % of DM intake	24.6	22.2	22.6	24.2	1.21	0.20	0.36
OM							
Intake, kg/d	26.0	26.9	26.5	26.3	0.74	0.10	0.76
Omasal flow, kg/d	18.8	20.3	19.9	19.2	0.74	0.09	0.38
Apparent digestion, kg/d	7.16	6.55	6.56	7.15	0.48	0.28	0.29
Apparent digestion, % of OM intake	27.7	24.3	24.8	27.1	1.69	0.15	0.31
True digestion, kg/d	14.0	14.9	14.4	14.5	0.53	0.12	0.95
True digestion, % of OM intake	54.1	55.4	54.5	54.9	1.52	0.56	0.84
NDF							
Intake, kg/d	9.79	9.35	9.66	9.48	0.21	0.06	0.38
Omasal flow, kg/d	5.13	5.30	5.44	5.00	0.35	0.68	0.30
Apparent digestion, kg/d	4.66	4.05	4.22	4.49	0.36	0.09	0.41
Apparent digestion, % of NDF intake	47.8	43.3	43.8	47.3	3.54	0.24	0.36
ADF							
Intake, kg/d	5.33	4.98	5.30	5.02	0.12	0.02	0.05
Omasal flow, kg/d	2.66	2.71	2.79	2.58	0.22	0.84	0.32
Apparent digestion, kg/d	2.67	2.28	2.51	2.44	0.25	0.03	0.63
Apparent digestion, % of ADF intake	49.9	45.7	47.3	48.3	4.40	0.22	0.76

Table 4.5 Intake, digestibility, and omasal flow of N constituents in cows fed low or high CP diets containing either wheat-based dried distillers grains with solubles (DDGS) or blend DDGS as the major protein source (n = 4)

	CP		DDGS			*P* value	
Variable	Low	High	Wheat	Blend	SEM	CP	DDGS
N intake, g/d	679	787	730	737	19	<0.01	0.72
N apparently digested in the rumen							
g/d	-189	-194	-207	-177	17	0.84	0.24
% of N intake	-27.8	-24.8	-28.4	-24.2	2.02	0.32	0.17
N truly digested in the rumen							
g/d	409	513	476	446	29.0	0.03	0.49
% of N intake	60.2	65.1	64.8	60.5	3.33	0.33	0.39
RDP supply							
g/d	2,741	3,445	3,193	2,993	192	0.03	0.48
% of DMI	9.9	12.0	11.3	10.7	0.61	0.03	0.50
Flow at omasal canal							
N							
g/d	869	981	936	914	31	0.01	0.54
% of N intake	128	125	128	124	2.0	0.32	0.17
NH_3-N, g/d	29.2	38.2	34.9	32.5	3.07	0.07	0.59
NAN[1]							
g/d	840	943	901	881	29	0.02	0.57
% of N intake	124	120	124	120	2.0	0.24	0.20
NANBN[2]							
g/d	241	236	219	258	25.8	0.89	0.31
% of NAN flow	28.7	25.3	24.6	29.5	3.07	0.45	0.29

% of N intake	35.5	30.0	30.5	35.0	3.48	0.29	0.38
% of DM intake	0.870	0.829	0.778	0.921	0.0910	0.76	0.29
RUP							
g/d	1,505	1,472	1,367	1,610	161	0.89	0.31
% of DM intake	5.44	5.18	4.86	5.76	0.569	0.76	0.29
Total bacterial NAN							
g/d	599	707	683	624	41	0.09	0.33
% of NAN	71.3	74.7	75.4	70.5	3.07	0.45	0.29
Microbial efficiency							
g of microbial N/kg OMTDR[3]	42.7	46.6	46.2	43.1	2.00	0.24	0.32

[1]NAN = non-NH_3 N

[2]NANBN = non-NH_3 nonbacterial N.

[3]OMTDR = OM truly digested in the rumen.

rumen. The supply of RDP, when expressed as g per day or as a proportion of DMI, increased (P = 0.03) when cows were fed the high compared to the low CP diet. Similarly, the amounts of N (P = 0.01) and total NAN (P = 0.02) flowing at the omasal canal increased, whereas NH_3-N flow tended to increase (P =0.07), when dietary CP content was increased. There was no diet effect (P > 0.05) on omasal flow of NANBN and RUP. When cows were fed the high compared to the low CP diet, omasal flow of bacterial NAN (g per day) tended to increase (P = 0.09). The efficiency of microbial protein synthesis did not differ (P > 0.24) among diets. The source of dietary protein did not influence omasal flows of microbial NAN or total NAN.

4.4.6 Nitrogen Balance and Apparent Total Tract Nutrient Digestibilities

Cows fed the high CP diet had greater N intake (P < 0.01) and milk N content (P = 0.03) when compared to those fed the low CP diet (Table 4.6). Urinary N and total N excretion were also higher (P < 0.01) in cows fed the high CP compared to the low CP diet. Although fecal N excretion did not differ (P > 0.05), fecal N excretion expressed as a percent of N intake was greater (P < 0.01) on the low compared to the high CP diet. There were no diet effects (P > 0.05) on apparent N balance. Apparent total tract DM (P = 0.08) and OM (P = 0.06) digestibilities tended to be higher when cows were fed the high compared to the low CP diet. Feeding the high CP diet increased (P < 0.01) apparent total tract N digestibility when compared to feeding the low CP diet. Feeding B-DDGS tended to increase (P = 0.07) apparent total tract N digestibility compared to feeding W-DDGS. Apparent total tract NDF, ADF and fat digestibilities did not differ (P > 0.05) with diet.

4.4.7 Urea-N Recycling Kinetics

Urea-N synthesis (i.e., urea-N entry rate; UER), urea-N loss in urine (UUE), and urea-N returned to the ornithine cycle (ROC) were higher (P < 0.05) in cows fed the high CP compared to those fed the low CP diet (Table 4.7). Feeding the high CP diet tended (P = 0.06) to increase urea-N that was transferred to the GIT (GER) when compared to feeding the low CP diet. There were no diet effects (P > 0.05) on the quantity of urea-N that was used for anabolic purposes (UUA) or lost in feces (UFE). Dietary treatment largely had no effects (P > 0.05) on fractional urea-N transfers, but the proportion of GER that was lost in feces tended to be higher (P = 0.07)

Table 4.6 Nitrogen balance and apparent total-tract nutrient digestibility in cows fed low or high CP diets containing either wheat-based dried distillers grains with solubles (DDGS) or blend DDGS as the major protein source (n = 4)

	CP		DDGS			*P* value	
Variable	Low	High	Wheat	Blend	SEM	CP	DDGS
N intake, kg/d	666	772	713	726	18.8	<0.01	0.61
Urinary excretion							
Total, kg/d	39.4	40.2	37.8	41.9	5.76	0.84	0.30
Total N, g/d	212	271	244	239	16.8	<0.01	0.64
Total N, % N intake	32.1	35.0	34.4	32.7	2.48	0.20	0.45
Fecal excretion							
DM, kg/d	9.43	9.51	9.48	9.45	0.397	0.67	0.87
N, g/d	252	261	260	254	12.8	0.30	0.47
N, % N intake	37.9	33.8	36.6	35.0	1.15	< 0.01	0.07
Total N excretion							
g/d	465	532	504	492	23.4	<0.01	0.47
% N intake	69.9	68.8	71.0	67.7	2.86	0.69	0.24
Milk N, g/d	182	207	193	196	11.3	0.03	0.69
Milk NE_L[1], Mcal/d	27.3	29.9	28.4	28.8	0.96	<0.01	0.53
Apparent N balance, g/d	19.7	33.6	16.5	36.8	20.0	0.57	0.42
Productive N[2], g/d	202	241	209	233	20.8	0.14	0.33
Apparent total-tract digestibility, %							
DM	65.3	66.1	65.6	65.7	0.67	0.08	0.77
OM	66.9	67.8	67.3	67.4	0.65	0.06	0.85
N	62.1	66.2	63.4	65.0	1.14	<0.01	0.07
NDF	45.6	43.9	44.2	45.4	1.08	0.15	0.28

ADF	39.6	38.1	38.6	39.1	1.18	0.31	0.73
Ether extract	85.4	84.9	85.2	85.1	0.84	0.64	0.94

[1] Milk NE_L (Mcal/d) = kg of milk × (0.0929 × % fat + 0.0563 × % true protein + 0.0395 × % lactose) (NRC, 2001).

[2]Calculated as N secreted in milk plus N apparently retained by the cow.

Table 4.7 Urea-N recycling kinetics in cows fed low or high CP diets containing either wheat-based dried distillers grains with solubles (DDGS) or blend DDGS as the major protein source (*n* = 4)

	CP		DDGS			*P* value	
Variable	Low	High	Wheat	Blend	SEM	CP	DG
Urea-N kinetics, g/d							
Production, UER	407	495	467	435	15.5	<0.01	0.20
Entry to GIT, GER	328	383	369	342	17.1	0.06	0.31
Return to ornithine cycle, ROC	231	271	264	238	11.8	0.05	0.18
Loss to feces, UFE	14.1	14.3	14.5	14.0	0.63	0.82	0.54
Loss to urine, UUE	79	112	97.7	93.2	4.63	<0.01	0.52
Reuse for anabolism, UUA	83.0	97.6	90.7	89.9	6.21	0.15	0.94
Fractional urea-N transfers							
UER to urine, u	0.196	0.230	0.211	0.215	0.0135	0.12	0.88
UER to GIT, (l - u)	0.804	0.770	0.789	0.785	0.0135	0.12	0.88
GER to ROC, r	0.705	0.708	0.716	0.697	0.0086	0.80	0.17
GER to feces, f	0.043	0.038	0.040	0.042	0.0017	0.07	0.47
GER to UUA, a	0.252	0.254	0.244	0.261	0.0093	0.88	0.25
Ruminal microbial capture of recycled N							
g N/d	68.9	58.8	67.6	60.0	4.60	0.15	0.27
% of total microbial NAN	11.5	8.5	10.1	10.0	0.71	0.01	0.92

in cows fed the low compared to those fed the high CP diet. The amount of recycled urea-N that was incorporated into microbial N (g/d) was unaffected by dietary treatment; however, when expressed as a percentage of total microbial NAN, the proportion of recycled urea-N that was incorporated into microbial N was greater (P = 0.01) on the low CP as compared to the high CP diet.

4.5 Discussion

In lactating dairy cows, MP requirements are met by a combination of microbial protein and dietary RUP arriving at the small intestine (NRC, 2001). Maximizing the supply of microbial protein is important as it can contribute more than 60% of MP and, qualitatively, it has an AA profile that closely matches AA requirements for milk protein synthesis (NRC, 2001). Among the numerous factors that alter microbial growth, it is important to ensure a sufficient supply of RDP as its ruminal degradation yields NH_3-N and preformed AA that are essential N sources for microbial protein synthesis (Clark et al., 1992; NRC, 2001). Manipulating dietary RDP content, therefore, is a recognized strategy to alter ruminal microbial protein synthesis and optimize N utilization in dairy cows. Another strategy to optimize N utilization that has received attention is manipulating dietary CP content (Leonardi et al., 2003; Reynal and Broderick, 2005; Olmos Colmonero and Broderick, 2006b; Gressley and Armentano, 2007). In the present study, our aim was to determine how changing dietary CP and RDP contents would alter N utilization and production performance in dairy cows fed W-DDGS or B-DDGS. In western Canada, W-DDGS and B-DDGS are widely available for feeding dairy cows and were included as the major protein sources to alter dietary RDP content. Previous studies (Boila and Ingalls, 1994a; Nuez-Ortín and Yu, 2010a, b) observed that the RDP content in W-DDGS was greater compared to B-DDGS. Because W-DDGS and B-DDGS differ in their RDP contents, their inclusion as the major protein source in dairy diets could potentially alter ruminal microbial protein synthesis and MP reaching the small intestine and, as a result, production responses. In addition, it is known that for a given diet, the amount of CP that is degraded in the rumen (i.e., RDP) decreases as the dietary CP content decreases (Olmos Colmonero and Broderick, 2006b), so we were interested in determining how concomitant changes of dietary CP content and RDP supply (by feeding W-DDGS or B-DDGS) interact to alter N utilization and production responses.

Altering dietary CP content had major effects on N utilization, which were largely reflective of the difference in N intake as has been reported by others (Brito et al., 2007b; Brito et al., 2009; Olmos Colmonero and Broderick, 2006a). The amount of dietary N that was apparently digested in the rumen was negative for all diets, indicating that omasal N flow was greater than N intake. Broderick et al. (2010) reported that zero ruminal N balance (i.e., when omasal N flow = N intake) occurred when dietary CP and RDP contents were ~14.7 and ~10.6%, respectively, and that ruminal N balance became more positive as dietary CP and RDP contents increased. In the present study, dietary CP and RDP contents were ≥15% and ≥9.9%, respectively, so ruminal N balance would be expected to be largely positive. Broderick et al. (2008) attributed the positive ruminal N balance to the N contribution of recycled urea-N to the ruminal NH_3-N pool and its subsequent utilization for microbial protein synthesis. In the present study, urea-N entry to the GIT was substantial, ranging from 328 to 383 g/d, so it is not surprising that ruminal N balance was positive. Others (Reynal et al., 2003; Olmos Colmenero and Broderick, 2006a) have also reported negative apparent N digestion in the rumen in dairy cows.

Feeding the high CP diet increased RDP supply by 704 g/d when compared to feeding the low CP diet. This was expected because, for a given diet, the amount of CP that is degraded in the rumen (i.e., RDP) increases as dietary CP content increases (Olmos Colmonero and Broderick, 2006b). Dietary RDP content should range from 9.5 to 10.5% (as % of DM) in order to ensure a sufficient supply of NH_3-N and preformed AA (NRC (2001), which are important N substrates for ruminal microbial growth (Broderick et al., 2007). In the present study, RDP supply in cows fed the low CP diet was 9.9%, which is very close to the minimum requirements and could be considered borderline deficient. Indeed, this low RDP supply depressed ruminal NH_3-N concentrations in cows that were fed the low CP when compared to those fed the high CP diet. It has been suggested that a ruminal NH_3-N concentration between 11.0 and 11.8 mg/dL is required to support maximum microbial protein synthesis (Balcells et al., 1993; Reynal and Broderick, 2005). In the present study, ruminal NH_3-N concentration on the 15.2% CP diet was below this threshold by >20%. Although we did not measure ruminal concentrations of free AA in the present study, we can surmise that the lower RDP supply from feeding the 15.2% when compared to the 17.3% CP diet decreased ruminal availability of preformed AA as has been

reported by others (Reynal and Broderick, 2005). The increased availability of preformed AA can stimulate microbial growth in vivo (Chikunya et al., 1996) and in in vitro cultures (Argyle and Baldwin, 1989; Carro and Miller, 1999). Also, the increased ruminal availability of BCFA with the high CP diet could also have stimulated microbial growth (Russell and Sniffen, 1984). Together, the ruminal deficiencies of NH_3-N, preformed AA, and BCFA suppressed microbial growth in cows fed the low CP diet, such that the omasal flow of microbial NAN was 108 g/d lower when compared to cows fed the high CP diet. Olmos Colmonero and Broderick (2006a) reported an increase in microbial NAN flow with dietary CP content; however, although other studies (Cunngingham et al., 1996; Korhonen et al., 2002) have reported an increase in total NAN flow to the small intestine with dietary CP content, this has been attributed to a greater flow of RUP as microbial NAN flow was unaltered. In the present study, total NAN flow increased with dietary CP content due to an increase in microbial NAN supply.

The choice of W-DDGS or B-DDGS as the major source of dietary protein had no influence on omasal flows of microbial NAN and N fractions. Intakes of N were similar in cows fed W-DDGS and B-DDGS, so it is not surprising that total N flow at the omasal canal was similar because it is primarily dictated by N intake (Reynal and Broderick, 2005; Olmos Colmonero and Broderick, 2006b). Based on observed differences in the RDP contents of W-DDGS and B-DDGS (Boila and Ingalls, 1994a; Nuez-Ortín and Yu, 2010a, b), we had anticipated that the ruminal availability of NH_3-N and other microbial growth factors (e.g., BCFA) would be different when W-DDGS or B-DDGS was included as the major source of dietary protein, thus influencing microbial NAN and RUP supply. However, of the major factors that influence ruminal microbial growth as discussed previously, only ruminal NH_3-N concentration tended to be affected by the source of dietary protein; therefore, it is not surprising that the omasal flow of microbial NAN was unaffected by source of dietary protein. Those previous studies (Boila and Ingalls, 1994a; Nuez-Ortín and Yu, 2010a, b) that have reported differences in RDP content between W-DDGS and B-DDGS used in situ techniques, and true in vivo ruminal degradabilities of these protein sources could be different due to methodological differences and associative effects in the rumen, among other factors. Although not significant, the omasal flow of RUP in cows that were fed B-DDGS was 243 g/d greater when compared to those fed W-DDGS, thus possibly reflecting potential differences in the ruminal degradabilities

of W-DDGS and B-DDGS. In support of this assertion, ruminal NH_3-N concentration tended to be lower in cows fed B-DDGS than W-DDGS. Abdelqader and Oba (2012) also reported a decrease in ruminal NH_3-N concentration when incremental amounts of C-DDGS replaced W-DDGS in isonitrogenous diets fed to dairy cows. To our knowledge, this is the first study that has compared omasal flows of N fractions and microbial NAN in cows fed W-DDGS or B-DDGS as the major protein source, so direct comparisons of our data with other published data are not possible.

Across diets, microbial NAN was a major contributor to total NAN flow at the omasal canal, and accounted for 70.5 to 75.4% of total NAN flow at the omasal canal as has been reported by others (Korhonen et al., 2002; Olmos Colmonero and Broderick, 2006a; Brito et al., 2007b). These data emphasize the importance of maximizing microbial NAN production in the rumen in order to meet the AA requirements for milk protein synthesis (NRC, 2001). Across diets, omasal flow of NH_3-N accounted for only 3 to 4% of omasal flow of total N, supporting previous studies (Brito et al., 2007b). Omasal flow of NH_3-N tended to be greater in cows fed the high CP compared to the low CP diet, which was expected as omasal flow of NH_3-N is reflective of ruminal NH_3-N concentration (Brito and Broderick, 2007); however, feeding W-DDGS or B-DDGS did not influence the omasal flow of NH_3-N as ruminal NH_3-N concentrations were similar.

Another major objective of the current study was to determine how changes in dietary CP and RDP content interact to alter urea-N recycling in dairy cows. When dairy cows are fed diets that are deficient in CP or RDP, they respond by increasing the proportion of endogenous urea that is recycled to the forestomachs (Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008). Recycled urea-N buffers the forestomachs from the low ruminal NH_3-N concentration that usually prevails when dietary CP or RDP content is low, thus providing additional N that can be used to maintain microbial protein synthesis. Feeding the high CP diet increased endogenous urea production (i.e., UER) when compared to the low CP diet, which is reflective of the greater N intakes in cows fed the high CP diet as UER is positively correlated with N intake (Lapierre and Lobley, 2001). The UER:N intake ratio was 0.61 to 0.64, which falls within the range of 0.43 to 1.23 (mean of 0.88) that was reported previously (Lapierre and Lobley, 2001) for

lactating cows at various levels of production and fed a wide variety of diets. Clearly, these ratios indicate the magnitude of the transit of N into the urea pool and the perpetual reliance of ruminants on urea-N recycling to the GIT in order to maintain a positive N balance.

Many dietary and intra-ruminal factors have been reported to influence the magnitude of urea-N transfer from blood to the GIT (Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008). Among those factors, there is evidence that ruminal NH_3-N concentration is negatively correlated with urea-N transfer into the rumen (Kennedy and Milligan, 1980). The exact mechanisms for this response are unknown, but could be related to a decrease in the ruminal epithelium's permeability to urea (Egan et al., 1986) or a decrease ruminal urease activity (Cheng and Wallace, 1979), which helps to maintain a favorable blood-rumen urea concentration gradient. Recently, Abdoun et al. (2009) demonstrated that high NH_3-N concentrations decrease urea-N transfer across the rumen in a pH-dependent manner, thus suggesting that the mechanism could involve the stimulation of the Na^+/H^+ exchanger as a means of regulating intracellular pH. In the present study, feeding the low CP diet lowered ruminal NH_3-N concentration, and this was associated with a numerically greater GER:UER ratio (i.e., a greater proportion of endogenous urea-N being transferred to the GIT) as has been reported by others (Marini and Van Amburgh, 2003; Marini et al., 2004). Although cows fed the high CP diet tended to have greater GER when compared to those fed the low CP diets, the proportion (in absolute amounts or as a proportion of GER) that was used for anabolic purposes (i.e., UUA) was unaffected by dietary CP content. Consequently, urea-N that was returned to the ornithine cycle (i.e., ROC) was higher in dairy cows that were fed the high CP diet. The anabolic utilization of recycled urea-N is primarily via microbial incorporation of NH_3-N arising from urea-N degradation, although other potential anabolic uses within the body include amination and transamination reactions (Lobley et al., 2000). Our observations indicate that the amount of recycled urea-N that was recovered as microbial N was low, ranging from 60.0 to 68.9 g/d, or 8.5 to 11.5% when expressed as a proportion of microbial NAN. Comparative data on the use of recycled urea-N for microbial NAN synthesis in dairy cows are scarce. In dairy cows consuming 350 to 414 g N/d, estimates obtained using ^{15}N isotope dilution techniques indicated that 34 to 41 g (or 12 to 25% when expressed as a proportion of microbial NAN) of duodenal microbial NAN was derived from recycled urea-N (Ouellet et al. 2002; Lapierre et al., 2008; Ouellet et al., 2010). When expressed

as a proportion of microbial NAN, our estimates fall below the range of 12 to 25% reported in these other studies with dairy cows, but it is important to note that mean N intake in our study (~719 g N/d) was ~337 g/d greater than the mean N intake (~382 g/d) in those previous studies. Apart from recycled urea-N, ruminal NH_3-N for microbial NAN synthesis can also be derived from the degradation of dietary N. As dietary N intakes increases, therefore, ruminal bacteria become less dependent on recycled urea-N as a source of NH_3-N. In our study, greater N intakes would result in a greater ruminal NH_3-N pool, thus reducing the dependence on recycled urea-N as a source of NH_3-N. Thus, differences in ruminal NH_3-N pool sizes could explain the discrepant results between studies in the proportion of recycled urea-N that is used for microbial NAN production. Feeding W-DDGS or B-DDGS as the major source of protein did not influence urea-N kinetics, presumably because RDP supply was similar in cows fed the two protein sources. Although there was a tendency for ruminal NH_3-N concentration to be higher in cows fed W-DDGS when compared to those fed B-DDGS, we can surmise that the difference in ruminal NH_3-N concentration to elicit the inhibitory effects of NH_3-N on urea-N transfer into the rumen. It is noteworthy that the difference in ruminal NH_3-N concentration that was elicited by dietary CP content (2.5 mg/dL) was double that elicited by the source of protein (1.2 mg/dL), and this might partly explain the variable responses on urea-N recycling that we obtained between the effects of dietary CP and source of dietary protein.

Our results showed that most of the positive responses in animal performance (i.e., milk yield and composition, and N efficiency) that we observed were due to changes in dietary CP content, rather than changes in the major source of dietary CP. Perusal of literature indicates that reducing dietary CP content is beneficial in reducing feed costs and N losses to the environment (Olmos Colmonero and Broderick, 2006b; Hristov et al., 2011a); however, responses in milk production to reducing dietary CP content have been equivocal. It appears that the magnitude of the decrease in dietary CP content, dietary RDP level, and the level of milk production are some of the major factors that determine responses in terms of DMI and milk yield. Feeding diets containing 16.5 to 18.5% CP (Cunningham et al., 1996) or 16.1 to 18.8% CP (Leornadi et al., 2003) resulted in similar yields of milk and milk protein. Olmos Colmonero and Broderick (2006b) reported a tendency for a quadratic increase in milk yield when dietary CP contents of 13.5, 15.0, 16.5, 17.9, and 19.4% were tested, with increases in milk yield being observed

between 13.5 and 16.5% CP and then declining thereafter. In other studies, decreasing dietary CP content (by approximately 2% units as in the present study) from 16 to 14% (Cabrita et al., 2011) or 16.6 to 14.4% (Lee et al., 2011a) reduced milk and milk protein yields. In our study, reducing dietary CP content from 17.3 to 15.2% reduced milk yield by 3 kg/cow per day. Also, milk protein content and yield decreased by 0.12 percentage units and 140 g/d, respectively. This decrease in production performance that we observed with the low CP diet is undesirable for economic reasons, and it could primarily be attributed to a deficiency in MP supply at the small intestine, which was 103 g/d lower when compared to the high CP diet. As discussed previously, the decreased RDP supply in cows fed the low CP diet could be responsible for the lower MP supply. It should be noted, however, that production responses to changes in dietary RDP supply have been inconsistent. Gressley and Armentano (2007) reported similar milk yields in dairy cows fed a 10.1 or 7.4% RDP, even though the lower RDP diet was about 28% below NRC (2001) recommendations. They did not measure ruminal microbial protein synthesis or MP supply in that study, so dietary effects on these parameters are unknown. Also, cows that were used in that study produced ~11 kg/d less milk than cows that were used in the present study, suggesting that the MP requirements for cows in the study by Gressley and Armentano (2007) were lower and could be met even at 7.4% dietary RDP. Reynal and Broderick (2005) compared dietary RDP levels ranging from 13.2 to 10.6% and observed similar milk yields; however, milk protein decreased linearly as dietary RDP content declined and this was attributed to linear decreases in both microbial NAN and MP supply. More recently, Cyriac et al. (2008) compared dietary RDP levels ranging from 11.3 to 7.6% and reported that the lowest RDP level resulted in reduced DMI and milk yield, but they did not measure microbial NAN or MP supply. These discrepant results indicate that production responses to dietary RDP level depend on numerous factors, primarily milk production level of the cows and dietary characteristics. Others (Firkins et al., 1986; Allen, 2000) have reported that an insufficient RDP supply can also decrease milk production by reducing fiber digestion which, in turn, can result in reduced DMI and nutrient supply for milk production. In the present study, DMI was unaffected by dietary RDP content, and apparent ruminal fiber digestion was greater in cows fed the low CP compared to those fed the high CP diet; however, total-tract fiber digestion was similar across RDP levels, thus indicating that the lower ruminal fiber digestion with the high RDP diet was offset by a higher

post-ruminal fiber digestion. Apparent total-tract digestibility of DM and N were actually lower in cows fed the low RDP compared to those fed the high RDP diet, suggesting that the decrease in milk yield with the low RDP diet could partly be attributed to a reduction in nutrient supply.

In the present study, feed efficiency (i.e., milk yield/DMI) was improved when dietary CP content was increased, which resulted from the increase in milk yield as DMI did not change. As expected, increasing dietary CP content increased N excretion, with this response being attributed to an increase in urinary N excretion (rather than fecal N excretion) as has been reported by others (Olmos Colmonero and Broderick, 2006b; Lee et al., 2011a). However, milk N efficiency (i.e., milk N/N intake) did not improve when we reduced dietary CP content because the decrease in N intake was matched by a decrease in milk N content, as has been reported previously (Haque et al., 2012). Across diets, total N excretion as a proportion of N intake ranged from 67.7 to 71.0%, which falls within the range that is typically reported in the literature (Castillo et al., 2001). Other indicators of N use that we measured were PUN and MUN concentrations. Feeding the low CP diet decreased PUN and MUN concentrations when compared to feeding the high CP diet, as has been reported in other studies in which dietary CP content has been manipulated (Olmos Colmonero and Broderick, 2006b, Lee et al., 2011a). Low concentrations of PUN and MUN suggest efficient use of dietary CP (Olmos Colmonero and Broderick, 2006b), and bulk tank MUN tests are commonly used as indicators of CP status in dairy cows.

Feeding W-DDGS or B-DDGS as the major protein source did not influence production responses. Because W-DDGS and B-DDGS are inherently different in their protein characteristics (Nuez-Ortín and Yu, 2009; CIGI, 2011), we had anticipated that feeding them as the major source of dietary protein would influence RDP supply for microbial growth and, consequently, MP supply for milk production. Our results indicate that RDP contents at 11.3 and 10.7% in diets containing W-DDGS and B-DDGS, respectively, were not different and were well above the recommended range of 9.5 to 10.5% for maximum microbial protein synthesis (NRC, 2001). It is not surprising, therefore, that microbial NAN and MP supply, and, as a consequence, milk production were unaffected by source of dietary protein.

4.6 Conclusions

Results from this study indicate that reducing dietary CP content from 17.3 to 15.2% decreased milk production. This response was partly attributed to an insufficient supply of RDP that suppressed microbial NAN synthesis in the rumen, thus decreasing intestinal MP supply. Our results also indicate that feeding up to 10% of dietary DM as B-DDGS or W-DDGS as the major source of protein does not have negative effects on MP supply and milk production in dairy cows, thus W-DDGS and B-DDGS are comparable as sources of protein in dairy cow diets.

In my second experiment (Chapter 4), dietary inclusion of B-DDGS compared to W-DDGS (10% of diet DM) did not compromise nutrient supply, and resulted in similar production responses. Therefore, based on my results, B-DDGS and W-DDGS are comparable as sources of protein in dairy cow diets. Although feeding B-DDGS and W-DDGS in low CP diets (15.2 vs. 17.3%) resulted in a beneficial reduction in N excretion, it also compromised production performance. Additionally, milk N efficiency (i.e., milk N/N intake) did not improve for cows fed the low CP diet as a result of the undesirable decrease in milk and milk protein yields. Nitrogen transactions that occur in the rumen have a huge impact on the efficiency of N utilization and production performance. A major factor that influences these N transactions is fermentable energy supply. Therefore, for my next experiment (Chapter 5) I focused on increasing dietary fermentability, a recognized strategy to enhance nutrient supply and, thus, productivity in high-yielding dairy cows. One way to increase ruminal fermentable energy supply is by partially substituting starch sources including barley and corn grain with sugar. Therefore the major goal of my third experiment was to determine whether partially replacing barley or corn starch with sugar (dry whey permeate as a source of lactose) in diets containing W-DDGS would improve ruminal N metabolism and reduce N excretion, without compromising production performance. A major concern when considering partial substitution of dietary starch with sugar is the increased risk of ruminal acidosis due to an increase in the rate of ruminal SCFA production. However there are indications that feeding sugar might not necessarily cause ruminal acidosis possibly because of an increase in ruminal SCFA absorption. However, this remains to be substantiated. Therefore, the second goal of my third experiment (Chapter 5) was to investigate the effects of partially replacing barley or corn starch with lactose on ruminal SCFA concentration and absorption, and ruminal acidosis.

5. EFFECTS OF PARTIAL REPLACEMENT OF DIETARY STARCH FROM BARLEY OR CORN WITH LACTOSE ON RUMINAL FUNCTION, OMASAL NUTRIENT FLOW, N UTILIZATION, AND LACTATION PERFORMANCE OF DAIRY COWS.

5.1 Abstract

Sugars are more rapidly fermented in the rumen than starch, and replacing dietary starch with sucrose in corn/alfalfa silage-based diets has been reported to improve milk production in dairy cows. Limited research is available with lactose as a partial replacement of starch from barley or corn, which differ in their rates and extents of ruminal fermentation. The objective of this study was to determine the effects of replacing barley or corn starch with lactose (as dried whey permeate; DWP) on ruminal function, omasal nutrient flow and lactation performance. Eight lactating dairy cows were used in a replicated 4 × 4 Latin square design with 28-d periods (18 d of dietary adaptation and 10 d of measurements) and a 2 × 2 factorial arrangement of dietary treatments. Four cows in one Latin square were ruminally-cannulated for the measurement of fermentation characteristics, short-chain fatty acid (SCFA) absorption, and N metabolism in the rumen, and omasal nutrient flow. The treatment factors were source of starch (barley vs. corn) and dietary inclusion level of DWP (0 vs. 6%; DM basis) as a partial replacement for starch. Diets were isonitrogenous (18% CP) and contained 3 or 8% total sugar. The starch content of the low sugar diet was 24% compared to 20% for the high sugar diet. Dry matter intake, and milk and milk component yields did not differ with diet. However, the dietary addition of DWP tended to increase ruminal butyrate concentration (13.6 vs. 12.2 m*M*), and increased the chloride-competitive absorption rates for acetate and propionate. There was no sugar effect on minimum ruminal pH, and the duration and area pH < 5.8. Although ruminal propionate concentration was higher in cows fed barley than corn, there were no differences in SCFA absorption rates. Minimum ruminal pH tended to be lower (5.47 vs. 5.61) when feeding barley than corn. The duration when ruminal pH was below pH 5.8 tended to be shorter (186 vs. 235 min/d), whereas the area (pH × min/d) that pH was below pH 5.8 was smaller (47 vs. 111) on the corn than barley diets. Cows fed the high compared to the low sugar diet had lower ruminal NH_3-N concentration. Microbial protein synthesis and omasal nutrient flow were not

affected by dietary sugar content. Feeding barley compared to corn tended to increase the omasal flow of N, total NAN and total bacterial NAN, and increased microbial efficiency. In conclusion, partially replacing dietary corn or barley starch with sugar up-regulated ruminal acetate and propionate absorption and reduced ruminal NH_3-N concentration, but had no effect on ruminal pH, microbial protein synthesis, omasal nutrient flow and production in dairy cows.

5.2 Introduction

Although it is recommended to feed at least 20% of dietary DM as starch for optimum production performance (Caccamo et al., 2012), dairy cow diets are usually not formulated for a targeted sugar content, which is typically less than 3% under most feeding conditions in North America (Hoover and Miller Webster, 2001). In recent years, there has been considerable interest in feeding sugar from sources such as molasses (sucrose) and dried whey permeate (DWP; lactose) as partial replacement for dietary starch to control rising feed costs (Golombeski et al., 2006; Martel et al., 2011). Compared to starch, sugar has a faster rate of ruminal fermentation (Sniffen et al., 1992; Chamberlain, 1993; Van Amburgh et al., 2010). Therefore, it is logical to expect that increasing dietary sugar content by partially replacing starch could result in the rate of short-chain fatty acid (SCFA) production exceeding its ruminal clearance rate. Consequently, accumulation of SCFA in the rumen could then lead to a decrease in pH (Oba, 2011), with associated negative effects on dry matter intake (DMI), fiber digestion, and milk production and composition (Allen, 1997; Zebeli et al., 2012). Contrary to these expectations, increasing dietary sugar content up to 13% of dietary DM by replacing a portion of dietary corn starch with either sucrose (Broderick and Radloff, 2004; Broderick et al., 2008) or lactose (DeFrain et al., 2004) did not cause a decrease in ruminal pH in lactating cows. Furthermore, ruminal pH tended to increase (Penner and Oba, 2009) or increased (Martel et al., 2011) when feeding up to 5% sucrose as a partial replacement for corn starch. Although desirable, these responses in ruminal pH are unexpected, and their etiology remains to be fully elucidated. Gaining a better understanding of the mechanism(s) involved is critical for the development of nutritional strategies to combat subacute ruminal acidosis (SARA).

Although several theories have been suggested (Penner et al., 2009a; Oba, 2011), butyrate-induced changes in ruminal epithelial cell metabolism could possibly explain the lack of

an induction of ruminal acidosis when sugar substitutes for dietary starch. Feeding up to 16% sugar by including lactose or sucrose has consistently been shown to increase ruminal butyrate concentration (Doreau et al., 1987; DeFrain et al., 2004, 2006). During its absorption into blood, up to 90% of ruminal butyrate is metabolized mainly to BHBA (Bergman, 1990). Therefore, the elevated plasma BHBA concentration observed by others (Doreau et al., 1987; DeFrain et al., 2004, 2006) when feeding sugar is an indirect reflection of increased ketogenesis from ruminal butyrate. This process is known to stimulate growth and proliferation of ruminal papillae by increasing cellular mitogenic rate, while decreasing apoptosis rate (Mentschel et al., 2001). Morphological surface enlargement resulting from an increase in papillae number and size as diet fermentability and ruminal butyrate concentration increases is well documented (Sakata and Tamate, 1978; Dirksen et al., 1985; Liebich et al., 1987; Baldwin et al., 2004; Naeem et al., 2012). This enhancement of epithelial absorptive area could potentially explain why dietary addition of sugar does not cause an accumulation of SCFA and, subsequently, a decrease in ruminal pH (Ordway et al., 2002; Penner et al., 2009b). However, there is increasing evidence suggesting that functional adaptation in ruminal epithelial cells that is characterized by changes in ion transport mechanisms as diet fermentability increases, also plays a key role in pH regulation. In a recent time course study, Etschamann et al. (2009) switched sheep from a low to high nonstructural carbohydrate (NSC) diet and measured (at 1, 2, 4, 6 and 12 weeks) the SCFA absorption-linked transepithelial movement of Na^+. Up to 73% of the upregulation in Na^+ absorption occurred within a week of diet change, hinting at the importance of functional changes that precede, and augment morphological adaptation known to require over 4 weeks to reach peak levels (Etschamann et al., 2009). Sehested et al. (2000) also measured short-term ruminal absorptive adaptation for SCFA in cows by feeding additional carbohydrate once daily and measuring epithelial butyrate absorption in vitro. Despite the absence of proliferative changes, butyrate absorption increased as ruminal SCFA concentration increased, possibly implying upregulation of cellular protein-mediated transport (Sehested et al., 2000). Moreover, Penner et al. (2009b) also observed that sheep that were less susceptible to SARA exhibited greater in vitro apical uptake of acetate and butyrate that was mediated via an upregulation in epithelial cell transporter activity. To our knowledge, there has been no study to determine whether dietary inclusion of sugar in dairy cow diets causes changes in SCFA absorption in vivo.

It is also not clear if any changes in absorptive function also alter the relative proportions of SCFA absorbed via passive diffusion or protein-mediated transport. Therefore, a major objective of the current study was to determine whether replacing dietary starch with sugar has an impact on total SCFA absorption in dairy cows and, if so, what mechanisms are involved in that response.

The growth rate of ruminal microbes is directly proportional to the rate at which carbohydrates are fermented to yield energy (ATP), provided N supply is not limiting (Nocek and Russell, 1988; Russell et al., 1992). Therefore, replacement of dietary starch with sugar could result in an increase in microbial N yield as sugar has a faster rate of ruminal fermentation compared to starch. Besides increased sequestration of both preformed AA and NH_3-N into microbial protein, the deamination rate of dietary AA to NH_3-N could also decrease with the addition of sugar in place of starch, as a result of its faster ruminal fermentation rate (Hristov et al., 2005). However, there are contradictions on the impact of replacing dietary starch with sugar on ruminal N metabolism. Substitution of starch DM with up to 7.5% sucrose (DM basis) had no effect on in vitro NH_3-N concentration and microbial protein synthesis (Vallimont et al., 2004), whereas Ribiero et al. (2005) reported an increase in bacterial OM after the addition of up to 8.0% sucrose to fermentation media. Despite a decrease in ruminal NH_3-N concentration when up to 7.5% sucrose (10% total dietary sugar) substituted for corn starch in cow diets, microbial N flow to the duodenum either decreased (Sannes et al., 2002) or remained unchanged (Broderick et al., 2008). However, supplementing grass silage-based diets with sucrose or lactose compared to starch led to a greater reduction in ruminal NH_3-N concentration and a greater increase in the estimated flow of microbial N at the small intestine in sheep (Chamberlain et al., 1993). Additionally, intra-ruminal infusion of sucrose in grass silage-based diets also caused a decrease in ruminal NH_3-N concentration and a concomitant increase in microbial protein synthesis in dairy cows (Kim et al., 1999; Kim et al., 2000). These discrepant responses could be explained by a number of factors, including the type of sugar (Chamberlain et al., 1993), forage source (Oelker et al., 2009), and the source of dietary starch that is being replaced with sugars. Corn and barley grain are the main starch sources that are fed to dairy cows in Canada and the United States. Corn contains more starch than barley (72 vs. 58%; Huntington, 1997). The rates and extent of ruminal starch also differ, with 55 to 70% of corn starch and 80 to 90% of barley starch

being digested in the rumen. As a result of these differences in their starch content and ruminal starch fermentation, the impact of replacing corn or barley starch with sugar on ruminal SCFA production and absorption, and ruminal acidosis and N utilization could also be different.

Therefore, the major objective of this study was to investigate the effects of partial replacement of barley or corn starch with lactose on ruminal SCFA concentration and absorption, and ruminal acidosis. The second objective was to delineate the effects of partial replacement of starch from barley or corn with lactose on ruminal N utilization, omasal flow of nutrients and production performance. We hypothesized that partial replacement of dietary starch with lactose would attenuate ruminal acidosis, improve efficiency of N use in the rumen, and lactation performance in dairy cows, and that the effects would be dependent on the source of starch being partially replaced (i.e., barley or corn).

5.3 Materials and Methods

5.3.1 Animals and Experimental Design

Eight lactating dairy cows (average DIM = 109 ± 36; average BW = 711 ± 37 kg) were used in a replicated 4 × 4 Latin square design with 28-d periods (18 d of dietary adaptation and 10 d of measurements) and a 2 × 2 factorial arrangement of dietary treatments. Four cows in one Latin square were ruminally-cannulated for the measurement of ruminal fermentation characteristics, SCFA absorption, ruminal N metabolism, omasal nutrient flow, and urea-N recycling kinetics. All cows were housed in individual tie-stalls at the Greenbrae Dairy Research Facility (University of Saskatchewan). The University of Saskatchewan Animal Care Committee approved the use of cows for this experiment (UCACS Protocol No. 20040048), and they were cared for in accordance with the Canadian Council of Animal Care (1993) regulations.

5.3.2 Experimental Treatments and Feeding Management

The treatment factors were source of starch (barley vs. corn) and dietary inclusion level of DWP (0 vs. 6%, DM basis) as a partial replacement for starch. Barley and corn grain were fed as dry rolled, which is the typical processing method used in Western Canada. The processing index for barley was 60.4%, whereas it was 67.9% for corn. Diets were isonitrogenous (18% CP)

and contained 3% (low) or 8% (high) total sugar (Table 5.1). The starch content of the low sugar diets was 24% compared to 20% for the high sugar diets. Cows were fed twice daily at 0900 and 1600 h as total mixed ration (TMR) for ad libitum intake. The forage:concentrate ratio of the TMR was 50:50 with the forage component of the TMR being a mixture of barley silage and chopped alfalfa hay (Table 5.1).

5.3.3 Data Collection and Sampling

On d 18 (0900 h) of each experimental period, the 4 ruminally-cannulated cows were fitted with temporary vinyl catheters (0.86 mm I.D. × 1.32 mm O.D.; Scientific Commodities Inc., Lake Havasu City, AZ) in the right and left jugular veins to facilitate continuous isotope infusion and blood sampling. Whole body N balance and ruminal microbial production using ^{15}N as a microbial marker were subsequently determined from d 19 to 23 as described by Lobley et al. (2000). Briefly, urine and fecal samples were collected on d 18 for analysis of ^{15}N natural abundance. Thereafter, continuous infusion of double-labeled urea ([$^{15}N^{15}N$]-urea, 99.8 atom % ^{15}N; Cambridge Isotope Laboratories, Andover, MA) prepared in 0.15 *M* sterile saline started at 0800 h on d 19 of each experimental period and ended at 0600 h on d 25. Individual cow N intake from d 11 to d 18 was used to determine the daily [$^{15}N^{15}N$]-urea dosage rate. Total fecal and urine collections were conducted from 0800 h on d 19 to 0800 h on d 23 (96 h). Large steel trays placed behind each stall were used to collect feces. On each day, fecal output for each cow was thoroughly mixed and quantitatively transferred to pre-weighed plastic tubs for weighing. Subsequently, a 2.5% fecal subsample was collected and stored at -20°C for later chemical analysis. Indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) used for total urine collection were inserted at 0800 h on d 18 before connection to urine collection tubing at the start of [$^{15}N^{15}N$]-urea infusion (0800 h on d 19). Urine was collected into 20-L carboy polyethylene containers containing 150 mL of concentrated HCl, and this acidification (pH < 3) of urine during collection was necessary to prevent microbial growth and volatilization of NH_3-N. Daily urine output was recorded, before collection of a 5% subsample that was pooled by cow for each period and stored at -20°C for later N analysis. A 2-mL urine sample was diluted with 8 mL of distilled water, stored at -20°C and later analyzed for urea-N.

Table 5.1 Ingredient and chemical composition of diets

	Low Sugar		High Sugar	
	Barley	Corn	Barley	Corn
Ingredient Composition, % of diet DM				
Alfalfa hay	15.0	15.0	15.0	15.0
Barley silage	34.4	34.4	34.4	34.4
Soybean hulls	0.25	3.42	0.17	2.60
Barley (rolled)	27.0	_	19.3	_
Corn (rolled)	_	21.9	_	15.8
Whey permeate (dry)	_	_	6.0	6.0
Wheat-based DDGS[1]	10.0	10.0	10.0	10.0
Canola meal	6.2	8.5	9.1	9.3
Corn gluten meal	0.50	0.33	0.58	0.42
Soybean meal	2.5	2.3	1.3	2.3
Fat canola oil	0.60	0.60	0.60	0.60
Mineral-vitamin mix	1.8	1.8	1.8	1.8
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[2]	0.17	0.17	0.17	0.17
Nutrient composition, % of DM				
DM	55.8	56.0	56.3	55.8
OM	92.3	92.5	91.8	91.9
CP	17.9	17.9	17.9	18.0
Ether extract	3.0	3.5	3.0	3.6
ADF	18.9	19.6	18.4	19.0
NDF	33.4	33.7	32.0	32.2
Starch	24.3	24.4	19.9	19.8
Total ethanol-soluble carbohydrates[3]	2.9	3.3	7.6	8.1
NSC[4]	38.0	37.5	38.6	38.1
NE_L[5], Mcal/kg	1.64	1.65	1.64	1.64

[1]Dried distillers grains with solubles.

[2]Dynamate (Eastern Minerals Inc., Henderson, NC) contained 18% K, 11% Mg, and 22% S.

[3]Determined according to Hall et al. (1999), using sucrose as a standard.
[4]NFC = 100 – (%NDF + %CP + %ether extract + %ash.
[5]Estimated using CPM-Dairy (v 3.0.8, Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; William H. Miner Agricultural Research Institute, Chazy, NY) using the chemical analysis of feed ingredients.

To measure the flow of nutrients at the omasal canal, Cr-EDTA (Udén et al., 1980) was used as a digesta marker. On d 17 of each experimental period, a 500-mL omasal digesta sample was collected for determination of background ^{15}N (^{15}NB) abundance. A priming dose of Cr-EDTA that was equivalent to half the daily dose was then administered into the rumen via the ruminal cannula. Subsequently, a Cr-EDTA solution was continuously infused into the rumen using a peristaltic pump (Model: 205U, Watson and Marlow, Cornwall, UK) for the next 7 d (d 18 to 25) at a constant rate of 1 L/d, providing 2.77 g of Cr per day. The amount of marker solution infused each day (~1 L) was recorded. During each period, a 50-mL sub-sample of the marker solution that was infused for each cow was collected and stored at room temperature for Cr analysis. The omasal sampling technique as described by Huhtanen et al. (1997) was used to collect omasal digesta. Briefly, this technique involved locating the omasal canal by hand and then inserting a sampling tube via the ruminal cannula. Sampling tubes were inserted at each sampling time in order to ensure correct positioning in the omasal canal and to minimize the potential negative impact on digesta passage and normal digestive function if the sampling tubes were left in place between samplings. Once the sampling tube had been inserted in the omasal canal, a 600-mL omasal digesta sample was collected from each cow at 0900, 1500 and 2100 on d 23; 0300, 1200 and 1800 h on d 24; and 0000, and 0600 h on d 25, such that the collected samples were representative of a 24-h feeding cycle. Omasal samples were stored at -20ºC and pooled by cow per period over the 24-h feeding cycle.

To measure ruminal SCFA and NH_3-N concentrations, and bacterial ^{15}N enrichment, approximately 400 mL of ruminal digesta was collected at the same time points as omasal sampling from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula. The ruminal contents were strained through 4 layers of cheesecloth. Two 10-mL sub-samples of ruminal fluid were then collected and mixed with chilled 25% (wt/vol.) meta-phosphoric acid (H_2PO_4) or 1% H_2SO_4 and stored at -20°C for later determination of SCFA and NH_3-N, respectively. After straining through cheesecloth, the remaining solid digesta was mixed with 500 mL of 0.15 *M* saline solution, and homogenized in a blender (NuBlend, Waring Commercial, Torrington, CT) for 60 s to dislodge particle-associated bacteria. The blended mixture was squeezed through four layers of cheesecloth to obtain a second filtrate. The two filtrates were mixed and 375 mL of the mixture were used to isolate mixed ruminal bacteria by

differential centrifugation. Briefly, filtrates were centrifuged at 500 × *g* for 20 min at 4°C to remove protozoa and residual feed particles. The supernatant was collected and centrifuged at 20,000 × *g* for 20 min at 4°C to obtain a ruminal bacterial pellet. Bacterial pellets were then pooled by cow per period and stored at -20°C. Blood samples were also collected at each omasal sampling time. Plasma was harvested by centrifugation at 1,500 × *g* for 15 min at 4°C. Plasma samples were subsequently pooled by cow for each 24-h feeding cycle before storage at -20°C for later BHBA, glucose, insulin and urea-nitrogen (PUN) analysis.

On d 28, the temporarily isolated and washed reticulo-rumen (WRR) technique as described by Kramer et al. (1996) was used to determine SCFA absorption. Briefly, reticulo-ruminal contents were evacuated through the cannula and were stored in insulated tubs. After evacuation, the reticulo-rumen was washed 3 times with lukewarm water (30 L; 38°C), followed by 3 washes using a buffer solution (24 L; 38°C). The wash buffer contained (in m*M*) 100 NaCl, 25 $NaHCO_3$, and 30 sodium acetate. Osmolality of the wash buffer was 330 mosmol/kg and its pH was adjusted to 6.2 by addition of HCl. Isolation of the reticulo-rumen was achieved by placement of an esophageal-occluding device in the distal esophagus, which suctioned saliva and prevented its entry into the reticulo-rumen. To prevent passage of experimental buffer from the reticulo-rumen, an indwelling Bardex Foley bladder catheter (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) was placed in the omasal orifice before the balloon was inflated. Following isolation of the reticulo-rumen, a final wash (8 L of wash buffer) was conducted and the remaining buffer was removed completely from the reticulo-rumen. Subsequently, 20 L of pre-warmed (38°C) low and high Cl^- experimental buffers containing Cr-EDTA (1 m*M*) as a volume marker (Table 5.2) were sequentially incubated in the rumen. The order in which the experimental buffers were incubated was randomly assigned and balanced for residual effects. Before incubation of the second experimental buffer, what remained of the first experimental buffer was removed completely from the rumen. This was followed by another wash (8 L of wash buffer) to prevent buffer carryover. To ensure mixing in the reticulo-rumen, incubated buffer was continuously gassed with 100% CO_2 through the use of tubing fitted with an air stone. A 15-mL sample of each experimental buffer was collected prior to incubation and stored -20°C for later determination of osmolality. Experimental buffer samples (35 mL) were also collected into 50-mL centrifuge tubes containing 7 mL of 25% (wt/vol) H_2PO_4 prior to

Table 5.2 Composition of experimental buffers

	Buffer	
	Low Chloride	High Chloride
Ingredient, mmol/L		
Ca gluconate	2	-
Mg gluconate	2	-
Na gluconate	5	-
Ca chloride	-	2
Mg chloride	-	2
Na chloride	-	5
Potassium acetate	20	20
Sodium acetate	40	40
Mannitol	84	-
Choline chloride	-	40
Sodium propionate	25	25
Butyric acid	15	15
Lactic acid	5	5
$NaHCO_3$	20	20
Cr-EDTA	1	1
Chloride concentration, mmol/L	0	49
Osmolality, mosmol/kg	332	332
pH	6.2	6.2

incubation, and at 5 and 50 min after incubation in the rumen. Samples were immediately stored at -20°C for later Cr and SCFA analyses. Following collection of the last sample, residual ruminal buffer was vacuumed out and reticulo-ruminal contents transferred back into the rumen.

Ruminal pH was also measured in the 4 ruminally-cannulated cows at 2-minute intervals over a 96-h period (d 19 to 23) using the Lethbridge Research Center Ruminal pH Measurement System (Dascor, Escondido, CA) as described by Penner et al. (2006).

Feed intake was recorded daily throughout the experiment. Samples of the TMR and orts were collected from d 21 to d 23 and were stored at -20°C for later analysis. Cows were milked three times daily at 0430, 1230, and 1900 h, and milk weights were recorded throughout the experiment. Milk samples were collected daily on 3 consecutive days (d 20, 21, and 22) from all three milkings into vials containing 2-bromo-2-nitropropane-1-2-diol as a preservative. Samples were submitted to the Alberta Central Milk Testing Laboratory (Edmonton, Alberta, Canada) for CP, fat, lactose, and MUN analyses.

5.3.4 Sample Analyses

After the experiment, frozen TMR and fecal samples were thawed overnight at room temperature, pooled per collection period for each cow, and subsequently dried in an oven at 60°C for 48 h (AOAC, 1990; method 930.15). Dried TMR and fecal samples were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Samples were analyzed for DM (AOAC, 1990; method 930.15), OM (AOAC, 1990; method 942.05), CP using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05), ether extract (AOAC, 1990; method 920.39), and ADF and NDF (Van Soest et al., 1991). Amylase and sodium sulfite were used for NDF determination. Total starch in ground feed samples was determined using the Megazyme Total Starch Assay Kit (McCleary et al., 1997; Megazyme International Ireland Ltd., Wicklow, Ireland) whereas total ethanol-soluble carbohydrates were determined as described by Hall et al. (1999) and Dubois et al. (1956). Frozen urine samples were thawed overnight at room temperature and then analyzed for N using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05). The plasma and dilute urine samples were analyzed for urea-N by the diacetyl monoxime method (Marsh et al., 1957) using a colorimetric urea-N kit

(Stanbio Urea Nitrogen Kit, Procedure No. 0580; Stanbio Laboratory, Boerne, TX, USA). The concentration of glucose in plasma was determined colorimetrically using the glucose oxidase method (Procedure No. 1070; Stanbio Laboratory, Boerne, TX). Plasma insulin was measured using a commercial bovine ELISA kit (Mercodia AB, Uppsala, Sweden). To quantify plasma BHBA, a coupled enzymatic oxidation of BHBA to acetoacetate with 3-hydroxybutyrate dehydrogenase (No. H6501; Roche, Mississauga, Ontario, Canada) and reduction of NAD to NADH was measured using a plate reader at 340 nm. Milk samples were analyzed for fat, CP, lactose and MUN using infrared spectroscopy (MilkoScan 605; Foss Electric, Hillerød, Denmark; AOAC, 1990; method 972.16).

In preparation for non-ammonia nitrogen (NAN) and ^{15}N analyses, the omasal ^{15}NB and bacterial pellet samples were freeze-dried. Freeze-dried ^{15}NB samples were then ground through a 1-mm screen (Christy-Norris Ltd.) before being pulverized with a ball mill. Freeze-dried bacterial pellet samples were ground using a mortar and pestle. The composited omasal digesta samples were freeze-dried and ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, UK). To determine Cr concentration, a 1-g sample was combusted at 550°C for 8 h in a muffle furnace (AOAC, 1990) before nitric acid digestion as described by Vicente et al. (2004). Subsequently, Cr concentration was measured by atomic absorption spectrophotometry (iCE 3000 series, Thermo Fisher Scientific Inc., Waltham, MA). The Cr concentration in omasal digesta was then used to determine omasal DM flow (France and Siddons, 1986). To determine the flow of nutrients to the omasum, the freeze-dried omasal digesta samples were analyzed for OM by combustion in a muffle furnace at 550°C for at 8 h, total N (Leco 2000; Leco Instruments, Inc., St. Joseph, MI), ADF and NDF. To quantify NH_3-N, 10 mL of 0.07 *M* sodium citrate (pH 2.2) was added to 0.5 g of omasal digesta and the mixture vortexed and subsequently held at 39°C for 30 min in a forced-air oven. The extracts were then centrifuged at 18,000 × *g* for 15 min at 4°C, and the resultant supernatant was analyzed for NH_3-N using the phenol-hypochlorite method as described by Broderick and Kang (1980). Samples of ^{15}NB, bacterial pellet and omasal digesta were prepared for ^{15}N analysis as described by Brito et al. (2009). Briefly, to volatize NH_3-N prior to ^{15}N analysis, ^{15}NB, bacterial pellet and omasal digesta samples containing approximately 100 µg of N were weighed into 5 × 9-mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK). Subsequently, 50 µL of 72 m*M* K_2CO_3

was added to each capsule before incubation in a forced air oven at 60°C for 24 h. Enrichment of ^{15}N in NAN of the samples was then measured by combustion to N_2 gas in an elemental analyzer and continuous flow isotope ratio-mass spectrometry.

Ruminal fluid and buffer samples were analyzed for SCFA using gas chromatography (GC) as described by Khorasani et al. (1996) with modifications. Briefly, samples were thawed and centrifuged (6,000 × *g*, 10 min, 4°C) before 1.5 mL of supernatant was transferred to micro-centrifuge tubes for additional centrifugation (14,000 × *g*, 10 min, 4°C). A 1-ml subsample of the resultant supernatant was mixed with 200 µL of isocaproic acid as an internal standard. Separation of compounds was conducted on an Agilent GC system (Agilent 6890 Series, Agilent Technologies, Waldbronn, Germany) using a column (30.0 m × 320 µm × 0.25 µm; model 7HM-G009-11, Zebron, Phenomenex, Torrance, CA) flow rate of 35 mL/min. Column conditions were an initial temperature of 90°C held for 0.1 min before an increase of 10°C/ min to 170°C. Injector temperature was set at 170°C, whereas detector temperature was 250°C. Ruminal samples for NH_3-N analysis were thawed and centrifuged (18,000 × *g*, 10 min, 4°C), with the resultant supernatant analyzed for NH_3-N using the phenol-hypochlorite assay described by Broderick and Kang (1980). Ruminal buffers were also centrifuged (6,000 × *g*, 10 min, 20°C) and the supernatant used for Cr analysis by atomic absorption spectroscopy (iCE 3000 series, Thermo Fisher Scientific Inc., Waltham, MA) as described by Williams et al. (1962). An osmometer (Model 3250, Advanced Instruments Inc. Norwood, MA) was used to measure osmolality of thawed experimental buffers.

5.3.5 Calculations and Statistical Analyses

Omasal true digesta NAN content was calculated as total N – NH_3-N. Omasal digesta and bacterial pellet ^{15}N enrichment (APE) was calculated as: ^{15}N APE = sample ^{15}N percent – mean background sample ^{15}N percent. Bacterial NAN flow was then calculated by multiplying omasal NAN flow by the ratio of omasal digesta ^{15}N enrichment to bacterial pellet ^{15}N enrichment (Wickersham et al., 2009b). Apparent ruminal nutrient digestibilities were calculated as nutrient intake – omasal flow of nutrient, with flows expressed as kilograms per day (Reynal and Broderick, 2005). Organic matter truly digested in the rumen (OMTDR) was determined as OM intake – (omasal OM flow – microbial OM flow). Omasal flow of nonammonia nonmicrobial N

(NANMN), RUP, and RDP were also calculated as described by Reynal and Broderick (2005) as follows: NANMN flow = total NAN flow – microbial NAN flow; RUP flow = total CP flow – microbial CP flow; and RDP supply = total CP intake – RUP flow.

The recorded ruminal pH data was summarized daily as minimum, mean, and maximum pH. Ruminal acidosis was considered to occur when ruminal pH was <5.8. The duration (min/d) and total area (pH × min) that ruminal pH was <5.8 were also calculated (Penner and Oba, 2009).

The Cr concentration of samples collected following 5 and 50 min of incubation were used to determine the actual volume of ruminal buffer. The disappearance or absorption rates of acetate, propionate and butyrate were then calculated according to the following formula:

Absolute SCFA absorption rate (mmol/h) = $V_{5min} \times C_{5min} - V_{50min} \times C_{50min}$

Fractional SCFA absorption rate (%/h) = $(V_{5min} \times C_{5min} - V_{50min} \times C_{50min})/ (V_{5min} \times C_{5min}) \times 100$

Where V = volume of buffer and C = concentration of acetate, propionate or butyrate at the respective time.

All data on nutrient digestibilities, omasal flow, and excretion, ruminal pH, SCFA absorption and blood metabolites were analyzed as a 4 × 4 Latin square using the PROC MIXED procedure of SAS (SAS Institute, 2004) according to the following model: $Y_{ijk} = \mu + P_i + C_j + T_k + E_{ijk}$ where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the fixed effect of period i, C_j is the random effect of cow j, T_k is the fixed effect of dietary treatment k, and E_{ijk} is the residual error. Production data for the 8 cows were analyzed using the MIXED procedure of SAS (SAS Institute, 2004) for a replicated 4 × 4 Latin square design according to the following model: $Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl}$ where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square i, P_j is the fixed effect of period j, $C_{k(i)}$ is the random effect of cow k (within square i), T_l is the fixed effect of dietary treatment l, ST_{il} is the interaction between square i and treatment l, and E_{ijkl} l is the residual error. Factorial contrasts were used to test the main effects of type of grain (barley vs. corn), level of dietary sugar (low vs. high) and their interaction (type of grain × level of dietary sugar). The interaction term was

removed from the model when $P > 0.15$. Treatment differences were considered significant when $P \leq 0.05$ and tendencies when $0.05 < P \leq 0.10$.

5.4 Results

5.4.1 Dietary Characteristics

Dietary ingredient and chemical compositions are presented in Table 5.1. The 6.0% inclusion level of dried whey permeate (DWP) targeted a total sugar concentration of approximately 7.0% based on previous studies showing improved lactation performance with dietary sugar levels ranging between 4.5 and 8.7% (Penner and Oba, 2009; Penner et al., 2009a). Nonstructural carbohydrate (NSC) analysis of TMR showed that partial replacement of an equivalent amount of barley or corn starch with 6% DWP (DM basis) increased total sugar from 2.9 and 3.3% (DM basis) in low sugar diets to 7.6 and 8.1% in high sugar diets. The starch content of the low sugar diets was 24.4% compared to 19.9% for the high sugar diets, and there were marginal deviations in total NSC concentration (within ±0.6 percentage units of the targeted 38.0%; Table 5.1). These results indicate that the 4 diets conformed to our experimental plan.

5.4.2 Production Parameters

There was no grain or sugar effect ($P > 0.05$) on DMI, milk yield, ECM and feed efficiency (Table 5.3). Although there was no diet effect ($P > 0.05$) on milk fat concentration, milk protein concentration tended to be higher ($P = 0.08$) in cows fed the barley diet compared to those fed the corn diet. Increasing dietary sugar content tended to decrease ($P = 0.08$) milk lactose concentration. There was no diet effect ($P > 0.05$) on milk fat, protein, and lactose yields. Cows fed the barley diet had a higher ($P = 0.04$) MUN concentration compared to those fed the corn diet, whereas increasing the sugar content of the diet decreased ($P = 0.02$) MUN concentration.

5.4.3 Ruminal Fermentation Characteristics

Ruminal NH_3-N concentration was lower ($P = 0.01$) in cows fed the high compared to the low sugar diet (Table 5.4). Increasing dietary lactose content tended to increase ($P = 0.08$)

Table 5.3 Dry matter intake (DMI), and milk yield and composition of cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (*n* = 8)

	Lactose		Grain			*P* value	
Variable	Low	High	Barley	Corn	SEM	Lactose	Grain
DM intake, kg/d	29.2	29.7	29.2	29.7	0.91	0.28	0.28
Milk yield, kg/d	40.6	40.7	40.7	40.6	2.31	0.95	0.79
ECM[1], kg/d	41.1	41.0	41.2	40.9	2.14	0.92	0.96
Feed efficiency[2]	1.40	1.38	1.41	1.37	0.051	0.39	0.22
Milk fat, %	3.53	3.50	3.50	3.53	0.118	0.75	0.75
Milk fat yield, kg/d	1.40	1.40	1.39	1.40	0.077	0.99	0.82
Milk protein, %	3.37	3.31	3.37	3.31	0.091	0.16	0.08
Milk protein yield, kg/d	1.35	1.34	1.36	1.32	0.069	0.73	0.21
Milk lactose, %	4.58	4.53	4.57	4.56	0.058	0.08	0.42
Milk lactose yield, kg/d	1.86	1.85	1.85	1.85	0.117	0.75	0.99
MUN[3], mg/dL	17.8	16.5	17.5	16.8	0.80	0.02	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/DMI.

[3]Milk urea-N.

Table 5.4 Ruminal fermentation characteristics of cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable	Low	High	Barley	Corn	SEM	Lactose	Grain
Ammonia, mg/dL	14.0	11.2	12.3	12.9	0.95	0.01	0.49
Acetate, m*M*	68.0	66.2	67.7	66.5	1.74	0.38	0.55
Propionate, m*M*	25.6	24.4	26.5	23.5	2.35	0.32	0.03
Isobutyrate, m*M*	1.05	0.88	0.98	0.95	0.028	<0.01	0.55
Butyrate, m*M*	12.2	13.6	12.8	13.0	0.50	0.08	0.81
Isovalerate, m*M*	1.51	1.30	1.40	1.41	0.072	0.03	0.85
Valerate, m*M*	1.91	1.99	2.04	1.86	0.056	0.13	<0.01
Acetate:Propionate	2.71	2.81	2.68	2.85	0.211	0.45	0.22
Total SCFA[1], m*M*	110	108	111	107	3.3	0.46	0.13
Total BCFA[2], m*M*	2.56	2.17	2.37	2.36	0.092	0.01	0.94

[1]BCFA = branched-chain fatty acids.

[2]SCFA = short-chain fatty acids.

ruminal butyrate concentration. However, ruminal isobutyrate, isovalerate, and total branched-chain fatty acids (BCFA) concentrations were higher ($P < 0.05$) on the low sugar compared to the high sugar diet. Feeding barley compared to corn increased ruminal propionate ($P = 0.03$) and valerate ($P < 0.01$) concentrations, whereas ruminal acetate and total SCFA concentrations, and acetate:propionate ratio did not differ ($P > 0.05$) with diet.

There was no sugar or grain effect on daily mean ($P \geq 0.15$) and maximum pH ($P \geq 0.78$) (Table 5.5). In addition, dietary inclusion of lactose had no effect ($P \geq 0.52$) on the duration and area pH < 5.8. Daily minimum pH tended to be higher ($P = 0.07$) on the corn compared to the barley diet. However, the duration and area pH < 5.8 tended to be higher ($P = 0.06$), and was higher ($P = 0.04$) in cows fed barley compared to corn, respectively.

5.4.4 Short-chain Fatty Acid Absorption

Both total and chloride-insensitive absolute absorption rates (mmol/h; Table 5.6) and fractional rates (%/h; Table 5.7) for acetate, propionate, butyrate and total SCFA did not differ ($P > 0.05$) with diet. However, the chloride-competitive absolute absorption rate (mmol/h) increased for acetate ($P = 0.02$) and total SCFA ($P = 0.04$), and tended to increase ($P = 0.08$) for propionate in cows fed the high compared to the low sugar diet. The chloride-competitive fractional absorption rate was higher for acetate ($P = 0.02$) and tended to be higher ($P \leq 0.09$) for propionate and total SCFA on the high compared to the low sugar diet. However, both absolute and fractional chloride-competitive absorption rates for butyrate did not differ ($P > 0.05$) with diet.

5.4.5 Ruminal Digestion and Nutrient Flow

Intake, ruminal digestion and omasal flow of DM, OM, NDF and ADF were not affected ($P > 0.15$) by dietary sugar content (Table 5.8). Although starch intake was higher ($P < 0.01$) for cows fed the low compared to those fed the high sugar diet, there was no sugar effect ($P = 0.99$) on omasal flow of starch. Therefore, the amount of starch apparently digested in the rumen was higher ($P < 0.01$) for cows fed the low when compared to those fed the high sugar diet. Although there was no grain effect ($P \leq 0.67$) on DM, OM, NDF and ADF intake, there was a tendency for an increase ($P \leq 0.10$) in the omasal flow of DM, OM and ADF on the barley than

Table 5.5 Ruminal pH of cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable	Low	High	Barley	Corn	SEM	Lactose	Grain
Ruminal pH							
Daily minimum	5.49	5.59	5.47	5.61	0.127	0.20	0.07
Daily mean	6.06	6.11	6.04	6.13	0.136	0.39	0.15
Daily maximum	6.64	6.65	6.64	6.65	0.123	0.78	0.94
Duration pH < 5.8, min/d	366	304	434	235	186	0.52	0.06
Area pH < 5.8, pH × min/d	82.2	75.2	110.8	46.7	49.3	0.80	0.04

Table 5.6 Absolute ruminal absorption rate of short-chain fatty acids in cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable (mmol/h)	Low	High	Barley	Corn	SEM	Lactose	Grain
Total absorption[1], mmol/h							
Acetate	538	609	589	558	52.9	0.32	0.65
Propionate	251	263	261	253	40.6	0.72	0.79
Butyrate	158	169	166	162	16.2	0.60	0.85
Total SCFA[2]	947	1,041	1,016	973	107	0.45	0.72
Cl^--insensitive absorption[3], mmol/h							
Acetate	540.0	501.3	504.5	536.8	46.2	0.56	0.62
Propionate	257.3	226.4	239.6	244.0	21.7	0.30	0.88
Butyrate	161.4	156.5	153.1	164.8	14.0	0.80	0.55
Total SCFA	959	884	897	946	81.0	0.51	0.67
Cl^--competitive absorption[4], mmol/h							
Acetate	-1.51	107.84	84.84	21.48	28.87	0.02	0.15
Propionate	-6.50	36.50	21.45	8.52	24.45	0.08	0.55
Butyrate	-3.17	12.68	12.64	-3.13	7.64	0.17	0.17
Total SCFA	-11.17	157.02	118.97	26.87	51.75	0.04	0.24

[1]Total absorption = SCFA absorption measured with the low chloride buffer.

[2]Total SCFA = Acetate + Propionate + Butyrate

[3]Cl^--insensitive absorption = SCFA absorption measured with the high chloride buffer (HCB).

[4]Cl^--competitive absorption = Total absorption - Cl^--insensitive absorption.

Table 5.7 Fractional ruminal absorption rates of short-chain fatty acids in cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable (mmol/h)	Low	High	Barley	Corn	SEM	Lactose	Grain
Total absorption[1], mmol/h							
Acetate	48.3	54.5	50.9	52.0	4.95	0.31	0.84
Propionate	51.6	54.4	52.4	53.7	7.95	0.66	0.84
Butyrate	57.4	61.6	58.4	60.7	6.53	0.59	0.76
Total SCFA[2]	50.6	55.6	52.3	53.8	5.86	0.43	0.82
Cl^--insensitive absorption[3], mmol/h							
Acetate	48.2	46.1	44.8	49.5	4.70	0.71	0.42
Propionate	54.8	50.6	51.1	54.3	5.34	0.49	0.61
Butyrate	57.9	57.7	54.5	61.2	5.49	0.97	0.35
Total SCFA	51.3	48.9	47.8	52.5	4.92	0.69	0.44
Cl^--competitive absorption[4], mmol/h							
Acetate	-0.24	9.56	7.31	2.01	2.597	0.02	0.18
Propionate	-1.57	7.47	4.53	1.37	5.086	0.09	0.52
Butyrate	-1.17	4.60	4.63	-1.20	2.811	0.18	0.17
Total SCFA	-0.76	6.62	4.55	1.31	2.670	0.08	0.41

[1]Total absorption = SCFA absorption measured with the low chloride buffer.

[2]Total SCFA = Acetate + Propionate + Butyrate

[3]Cl^--insensitive absorption = SCFA absorption measured with the high chloride buffer (HCB).

[4]Cl^--competitive absorption = Total absorption - Cl^--insensitive absorption.

Table 5.8 Nutrient flow from and digestion in the rumen of cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable	Low	High	Barley	Corn	SEM	Lactose	Grain
DM							
Intake, kg/d	28.2	28.7	28.2	28.7	1.32	0.39	0.38
Omasal flow, kg/d	18.3	18.7	19.4	17.6	0.65	0.59	0.07
Apparent digestion, kg/d	10.1	10.0	9.0	11.1	0.97	0.88	0.04
Apparent digestion, % of DM intake	36.2	34.9	32.6	38.5	1.96	0.62	0.08
OM							
Intake, kg/d	26.2	26.3	26.1	26.4	1.25	0.84	0.62
Omasal flow, kg/d	15.4	15.8	16.3	14.9	0.50	0.63	0.07
Apparent digestion, kg/d	10.8	10.6	9.8	11.6	0.97	0.79	0.04
Apparent digestion, % of OM intake	41.7	40.2	38.1	43.9	1.95	0.55	0.07
True digestion, kg/d	16.8	16.7	16.3	17.2	1.05	0.91	0.34
True digestion, % of OM intake	64.4	63.3	63.0	64.7	1.62	0.60	0.42
Starch							
Intake, kg/d	6.91	5.67	6.27	6.31	0.353	<0.01	0.77
Omasal flow, kg/d	1.16	1.16	1.19	1.14	0.107	0.99	0.64
Apparent digestion, kg/d	5.74	4.50	5.07	5.17	0.323	<0.01	0.47
Apparent digestion, % of DM intake	83.3	79.6	81.1	81.8	1.64	0.14	0.75
NDF							
Intake, kg/d	9.57	9.21	9.32	9.45	0.397	0.25	0.67
Omasal flow, kg/d	6.28	6.52	6.90	5.89	0.368	0.67	0.13
Apparent digestion, kg/d	3.40	2.80	2.53	3.67	0.270	0.16	0.02

Apparent digestion, % of NDF intake	35.5	29.9	27.5	38.0	2.45	0.15	0.02
ADF							
Intake, kg/d	5.49	5.39	5.31	5.57	0.226	0.58	0.22
Omasal flow, kg/d	3.22	3.28	3.50	2.99	0.193	0.82	0.10
Apparent digestion, kg/d	2.32	2.16	1.86	2.63	0.177	0.53	0.02
Apparent digestion, % of ADF intake	42.2	39.6	35.3	46.5	2.72	0.52	0.02

the corn diet. The amount of DM and OM apparently digested in the rumen was higher (P = 0.04), whereas the percentage of DM and OM apparently digested in the rumen tended to be higher ($P \leq 0.08$) in cows fed corn compared to those fed barley. Cows fed corn compared to those fed barley also had a greater amount and percentage of NDF and ADF apparently digested in the rumen (P = 0.02).

5.4.6 Intake, Digestibility, and Omasal Flow of Nitrogen Constituents

Intake of N was unaffected by diet ($P > 0.05$), and it averaged 821 g/d (Table 5.9). There was no sugar effect ($P > 0.05$) on N digestibility and flow of N constituents at the omasal canal. However, N apparently digested in the rumen, when expressed as grams per day (P = 0.07) or as a proportion of N intake (P = 0.08), tended to be higher in cows fed corn compared to those fed barley. Although there was no grain effect ($P > 0.05$) on RDP supply, cows fed barley compared to those fed corn tended to have a greater ($P < 0.08$) omasal flow of N and NAN expressed as grams per day or as a proportion of N intake. Feeding cows barley compared to corn also tended to increase ($P < 0.09$) the omasal flow of total bacterial NAN expressed as grams per day, and increased (P = 0.04) microbial efficiency. However, there was no grain effect ($P > 0.05$) on the flow of NANBN and RUP at the omasal canal.

5.4.7 Apparent Nitrogen Balance and Total Tract Nutrient Digestibility

There was no diet effect ($P > 0.05$) on N intake, urinary and fecal N excretion, and subsequently, apparent N balance (Table 5.10). Feeding a high sugar diet tended to increase apparent total tract DM (P = 0.10) and OM (P = 0.08) digestibility, and increased (P = 0.02) apparent total tract fat digestion. Apparent total tract N digestibility tended to be higher (P = 0.09) when cows were fed barley compared to corn, whereas feeding corn compared to barley increased apparent ADF digestibility (P = 0.04).

5.4.8 Blood Metabolites

Although increasing the sugar content of the diet through the addition of lactose tended to reduce PUN (P = 0.07), diet had no effect ($P > 0.05$) on plasma glucose, insulin and BHBA (Table 5.11).

Table 5.9 Intake, digestibility, and omasal flow of N constituents in cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable	Low	High	Barley	Corn	SEM	Lactose	Grain
N intake, g/d	810	832	817	824	36	0.38	0.77
N apparently digested in the rumen							
g/d	10.7	-12.3	-46.9	45.3	32.06	0.58	0.07
% of N intake	1.28	-2.11	-5.63	4.80	3.846	0.48	0.08
N truly digested in the rumen							
g/d	541	541	534	548	40	0.98	0.69
% of N intake	66.7	64.7	65.4	66.1	2.82	0.47	0.79
RDP supply							
g/d	3,464	3,449	3,413	3,499	253	0.94	0.70
% of DMI	12.2	12.0	12.1	12.1	0.53	0.67	0.88
Flow at omasal canal							
N							
g/d	803	847	868	783	27	0.30	0.08
% of N intake	98.7	102.1	105.6	95.2	3.85	0.48	0.08
NH_3-N, g/d	12.8	11.1	11.9	12.0	1.02	0.98	0.13
NAN[1]							
g/d	790	837	856	771	28	0.28	0.07
% of N intake	97.1	100.7	104.1	93.7	3.77	0.45	0.08
NANBN[2]							
g/d	257	281	272	266	23	0.24	0.73
% of NAN flow	32.3	33.7	31.8	34.2	2.52	0.59	0.35

% of N intake	31.7	33.9	33.2	32.4	2.87	0.43	0.79
% of DM intake	0.906	0.983	0.957	0.932	0.0818	0.35	0.74
RUP							
g/d	1,606	1,757	1,702	1,661	141	0.24	0.73
% of DM intake	5.66	6.14	5.98	5.82	0.511	0.35	0.74
Total bacterial NAN							
g/d	533	555	583	505	29	0.58	0.09
% of NAN	67.7	66.3	68.2	65.8	2.52	0.59	0.35
Microbial efficiency							
g of microbial N/kg OMTDR[3]	32.3	33.8	35.8	30.3	2.09	0.48	0.04

[1]NAN = non-NH_3 N

[2]NANBN = non-NH_3 nonbacterial N.

[3]OMTDR = OM truly digested in the rumen.

Table 5.10 Nitrogen balance and apparent total-tract nutrient digestibility of cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable	Low	High	Barley	Corn	SEM	Lactose	Grain
N intake, g/d	812	830	826	817	38.8	0.45	0.69
Urinary Excretion							
Total, kg/d	40.0	38.4	38.4	40.1	3.58	0.68	0.66
Total N, g/d	304	315	313	305	15.3	0.23	0.36
Total N, % N intake	37.7	38.2	38.1	37.7	1.86	0.77	0.81
Urea-N, g/d	225	233	236	222	14.7	0.38	0.14
Urea-N, % urinary N	74.0	73.8	75.3	72.6	1.55	0.90	0.07
Fecal Excretion							
DM, kg/d	9.25	8.85	9.09	9.01	0.518	0.13	0.73
N, g/d	239	241	235	244	13.0	0.79	0.28
N, % N intake	29.4	29.0	28.5	30.0	0.76	0.61	0.04
Total N excretion							
g/d	542	555	549	549	26.4	0.07	0.94
% N intake	67.0	67.2	66.6	67.6	2.44	0.91	0.57
Milk N							
g/d	217	216	221	212	19.5	0.91	0.30
% N intake	26.6	26.0	26.7	25.9	1.80	0.41	0.34
Apparent N-balance, g/d	52.9	58.9	56.5	55.4	10.41	0.69	0.94
Productive N[1], g/d	270	275	277	268	26.3	0.80	0.63
Apparent total-tract digestibility, %							
DM	67.4	69.1	68.1	68.3	0.94	0.10	0.84

OM	68.0	70.1	69.1	68.9	0.87	0.08	0.86
N	70.5	71.0	71.4	70.0	0.78	0.51	0.09
Ether extract	84.5	88.3	86.6	86.2	1.01	0.02	0.81
NDF	46.5	45.3	44.3	47.5	1.48	0.58	0.15
ADF	42.7	41.2	39.9	44.0	1.25	0.38	0.04

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

Table 5.11 Blood metabolites of cows not supplemented (Low) or supplemented (High) with lactose as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Lactose		Grain			*P* value	
Variable	Low	High	Barley	Corn	SEM	Lactose	Grain
PUN[1], mg/dL	18.5	16.9	18.0	17.4	1.52	0.07	0.48
Plasma glucose, mg/dL	64.1	64.3	64.2	64.2	1.99	0.89	0.95
Plasma insulin, μg/L	1.12	1.32	1.11	1.33	0.279	0.23	0.19
Plasma BHBA[2], mg/dL	10.9	11.5	11.3	11.1	0.647	0.42	0.78

[1]Plasma urea-N.

[2]ß-hydroxybutyrate.

5.5 Discussion

Numerous studies (Sannes et al., 2002; DeFrain et al., 2004, 2006; Broderick and Radloff, 2004; Broderick et al., 2008; Oelker et al., 2009; Penner and Oba, 2009; Penner et al., 2009a; Martel et al., 2011) have been conducted to determine the effects of increasing diet fermentability by feeding sugar as a partial replacement of starch with varied responses in milking performance. Besides forage (Oelker et al., 2009) and sugar source (Chamberlain et al., 1993), the type of grain partially replaced by sugar also accounts for the equivocal results reported in literature. In Canada and the United States, barley and corn grain are the major starch sources used to supply the energy precursors that are required for ruminal microbial protein synthesis and milk production. However, these grains differ in their starch content. As expected, barley grain used in our study contained less starch compared to corn grain (61.3 vs. 70.2% of DM). The rate and extent of ruminal starch degradation of these grains also differ, and are influenced by numerous factors including grain-processing method (Patton et al., 2012). For instance, the rate of ruminal starch degradation for dry rolled barley is almost one-fold that of cracked corn (15.3 vs. 8.4% per hr) and, as a result, the extent of ruminal starch degradation of dry rolled barley is greater than that of cracked corn (69.0 vs. 54.6%). Given that barley was dry rolled, whereas corn was cracked (coarsely-rolled) in our study, we anticipated that there would be type of grain × level of dietary sugar interactions. However, contrary to our expectations, we did not observe any interactions for all variables that were measured. This possibly could be explained by the lack of type of grain (barley vs. corn) effect on apparent digestion of dietary starch in the rumen, which averaged 81% across diets. This was within the reported 55 to 90% range (Huntington, 1997; Huntington et al., 2006). The ruminal passage rate of digesta can have an impact on starch digestibility in the rumen (Huntington et al., 2006). Although type of grain did not affect DMI in the present study, DM passage at the omasal canal was lower in cows fed corn compared to those fed barley. Therefore, we can surmise that the longer residence time of digesta in the rumen in cows fed corn increased the extent of starch hydrolysis, compensating for its slower rate of fermentation when compared to barley.

Increasing dietary fermentability is a recognized strategy to enhance nutrient supply and, thus, productivity in high-yielding dairy cows (Broderick, 2003). However, it also increases the risk of ruminal acidosis, which can have a negative impact on animal health and production

performance (Broderick, 2003; Ferraretto et al., 2013). Therefore, this has been a major concern when considering partial substitution of dietary starch with sugar in lactating cow diets. Given its faster rate of ruminal fermentation compared to starch, it is logical to expect that the dietary inclusion of sugar could result in an accumulation of SCFA in the rumen (Firkins et al., 2008; Oba, 2010). However, feeding lactose (3 vs. 8% total dietary sugar) as a partial replacement of barley or corn starch in our study did not result in a decrease in ruminal pH or changes in the duration and area when pH was below 5.8. Similar to our findings, ruminal pH did not change when cows were fed up to 13% of dietary DM as lactose or sucrose (DeFrain et al., 2004; Broderick and Radloff, 2004; Broderick et al., 2008). Moreover, feeding up to 5% sucrose as partial replacement of corn starch tended to increase (Penner and Oba, 2009) or increased (Martel et al., 2011) ruminal pH. Although the mechanism(s) responsible for these responses are yet to be fully elucidated, a possible explanation could be related to SCFA-mediated changes in ruminal epithelial permeability when diet fermentability increases (Gäbel et al., 1991). Ruminal concentration of butyrate typically increases when dietary lactose or sucrose content increases (DeFrain, 2004, 2006; Golombeski et al., 2006; Ribiero et al., 2006; Martel et al., 2011; Eastridge et al., 2011). As expected, feeding lactose in the current study also resulted in a tendency for an increase in ruminal butyrate concentration. This reflects a shift in ruminal fermentation, as lactose is rapidly degraded to lactate which, upon further metabolism, predominantly yields butyrate. Butyrate is a potent modulator of numerous cellular processes and functions in the gut (Guilloteau et al., 2010). Therefore it is plausible that butyrate metabolism during ruminal absorption could trigger rapid functional changes in epithelial tissue geared towards preventing the luminal accumulation of SCFA (Etschamann et al., 2009; Penner et al., 2011). This process is subsequently augmented by the morphological changes in ruminal epithelial tissue that are known to occur at a much slower rate. It is estimated that over 50% of protons in the rumen of dairy cows are removed through absorption across the epithelial cells (Allen, 1997). Therefore, an increase in SCFA absorption rate could possibly counteract an increase in the rate of SCFA production, thereby preventing the expected proton accumulation and subsequent decrease in ruminal pH when diet fermentability increases (Penner et al., 2009b; Martel et al., 2011). However, the mechanisms responsible for absorption of SCFA across the stratified squamous epithelium of the reticulo-rumen are still not fully understood. Passive

diffusion and protein-mediated transport are thought to be the key modes of absorption of protonated or undissociated SCFA (HSCFA), and anions or dissociated SCFA ($SCFA^-$), respectively (Storm et al., 2012). There is limited information on ruminal SCFA absorption, especially on the type of SCFA transporters that are present, their functional significance, mechanisms of action, and contribution to regulation of ruminal pH. Several families of bicarbonate (HCO_3^-) exchange proteins including the putative anion transporter 1 (PAT), anion exchanger 2 (AE) and downregulated-in-adenoma (DRA) have been characterized and suggested to be involved in SCFA transport across the ruminal epithelium in ruminants (Bilk et al., 2005). Using the temporarily-isolated and WRR technique in sheep, Gäbel et al. (1991) showed the existence of an anion ($SCFA^-$, Cl^-, HCO_3^-) exchange system in ruminal epithelial cells. In the same study, an increase in diet fermentability led to an increase in luminal pH because there was an elevation of net HCO_3^- secretion and SCFA absorption by the $SCFA^-/HCO_3^-$ transporter (Gäbel et al., 1991). Additionally, Gäbel et al. (1991) noted that there was competition for transport between $SCFA^-$ and Cl^-. Kramer et al. (1996) and Stumpff et al. (2009) later corroborated these findings, leading to suggestions that the same HCO_3^- exchanger transports both $SCFA^-$ and Cl^-. In a recent study, transitioning calves from a milk replacer diet by supplementing grain or hay increased mRNA expression of DRA over 100-fold, suggesting that it might play a role in buffering the rumen as a result of its $SCFA^-/HCO_3^-$ counterexchange capability (Connor et al., 2010). Therefore, evidence from these studies points to the importance of the epithelial $SCFA^-/HCO_3^-$ counterexchange system in regulation of ruminal pH.

A major objective of the current study was to determine whether increasing dietary sugar content by replacing corn or barley starch with lactose in dairy cow diets causes changes in mucosal permeability. To measure total acetate, propionate and butyrate absorption, and the contribution of the anion ($SCFA^-$, Cl^-, HCO_3^-) exchange system, we used 2 experimental buffers formulated to be similar in composition except for their chloride concentration (i.e., a low [0 m*M*] versus high [40 m*M*] chloride buffer; Table 2). Ruminal chloride concentration is usually low, ranging from 16 to 20 m*M* (Duffield et al., 2004; Shen et al., 2012). Therefore, measuring WRR disappearance rates using a low chloride buffer gives total absorption estimates for acetate, propionate and butyrate. In a recent study, Aschenbach et al. (2009) showed that a chloride concentration >40 m*M* inhibits absorption of acetate and propionate, but not butyrate. Therefore,

to quantify the proportion of total absorption that was chloride-insensitive, we used a high chloride buffer. Chloride-competitive absorption of SCFA, which indirectly gives a measure of HCO_3^--dependent transport, was then determined by difference (as total absorption minus chloride-insensitive absorption).

In the present study, there was no lactose effect on total and chloride-insensitive absorption of acetate, propionate and butyrate. However, increasing dietary lactose content led to an increase in chloride-competitive absorption of acetate and propionate, suggesting an upregulation of carrier-mediated transport of dissociated acetate and propionate ($acetate^-/HCO_3^-$ and $propionate^-/HCO_3^-$ exchange). Since absorption of a mol of $SCFA^-$ through the $SCFA^-/HCO_3^-$ exchanger leads to luminal secretion of approximately 0.5 mol of HCO_3^- (Gäbel et al., 1991), upregulation of chloride-competitive absorption of acetate and propionate could have increased the influx of HCO_3^- into the rumen in the current study. This presumption is supported by our observation that increasing dietary lactose content did not cause a decrease in ruminal pH. Epithelial cell-derived HCO_3^- plays a critical role in ruminal pH regulation as it reacts with luminal protons (H^+) to form CO_2 and water (Aschenbach et al., 2009, 2011; Connor et al., 2010). Aschenbach et al. (2011) estimated that the anion exchange system in high-yielding dairy cows could contribute a comparable amount of HCO_3^- to the rumen as saliva. Although speculative, the fact that chloride-competitive absorption of acetate and propionate increased on the high sugar diet is suggestive of a functional adaptation mechanism that not only increases absorption of acetate and propionate, but also prevents the expected fall in ruminal pH due to the buffering effect of epithelial cell-derived HCO_3^-. Luminal SCFA, especially butyrate, increases epithelial cell metabolic and mitotic activity (Sakata and Tamate, 1978; Mentschel et al., 2001; Connor et al., 2010). Therefore, the increase in ruminal concentration of butyrate on the high sugar diet in our study could have led to its increased absorption and metabolism by epithelial cells, potentially explaining the upregulation of chloride-competitive absorption of acetate and propionate. The exact mechanism leading to this upregulation is yet to be fully elucidated. However, it could involve an increase in the activity and/or number of transport proteins, including DRA, PAT and AE, which are regulated through transcriptional, translational, or post-translational processes. Diet-dependent functional and metabolic changes in ruminal epithelial cells have also been reported in other studies. Connor et al. (2010) noted increased gene

expression for DRA in calves as diet fermentability increased, whereas Gäbel et al. (1991) reported an increase in both SCFA absorption and HCO_3^- secretion under WRR conditions in concentrate- compared to hay-fed sheep. Storeheier et al. (2003) showed that the documented seasonal decrease in ruminal surface area (decreased papillae length and perimeter, and papillae density) in Arctic reindeer is counteracted by an increase in SCFA transporter activity when they consume high sugar diets (lichens that contain mannose, glucose and galactose) in winter. Therefore, besides morphological adaptation, an increase in chloride-competitive uptake of acetate and propionate as observed in our study is a mechanism that could possibly prevent a deleterious decrease in ruminal pH when feeding high sugar diets. Other SCFA transport systems reported in literature (Penner et al., 2009b) including the HCO_3^--independent and nitrate-sensitive uptake of acetate, and HCO_3^--independent uptake of butyrate by epithelial tissue could also be involved.

Compared to acetate and propionate, there was no upregulation of chloride-competitive absorption of butyrate when feeding a high lactose diet. When compared to acetate and butyrate, passive diffusion is considered to be the major pathway for the ruminal absorption of butyrate, primarily because of its higher lipophilicity and steeper diffusion gradient due to its more extensive metabolism by ruminal epithelium (Aschenbach et al., 2011). Therefore, the marginal contribution of carrier-mediated transport to total absorption of butyrate could possibly explain our findings. Aschenbach et al. (2009) also noted that a chloride concentration of up to 80 m*M* did not inhibit butyrate absorption under WRR conditions. This further implies that chloride-competitive absorption might not be of significance for butyrate uptake.

As a result of the positive correlation between the rate of carbohydrate fermentation and sequestration of preformed AA and NH_3-N into microbial protein in the rumen (Nocek and Russell, 1986; Russell et al., 1992), feeding sugar has the potential to improve ruminal N utilization efficiency in dairy cows. In the current study, partially replacing barley and corn starch with lactose resulted in a decrease in ruminal NH_3-N concentration. Others reported similar findings following dietary inclusion of lactose (up to 16% of diet DM; DeFrain et al., 2004, 2006) and sucrose (3 to 8% inclusion level; Sannes et al., 2002; Ribiero et al., 2006). There is evidence that some ruminal bacterial species that ferment non-structural carbohydrates

have a preference for preformed AA as precursors for protein synthesis (Oh et al., 1999). Therefore, an increase in the sequestration rate of preformed AA into microbial protein would limit their ruminal deamination into NH_3-N in cows fed supplemental sugar, which could partly explain the decrease in ruminal NH_3-N concentration (Hristov et al., 2005). Additionally, an increase in dietary readily fermentable energy supply could also reduce ruminal NH_3-N concentration by enhancing the use of NH_3-N for microbial growth, especially by fibrolytic bacteria (Russell et al., 1992). In past studies (DeFrain et al., 2006; Sannes et al., 2002; Ribiero et al., 2006), feeding lactose or sucrose in place of starch also resulted in a reduction in ruminal isobutyrate, isovalerate and total BCFA concentrations. Our findings were in agreement with these earlier reports. This decrease in ruminal BCFA concentrations is also suggestive of an increase in microbial branched-chain AA synthesis as dietary fermentability increases (Firkins et al., 2006). However, the decrease in ruminal concentrations of NH_3-N and BCFA on the high sugar diet in our study did not result in an increase in total microbial NAN flow at the omasal canal. Similarly, a decrease in ruminal NH_3-N concentration following the inclusion of up to 7.5% sucrose in place of corn starch did not result in a concomitant increase in omasal microbial NAN flow (Broderick et al., 2008). On the contrary, intraruminal infusion of 1 kg of sucrose per day in cows fed grass silage resulted in a decrease in ruminal NH_3-N concentration and a corresponding increase in duodenal microbial protein supply (Kim et al., 1999, 2000). In addition, Chamberlain et al. (1993) also reported ruminal NH_3-N concentration to be lower, and duodenal supply of microbial NAN to be higher when supplementing grass silage with either lactose or sucrose compared to starch. However, the sole dietary ingredient in the aforementioned studies (Kim et al., 1999, 2000; Chamberlain et al., 1993) was grass silage, characterized by a low ruminally fermentable energy (water soluble CHO <13%; NSC content <22% of silage DM) and a high non-protein N (NPN >67% of silage N) content. Therefore, an increase in the fermentable energy to RDP ratio on the grass silage-based diets following sugar supplementation possibly explains the reported increase in microbial protein synthesis (Kim et al., 1999, 2000; Chamberlain et al., 1993). Although energy availability regulates microbial protein synthesis, an inadequate supply of RDP can be detrimental to production performance (Hoover and Stokes, 1991). According to NRC (2001) recommendations, dietary RDP content should range from 9.5 to 10.5% (as % of DM) to ensure a sufficient supply of NH_3-N and

preformed AA, which are important N substrates for ruminal microbial growth (Broderick et al., 2007). In a study by Sannes et al. (2002), replacing corn starch with sucrose in diets containing 17.2% CP resulted in a decrease in both ruminal NH_3-N concentration and microbial protein yield. The inability of the increased diet fermentability to stimulate microbial protein synthesis was attributed to a limited RDP supply (Sannes et al., 2002). This assertion was supported by the observation that mean ruminal NH_3-N concentration in cows fed supplemental sucrose was below 11.0 to 11.8-mg/dL, which is the range that has been suggested as being optimum for maximum microbial growth (Balcells et al., 1993; Reynal and Broderick, 2005). Additionally, ruminal NH_3-N concentration in cows fed sugar was also below this threshold earlier post-feeding, and for a substantially longer period compared to cows fed the other diets (Sannes et al., 2002). Across all diets in the current study, RDP supply was in excess of requirements (12% of DMI), whereas NSC content (average of 38% of DM) was within the suggested 35 to 41% range (CPM-Dairy, v 3.0.8). Presumably, both RDP and energy supply were not limiting in cows fed the low and the high sugar diets in our study and, therefore, this could partly explain why omasal flows of microbial NAN were similar.

Apart from the decrease in ruminal NH_3-N following the addition of lactose in cow diets in our study, there was also a decrease in PUN and MUN concentrations. Others (DeFrain et al., 2006; Charbonneau et al., 2006) also reported similar findings that are suggestive of an improvement in ruminal N utilization (NRC, 2001). A decrease in ruminal NH_3-N could possibly result in a decrease urinary urea-N (UUN) excretion by reducing endogenous urea-N production and/or increasing the proportion of PUN that is recycled to the GIT (Reynolds and Kristensen, 2008). This is supported by work from Sannes et al., (2002) and Broderick et al. (2008), who observed a decrease in both ruminal NH_3-N concentration and UUN excretion when feeding sucrose to cows. However, partial replacement of starch with lactose in the present study did not result in a decrease in UUN excretion. Therefore, it is possible that feeding cows the high compared to the low sugar diet in our study did not result in a substantial decrease in endogenous urea-N production and/or an increase in the repartitioning of PUN towards recycling to the GIT, rather than excretion as UUN. In fact, providing supplemental lactose did not alter UUN.

Lactation responses reported in studies that have been conducted to determine the effects of increasing dietary sugar content by replacing starch are equivocal. In the current study, substituting dietary starch with lactose had no effect on milk and milk protein yield. This is in agreement with past studies (DeFrain et al., 2006; Penner and Oba, 2009). However, feeding an incremental amount of sucrose (up to 10% total dietary sugar) resulted in a quadratic change in yields of milk and milk protein that mirrored changes in DMI (Broderick and Radloff, 2004). In the current study, we did not observe a sugar effect on DMI and this, perhaps, partly explains the similar yields of milk and milk protein in cows fed the low compared to the high sugar diet. Similarly, others also reported no changes in both DMI, and yields of milk and milk protein after partially replacing corn starch with lactose (DeFrain et al., 2006) and sucrose (Penner and Oba, 2009). Among the numerous factors that affect DMI, ruminal fiber digestion plays a prominent role (Allen, 2000). Changes in both ruminal pH and digesta passage rate have been reported to possibly determine the rate and extent of fiber digestion in the rumen when cows are fed high sugar diets (Piwonka et al., 1994; Oelker et al., 2009). In our study, partial replacement of barley and corn starch with lactose did not affect ruminal pH. Additionally, we did not observe a sugar effect on the passage rate of either DM or fiber at the omasal canal. These observations possibly explain the lack of differences in apparent ruminal and total tract NDF digestion, and DMI between cows fed the high compared to the low sugar diet. Our results are in accordance with Oelker et al. (2009) who also did not observe changes in ruminal pH, apparent total tract NDF digestibility and DMI when feeding sucrose. On the contrary, faster ruminal passage rates of fluid (Khalili and Huhtanen, 1991), particulate matter (Piwonka et al., 1994) and NDF (Broderick et al., 2008) have been reported when sucrose partially replaces starch in cow diets. Additionally, Murphy et al. (1997) noted an improvement in palatability of diets due to the sweetening effect of sugar. Therefore, these responses have been suggested to partially account for the reported increase in DMI when cows are fed sugar-containing diets (Penner and Oba, 2009). Discrepancies in the production responses to supplemental sugar can also be attributed to differences in ruminal microbial protein production. Microbial protein can contribute over 60% of MP, and it has an AA profile that closely matches AA requirements for milk protein synthesis (NRC, 2001). Therefore, it is not surprising that a decrease in microbial protein yield in cows fed sucrose in place of corn starch also resulted in a concomitant decrease in milk and milk protein

yield (Sannes et al., 2002). Others (Martel et al., 2011) also reported a decrease in production performance when feeding sucrose-containing diets resulted in inadequate MP supply in lactating cows. In our study, partial replacement of sugar with lactose had no impact on omasal flow of microbial NAN and RUP supply, thus, possibly accounting for the similar yields of milk and milk protein across diets. This is in agreement with Broderick et al. (2008) who also did not observe changes in MP supply after partially replacing corn starch with sucrose.

Increased milk fat secretion has been reported with the feeding of lactose (Schingoethe and Skyberg, 1981) and sucrose (Golombeski et al., 2006; Penner and Oba, 2009). This response has been attributed to an increase in ruminal butyrate production and its extensive metabolism by ruminal epithelial cells during absorption to form BHBA, which is a precursor for milk fatty acid synthesis. This is supported by the reported positive correlation that exists between plasma BHBA and milk fat yield (Penner and Oba, 2009). In the current study, feeding lactose resulted in a tendency for an increase in ruminal butyrate concentration. However, there was no sugar effect on milk fat yield, possibly because plasma BHBA concentration was similar across diets. Although, ruminal butyrate concentration did not change with the addition of sucrose to the diet, milk fat concentration and yield increased (Broderick et al., 2008). This increase in milk fat secretion was attributed to an increase in DMI, and thus, energy intake (Broderick et al., 2008). Presumably, energy intake was not different across diets in our study, as DMI was similar in cows fed the low or the high sugar diet. Possibly, this accounts for milk fat secretion being similar in cows fed the low and high sugar diets.

Given its slower rate and extent of ruminal starch degradation (Patton et al., 2012), we anticipated a less dramatic decrease in ruminal pH when feeding corn relative to barley. Therefore, the higher daily minimum pH, and the shorter duration and smaller area when pH was below 5.8 when feeding corn compared to barley were consistent with our expectations. In agreement with our findings, Khorasani et al. (2001) reported the postprandial decrease in ruminal pH to be slower and steadier for corn than barley. McCarthy et al. (1989) and Overton et al. (1995) also reported total SCFA concentration to be lower, and average ruminal pH to be higher when dietary corn was replaced with barley. The lower ruminal pH when cows were fed barley than corn in our study could partly explain the decrease in apparent ruminal fiber

digestion. A ruminal pH of 6.2 has been suggested as the minimum pH for optimum fiber digestion (Van Soest, 1994), and previous in vitro studies have reported that populations of the principal fiber-digesting bacteria such as *Fibrobacter* spp. and *Ruminococcus albus* decline rapidly when pH falls below 6.0 (Russell and Wilson, 1996). In the present study, mean ruminal pH was closer to 6.2 and the severity of ruminal acidosis (i.e, the duration and area when pH was <5.8) was lower in cows fed corn compared to those fed barley, thus contributing to the observed differences in ruminal fiber digestion. McCarthy (1989) and Overton et al. (1995) reported similar findings, and suggested that the decrease in ruminal pH and fibrolytic activity also resulted in the decrease in DMI they observed for cows fed barley than corn. Propionate induces hypophagia (Allen et al., 2000). Therefore the higher ruminal propionate concentration in cows fed barley compared to corn possibly contributed to the decrease in DMI (McCarthy, 1989; Overton et al., 1995). However, DMI did not change in our study despite ruminal pH and apparent ruminal fiber digestion being lower, and ruminal propionate concentration being higher, in cows fed barley compared to those fed corn. Others (Gozho and Mutsvangwa, 2008; Sadri et al., 2009) did not observe changes in DMI when cows were fed barley compared to those fed corn, presumably because there were no changes in ruminal pH or fiber digestion. The myriad of dietary factors that interact to regulate DMI (Allen, 2000) possibly explain these variable responses.

Research results on the relationship between dietary starch source (barley vs. corn), and milk production and composition are also equivocal (Silveira et al., 2007). An increase in DMI when feeding cows corn compared to barley resulted in an increase in the amount of substrates available for synthesis of milk and milk components (McCarthy et al., 1989; Overton et al., 1995). In the present study, feeding barley did not compromise DMI, possibly explaining the similar milk yields across diets. Our results are in agreement with Yang et al. (1997) and Sadri et al. (2009) who did not observe changes in either DMI or milk yield when feeding either barley or corn. Changes in ruminal fermentation patterns when cows are fed corn compared to barley starch could also alter milk composition and compositional yields (Gozho et al., 2008). In our study, milk composition was comparable on the corn and barley diets with the exception of a tendency for increased milk protein content with barley. This increase in milk protein content is likely related to the tendency for a increase in MP supply in cows fed barley than corn. Although

we did not observe the reported decrease in ruminal NH_3-N concentration, which is suggestive of its increased sequestration into microbial protein (McCarthy et al., 1989; Overton et al., 1995; Casper et al., 1999), omasal flow of microbial NAN was 13% higher in cows fed barley than corn in our study. The faster rate of ruminal fermentation of barley compared to corn possibly explains this increase in microbial protein synthesis. Duodenal microbial N supply was also higher in cows fed barley than corn (McCarthy et al., 1989). In the current study, microbial efficiency was also higher in cows fed barley compared to corn. Ruminal degradation of barley protein is higher than that of corn protein (Herrera-Saldana et al., 1990). Therefore, the increase in microbial efficiency in cows fed barley in our study hints to increased ruminal availability of preformed AA and peptides that can stimulate microbial growth (Argyle and Baldwin, 1989; Chikunya et al., 1996).

5.6 Conclusions

Results from this study indicate that partially replacing dietary corn or barley starch with lactose (as DWP) had no effect on ruminal pH in dairy cows. This response was partly attributed to an upregulation of ruminal chloride-competitive absorption of acetate and propionate. Our results also indicate that despite the improvement in some indicators of N utilization, including ruminal NH_3-N concentration, when cows were fed up to 6% of dietary DM as DWP (lactose), there was no increase in MP supply and milk production. Feeding barley compared to corn in our study resulted in a greater severity of ruminal acidosis, and had no effect on chloride-competitive absorption of acetate and propionate. Although milk production did not differ, feeding barley compared to corn resulted in a tendency for an increase in MP supply and milk protein content in this study.

6. GENERAL DISCUSSION

Ensuring economic viability and addressing the environmental costs of production are among the key challenges dairy producers faces today (VandeHaar and St-Pierre, 2006). Therefore, optimizing N use efficiency in dairy cows is central to economic and environmental sustainability (VandeHaar and St-Pierre, 2006; Ryan et al., 2011). In recent years, there has been increased interest in replacing traditional feed ingredients with co-products from the bio-ethanol and food processing industries as energy or protein sources in dairy cow diets as a result of their relatively lower cost and increased availability (Golombeski et al., 2006; Martel et al., 2011). These co-products include wheat-based (W-DDGS) and blend distillers grains with solubles (B-DDGS) and dried whey permeate (DWP). However, to ensure their judicious use, dietary inclusion of co-products should 1) optimize ruminal and whole body N metabolism, 2) maintain or improve milk production performance, 3) prevent an increase, or reduce N excretion, 4) enhance cow health and welfare, and 5) ensure high quality milk products (VandeHaar and St-Pierre, 2006). Therefore, the three experiments presented in this thesis were conducted with the overall objective of determining the impact of partial or complete replacement of traditional protein supplements including canola meal (CM) with W-DDGS and B-DDGS (Chapters 3a, 3b and 4), and partial substitution of energy sources (barley and corn grain) with DWP (Chapter 5) on ruminal function, nutrient supply, milk production performance, and N loss to the environment. The major focus was on manipulation of dietary CP and RDP content (Chapters 3a and 4) and carbohydrate fermentability (Chapter 5), which are recognized strategies to alter ruminal microbial protein synthesis and nutrient supply, and optimize N utilization in dairy cows.

The use of wheat as the principal feedstock for ethanol production in western Canada has led to an increase in the availability of wheat-based dried distillers grains with solubles (W-DDGS). Although numerous studies conducted mostly in the USA have demonstrated that corn-based dried distillers grains with solubles (C-DDGS) can be used as a suitable replacement for traditional protein supplements while maintaining or increasing levels of milk production (reviewed by Schingoethe et al., 2009), there has been limited research on use of W-DDGS as a protein source in dairy cow diets. Therefore, the first question I had was whether complete replacement of CM (as the major protein source) in barley-based rations with increasing amounts

of W-DDGS (0, 10, 15 and 20% inclusion levels) would have any impacts on ruminal fermentation characteristics, microbial protein production, omasal flow of nitrogenous fractions, and productivity of lactating dairy cows (Chapter 3a). Although substitution of CM with W-DDGS in dairy cow diets resulted in a decrease in RDP supply (from 12.7 to 11.6% of DMI), it did not negatively affect ruminal fermentation, microbial protein production, and omasal nutrient flow. More importantly, the inclusion of up to 20% W-DDGS by replacing CM linearly increased DM intake by 0.3 to 2.1 kg/d, and milk production by 1.2 to 1.8 kg/d. In addition, the overall impact of feeding W-DDGS as a substitute for CM on N loss to the environment was minimal.

This experiment (Chapter 3a) was important because it was one of the first studies to show that the widely available and cost-effective W-DDGS could be used to replace CM without compromising nutrient supply, productivity and health of dairy cows. This is critical for dairy producers because it provided a strategy they could use to improve on-farm profitability. My work also added to the body of knowledge that provides a basis for the successful marketing of W-DDGS as ruminant feed which, besides ethanol, provides additional revenue that is required to ensure viability of the federal and provincial government's biofuel programs in Canada. Despite the positive production responses (Chapter 3a), a major shortcoming of my study was that I fed cows diets that contained nearly 19% CP, primarily because of the high CP content of W-DDGS which made it difficult to formulate diets containing lower CP contents. Therefore, the high dietary CP levels could have masked responses to potential changes in RDP supply and, ultimately, milk production. Clearly, this needed to be addressed, and this became one of my goals for the next experiment (Chapter 4).

Compared to W-DDGS, there is limited information on the nutritional value of the increasingly available B-DDGS for dairy cows. The nutrient composition of B-DDGS is mainly influenced by the ratio of wheat to corn in the feedstock that is used for ethanol production (Canadian International Grains Institute [CIGI], 2011). Blend DDGS typically contains less CP and RDP than W-DDGS. Therefore, I wondered whether dietary inclusion of B-DDGS compared to W-DDGS would result in differences in ruminal and whole body N metabolism, and production performance. When dairy cows are fed diets that are deficient in CP or RDP, they

respond by increasing the proportion of endogenous urea-N that is recycled to the forestomachs, which can provide additional N for microbial protein synthesis (Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008). For this reason, I had another question on whether this adaptation mechanism could counteract a decrease in RDP content in diets containing W-DDGS and B-DDGS. Therefore, the primary objective of the second experiment (Chapter 4) was to determine how interactions between the type of distillers co-product (W-DDGS vs. B-DDGS) and diet CP content (15 vs. 17%) alter ruminal N utilization, urea-N recycling to the GIT, omasal nutrient flow, and production performance in lactating dairy cows. Based on my results (Chapter 4), feeding up to 10% of dietary DM as B-DDGS or W-DDGS as the major source of protein does not have negative effects on MP supply and milk production in dairy cows, thus W-DDGS and B-DDGS are comparable as sources of protein in dairy cow diets.

Reducing dietary CP content from 17.3 to 15.2% in the second experiment (Chapter 4) reduced milk yield by 3 kg/cow per day. Also, milk protein content and yield decreased by 0.12 percentage units and 140 g/d, respectively. This decrease in production performance for cows fed the low CP diet is undesirable for economic reasons, and it could primarily be attributed to a deficiency in MP supply at the small intestine, which was 103 g/d lower when compared to the high CP diet. Others (Cabrita et al., 2011; Lee et al., 2011a) also reported similar findings. Although feeding the low CP compared to the high CP diet reduced endogenous urea-N synthesis, it resulted in a numerically greater proportion of the endogenous urea-N being transferred to the GIT, as has been reported by others (Marini and Van Amburgh, 2003; Marini et al., 2004). More importantly, the amount of recycled urea-N used for microbial NAN synthesis (expressed as a proportion of microbial NAN) was higher in cows fed the low CP compared to the high CP diet. However, the absolute amount of recycled urea-N incorporated into microbial N was low (ranging from 60.0 to 68.9 g/d), thereby failing to substantially compensate for the inadequate RDP supply in cows fed the low CP diet.

Besides lowering feed costs, feeding medium to low CP diets to dairy cows is beneficial in limiting N losses to the environment (Olmos Colmonero and Broderick, 2006b; Hristov et al., 2011a). As expected, decreasing dietary CP content in my study (Chapter 4) decreased urinary N excretion (rather than fecal N excretion). Olmos Colmonero and Broderick (2006b) and Lee et al.

(2011a) reported similar responses. However, milk N efficiency (i.e., milk N/N intake) did not improve because the decrease in N intake was matched by a decrease in milk N content. Therefore, this desirable reduction in urinary N excretion in cows fed the low CP diet in my study was accompanied by the undesirable cost of lost production. However, my efforts were not futile, as I was able to show that urea-N recycling can avail N for microbial protein synthesis when diets fed to cows contain distillers grains and are deficient in RDP content. Therefore, there is need for additional research to determine optimal dietary conditions required to fully harness the urea-N recycling capacity of dairy cows when diets contain W-DDGS and B-DDGS. Additionally, there is also an opportunity for testing of other strategies to prevent the loss of production when feeding MP-deficient diets containing W-DDGS and B-DDGS, including supplementation with rumen-protected AA (Nichols et al., 1998; Lee et al., 2011a).

Increasing dietary fermentability is a recognized strategy to enhance nutrient supply and, thus, productivity in high-yielding dairy cows (Broderick, 2003). Several studies have shown an improvement in ruminal N metabolism (Kim et al., 1999, 2000) and a decrease in urinary N excretion (Sannes et al., 2002; Broderick et al., 2008) when diet fermentability is increased by partially replacing starch with sugar. I questioned whether I could observe similar responses by partially replacing barley or corn starch with lactose in diets containing W-DDGS. Therefore, the first objective of my third experiment (Chapter 5) was to delineate the effects of partial replacement of starch from barley or corn with lactose on ruminal N utilization, omasal flow of nutrients and production performance. Despite an improvement in some indicators of N utilization, including ruminal NH_3-N concentration, when cows were fed up to 6% of dietary DM as DWP (lactose), there was no increase in MP supply and milk production. In addition, feeding lactose did not reduce urinary N excretion in my study. These responses could be partially explained by the fact that all my experimental diets supplied an excessive amount of RDP and energy. For this reason, it will be prudent to conduct future research on whether feeding lactose to dairy cows could improve ruminal and whole N metabolism, and reduce N excretion when dietary RDP and energy are not in excess of requirements.

The risk of ruminal acidosis increases as dietary fermentability increases and, therefore, could have a negative impact on animal health and production performance (Broderick, 2003;

Ferraretto et al., 2013). Therefore, this has been a major concern when considering partial substitution of dietary starch with sugar in lactating cow diets. Although it is logical to expect that the dietary inclusion of sugar could result in an increase in production and accumulation of SCFA in the rumen (Firkins et al., 2008; Oba, 2010), ruminal pH has been shown not to decrease when cows are fed lactose or sucrose as a partial replacement of starch (Broderick and Radloff, 2004; Broderick et al., 2008; Penner and Oba, 2009; Martel et al., 2011). Since the mechanism(s) responsible for these responses are yet to be fully elucidated, I wondered whether a possible explanation could be related to SCFA-mediated changes in ruminal epithelial permeability when diet fermentability increases (Gäbel et al., 1991). Therefore the second objective of my third experiment (Chapter 5) was to investigate the effects of partial replacement of barley or corn starch with lactose on ruminal SCFA concentration and absorption, and ruminal acidosis. Results from this experiment (Chapter 5) indicate that partially replacing dietary corn or barley starch with lactose (as DWP) had no effect on ruminal pH in dairy cows, as reported in earlier studies (Broderick et al., 2008; Penner and Oba, 2009; Martel et al., 2011). However, I was able to show that this response could be related to an upregulation of ruminal chloride-competitive absorption of acetate and propionate. It is also possible that this response was a result of the increase in ruminal butyrate concentration in cows fed lactose, as butyrate is a potent modulator of numerous cellular processes and functions in the gut (Guilloteau et al., 2010). Although this remains to be substantiated, my study is an important step in understanding ruminal SCFA absorption, and how it is affected by dietary factors, including addition of sugar in place of starch. This is critical for the development of nutritional strategies to combat subacute ruminal acidosis (SARA) and, therefore, prevent the loss of production and improve cow health and welfare.

Although the major focus of my thesis research was on how dietary N and energy supply influences ruminal N metabolism and milk production, I was also able to address some key questions about whether ruminal fatty acid (FA) metabolism and milk FA composition change when W-DDGS is included in cow diets (Chapter 3b). When compared to CM, W-DDGS is greater in CF content (5.4 vs. 3.5%; CIGI 2009, 2011). Therefore, the replacement of CM with increasing amounts of W-DDGS can increase dietary content of CF, which might negatively affect DM intake, milk production, and milk fat concentration (NRC 2001). Additionally, wheat

oil is a rich source of PUFA, particularly linoleic acid (C18:2n-6; Becker 2008). Therefore, feeding increasing amounts of W-DDGS can disrupt ruminal BH of PUFA and result in the ruminal accumulation of various BH intermediates. Some of these intermediates including C18:1 *trans*-10 and conjugated linoleic acid (CLA) *trans*-10, *cis*-12, have been demonstrated to cause MFD in dairy cows (Bauman and Griinari 2001; Peterson et al. 2003). Milk fat depression in dairy herds fed W-DDGS would be undesirable for economic reasons because under the Canadian supply management system, milk quota is based on kg of milk fat, and the multiple-component pricing system puts a price premium on milk fat. However, there is very limited information on how feeding increasing amounts of W-DDGS can alter milk fat content. On the other hand, the high PUFA content of W-DDGS and its impact on ruminal BH can positively influence milk FA composition. Among the PUFA, oleic (C18:1), C18:2n-6, and linolenic (C18:3n-3) acids have been demonstrated to improve human health (reviewed by Wang et al. 2011). Of the ruminal BH intermediates that are of biological importance, some specific isomers of CLA (particularly *cis*-9, *trans*-11) and vaccenic acid (C18:1 *trans*-11) can also have potential benefits on human health (Wang et al. 2011). Given that there are no studies that have examined the effects of replacing CM with increasing amounts of W-DDGS on changes in ruminal BH and milk FA composition, the second objective of my first experiment (Chapter 3b) was to evaluate the effects of replacing CM with increasing amounts of W-DDGS on omasal flow of FA and milk FA composition in dairy cows. Inclusion of W-DDGS up to 20% of diet DM tended to cause MFD, possibly as a result of the increase in omasal flow of BH intermediates that suppress milk fat synthesis, including C18:1 *trans*-10. However, feeding greater amounts of W-DDGS also increased milk concentrations of C18:2n-6, C18:3n-3, total PUFA, total CLA, and CLA *cis*-9, *trans*-11, which are of interest because of their potential to promote better human health. Therefore, my study showed the potential of feeding W-DDGS in place of CM on making bovine milk more appealing to consumers. However, it also highlighted the potential problem of MFD occurring and, thus, affecting profitability on dairy farms.

7. OVERAL CONCLUSIONS

Results presented in this thesis provide some evidence that W-DDGS and B-DDGS can partially or completely replace traditional protein supplements, including CM, as the major protein source in dairy cow diets without negatively affecting ruminal function, nutrient supply, and production performance. Additionally, feeding W-DDGS and B-DDGS in medium to low CP diets, and partially replacing starch with lactose in diets containing W-DDGS can result in an improvement in some indicators of N utilization. This research also provides evidence that the addition of lactose to cow diets results in an upregulation of facilitated transport of acetate and propionate across the ruminal epithelial cells, which possibly prevents the occurrence of ruminal acidosis. As optimization of the efficiency of N use is central to economic and environmental sustainability, additional research is required to determine optimal dietary conditions that ensure a balance between maximum milk production and minimum loss of N to the environment.

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9. APPENDIX

9.1 Appendix Tables

Appendix Table I. Chemical composition of dietary ingredients for Experiment 2 (Chapter 4)

	Chemical composition, % DM basis			
Ingredient	DM	CP	ADF	NDF
Alfalfa hay	94.6	22.3	29.4	40.2
Barley silage	93.0	11.9	29.7	44.4
Cottonseed hulls	89.8	6.4	69.0	84.0
Soybean hulls	88.9	13.1	46.5	62.4
Barley grain	88.0	11.4	7.1	16.9
B-DDGS[1]	88.9	31.5	18.5	38.4
W-DDGS[2]	88.8	36.5	13.1	32.2
Canola meal	90.3	40.6	24.0	31.1
Soybean meal	89.6	50.6	6.8	10.1
Corn gluten meal	92.2	66.2	4.6	14.1

[1]Corn-wheat dried distillers grains with solubles (c:w = 85:15).
[2]Wheat-based dried distillers grains with solubles.

Appendix Table II. Chemical composition of dietary ingredients for Experiment 3 (Chapter 5)

	Chemical composition, % DM basis							
Ingredient	DM	CP	ADF	NDR[1]	Starch	ESC[2]	CF[3]	Ash
Alfalfa hay	94.4	18.5	36.1	47.3	1.5	7.4	1.2	9.0
Barley silage	94.2	9.5	29.5	45.1	29.2	1.9	2.5	7.2
Cottonseed hulls	93.2	5.2	61.7	80.4	0.6	2.0	2.7	3.3
Soybean hulls	89.3	10.9	48.9	70.1	1.1	2.7	1.2	7.6
Barley grain	87.2	11.7	6.6	17.2	61.3	3.6	2.5	2.7
Corn grain	87.1	8.1	3.9	11.3	70.2	3.8	3.5	1.4
W-DDGS[4]	92.6	39.5	10.2	38.2	1.8	7.2	6.8	6.2
Canola meal	90.5	40.7	18.8	27.6	2.6	10.6	3.4	7.6
Soybean meal	90.2	52.1	5.4	9.0	2.1	13.8	1.6	7.2
Corn gluten meal	91.2	54.4	4.1	8.4	30.3	2.5	1.8	3.2
Whey permeate (dry)	94.0	4.7	1.9	5.2	1.3	74.3	0.3	9.2

[1]NDF without sodium sulfite.

[2]Ethanol soluble carbohydrates.

[3]Crude fat.

[4]Wheat-based dried distillers grains with solubles.