Effects of feeding oscillating dietary ruminally-degradable protein levels on production, ruminal function, omasal nutrient flow, and N utilization in dairy cows

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Animal and Poultry Science,
University of Saskatchewan,
Saskatoon, SK, Canada

By

Audrey Yvette Makurumure

Spring 2020

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Master of Science degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of the material in this thesis in part or in whole should be addressed to:

Department Head
Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5A8, Canada
Or
Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9
Canada.

ABSTRACT

The objectives of this experiment were to determine the effects of feeding oscillating dietary ruminally-degradable protein (RDP) levels on: 1) feed intake, milk production and composition; and 2) ruminal fermentation characteristics, extent of ruminal nutrient digestion, omasal outflow of nutrients, and nitrogen (N) balance in lactating dairy cows. Eight Holstein dairy cows (days-in-milk = 110 ± 40; mean bodyweight = 734 ± 72 kg) were used in a replicated 4 × 4 Latin square design experiment with 4 dietary treatments. Each experimental period was 28 d long (14 d of dietary adaptation and 14 d of data and sample collection). Four cows in one Latin square were ruminally-cannulated and were used to study dietary effects on ruminal fermentation, nutrient digestion, and N balance. Heat-treated soybean meal (SoyPlus; Landus Cooperative, Ames, Iowa) and untreated soybean meal were used to manipulate dietary RDP levels of the experimental diets. The diets were initially formulated to be isonitrogenous at 16.5% CP; however, actual dietary CP contents ranged from 18.1 to 18.6% CP. Three diets were formulated to contain 9.34% (as % of dietary DM) RDP, 11.3% RDP, and 12.6% RDP. These three diets were then combined into 4 dietary treatments as follows: 1) feeding the 9.34% RDP diet on a continuous basis (designated LRDP); 2) feeding the 11.3% RDP diet on a continuous basis (MRDP); 3) feeding the 9.34 and 11.3% RDP diets on an oscillating (48-h) basis (LRDP/MRDP); and 4) feeding the 9.34 and 12.6% RDP diets on an oscillating (48-h) basis (LRDP/HRDP). Treatments 1 and 2 were designated STATIC RDP diets, whereas treatments 3 and 4 were designated OSC RDP diets. Experimental diets were offered to cows as total mixed rations (TMR) twice a day for ad-libitum intake. Dry matter intake (mean = 29 kg/d) was unaffected by diet ($P \geq 0.31$). Milk yield was greater ($P = 0.02$) in cows fed the LRDP/ MRDP diet compared to those fed the LRDP/HRDP diet. Milk protein content was greater ($P < 0.01$) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Milk protein yield was greater ($P = 0.02$) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Milk urea-N content was greater ($P < 0.01$) in cows fed the MRDP diet compared to those fed the OSC RDP diets. Milk urea-N content was greater ($P < 0.01$) in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Plasma urea-N concentration tended to be greater ($P = 0.10$) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Nitrogen intake tended to be greater ($P = 0.09$) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Total urinary excretion was unaffected by diet ($P \geq 0.15$). Urinary N excretion (expressed as g/d) was greater ($P = 0.04$) in cows fed the MRDP diet compared to those fed the OSC RDP diets. Also, urinary N

excretion (expressed as g/d) was greater (P = 0.03) in cows fed the LRDP/MRDP diet compared to those fed the LRDP/HRDP diet. Urinary N excretion (expressed as % of N intake) was unaffected by diet ($P \geq 0.30$). Urinary urea-N excretion (expressed as g/d) was unaffected by diet ($P \geq 0.11$); however, urinary urea-N excretion (expressed as % of N intake) was greater (P = 0.05) in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Total N excretion (expressed as % of N intake) was unaffected by diet ($P \geq 0.23$). Milk N secretion (expressed as g/d or % of N intake) was unaffected by diet ($P \geq 0.19$). Apparent N balance (expressed as g/d) was unaffected by diet ($P \geq 0.13$. Ruminal acetate concentration was greater ($P < 0.01$) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Ruminal concentrations of propionate were greater (P = 0.03) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Ruminal concentrations of total SCFA were greater (P = 0.01) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Ruminal ammonia-N concentration, acetate: propionate ratio and ruminal pH were not affected by diet ($P \geq 0.20$). Intake, omasal flow and apparent digestion of DM were not affected by diet ($P \geq 0.14$). Omasal flow of NH_3-N (expressed as g/d) was unaffected by diet ($P \geq 0.52$). In conclusion, oscillating RDP levels of dairy cow diets improved milk protein yield and content and tended to increase plasma urea-N concentration and N intake. Total N excretion, milk N secretion, and apparent N balance were unaffected by feeding OSC RDP diet. Generally, the dietary changes did not improve NUE as expected.

ACKNOWLEDGEMENTS

To my academic supervisor, Dr. Timothy Mutsvangwa, thank you so much for taking me as your student and for the patience, guidance and the constructive criticisms you provided throughout my studies. I will forever be grateful.

I would also like to thank my committee members, Dr. David Christensen and Dr. John McKinnon, for their valued input and criticisms during my studies. Thank you to Dr. Fiona Buchanan for serving as my academic chair.

This research would not have been possible without the funding from the Saskatchewan Ministry of Agriculture through the Agriculture Development Fund (ADF) and for that I am grateful.

To Morgan Hobin and the wonderful staff at the Rayner Dairy Research and Teaching Facility, thank you for assisting me in caring for my cows and doing sample collection, not forgetting the much-needed humor when I was tired and weary from long sleepless nights in the barn. Special mention goes to Peter for being a great friend.

Many thanks to my fellow grad students who helped me with animal care, sample collection and analyses. Tonderai, Karen, Eranga, Adriane, Elisabeth, Khalil, Samantha, Daneille, Sarah, Basim, you guys made the hard work much bearable!

Natalia Rudnitskaya, Sam Abeysakara, Niu Zhi and Enkra thank you for the lab orientation and walking me through the processing and analysis of my data.

Thank you so much to my family; Mum, Dad, Kuda, Stanley, Ashley, my grandparents and the Chisvos for your prayers and encouragement throughout my studies. To those from Saskatoon, the Mukuras, Del, Ngangas, Constance, Elaine, Louise, Pearl, Chisangas, Venetia, Solace and everyone who contributed in my daily livelihood during my stay In Canada, God bless you!

To my patient and wonderful husband, Munashe, thank you for waiting and bearing the distance. For enduring the long calls and for being my rock when I was beat down by my research. Your love, support and sacrifice will forever be cherished.

Finally, and most importantly, I would like to give much thanks to God Almighty for making all things possible beyond my strengths and imagination. Great is Thy faithfulness Oh God my Father!

DEDICATION

This is for you Mum and Dad. Thank you for teaching me to believe in myself and pursue my dreams regardless of my circumstances. I love you!!!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$[(^{15}NH_4)_2SO_4]$	^{15}N-labelled ammonium sulphate
AA	amino acid
ADF	acid detergent fiber
AOAC	association of official analytical chemists
BCFA	branched chain fatty acids
BW	body weight
CP	crude protein
Cr	chromium
Cr-EDTA	chromium- ethylenediaminetetraacetic acid
DM	dry matter
EE	ether extract
FAB	fluid associated bacteria
FPP	fluid particulate phase
GC	gas chromatography
GIT	gastrointestinal tract
H_2PO_4	metaphosphoric acid
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
HRDP	high degradable rumen protein
iNDF	indigestible NDF
LAB	liquid associated bacteria
LPP	large particle phase
LRDP	low ruminally-degradable protein
MRDP	medium ruminally-degradable protein
MUN	milk urea-N
MP	microbial protein

N	nitrogen
NAN	non-NH_3-N
NDF	neutral detergent fiber
NH3-N	ammonia nitrogen
NPN	non-protein N
OM	organic matter
OSC RDP	oscillating dietary RDP
OTD	omasal true digesta
P	phosphorus
PAB	particle associated bacteria
PF	particulate phase
PUN	plasma urea-N
RDP	ruminally-degradable protein
RUP	ruminally-undegradable protein
SCFA	short-chain fatty acid
SPP	small particle phase
TMR	total mixed ration
UT	urea transporter(s)
Yb	ytterbium
$YbCl_3$	ytterbium chloride

1. GENERAL INTRODUCTION

Dairy products such as milk, cheese and yogurt are ranked high as good sources of dietary protein supply for humans; however, the efficiency at which dairy cows (and ruminants in general) convert nitrogen (N) into edible protein is very low. Dairy cows utilize only about 20-35% of the N provided in their diet to produce milk protein (Tamminga, 1992; Reynolds et al., 2013), leaving the remaining 65-80% of this dietary N to be excreted in urine and faeces. Other ruminant animals (beef cattle, sheep and goats) have a low N efficiency that varies widely from 10-40% (Calsamiglia et al.,2010). This has caused a general concern on the environmental impacts of livestock production. According to United Nations (2019), the world population is growing exponentially each year and is expected to be above 9 billion by the year 2050 (FAO, 2006; Cleland, 2013; United Nations, 2017); consequently, there will be a need to produce more food to feed the growing population. This is concerning as there will be an increase in meat and milk products which make up an integral part of the human diet in many societies, yet the production of livestock products has been known to negatively influence the environment through nutrient pollution (Capper et al., 2009). In Canada, including Saskatchewan, the dairy industry is an intensive livestock production system in which dairy cows are raised in confinement due to the complex nature of the dairy production system from breeding to feeding and milking. This intensive farming system poses an environmental threat due to the accumulation of nutrients from waste such as N and phosphorus (P). Although N is an essential element in agriculture, it is not desirable when in excess as it is a major pollutant that causes eutrophication of water bodies from N runoff, and air and water pollution.

The nitrogen utilisation efficiency (NUE) of an animal can be measured in various ways including plasma urea-N (PUN) and milk urea-N (MUN) concentration (Guliński et al., 2016) and milk N efficiency (MNE) (Chase et al.,2007). Nitrogen utilization efficiency is influenced by various factors which are either dietary (e.g., type of feed) or animal related (e.g., type of rumen microbes present, pH). The presence of ruminal microbes that are proteolytic in nature greatly influences NUE (Calsamigliaet al., 2010; Hristov et al., 2011a). This is due to the indiscriminate degradation of protein by proteolytic microbes which produces NH_3-N as a by-product. This causes the supply of excess ruminal NH_3-N compared to the microbial requirements for NH_3-N (Tamminga, 1979; Belanche et al., 2012). The degradation of protein is highly dependent on the presence of fermentable energy in the rumen and the end products of the protein degradation (peptides, AA and NH_3-N) are incorporated into microbial protein

(Russell et al., 1983; Russell et al., 1992; Hristov et al., 2005). However, the indiscriminate degradation of protein results in excess production of NH_3-N which will not be incorporated into microbial protein (MP) synthesis but rather lost through absorption into the blood stream. This NH_3-N is then converted to urea in the liver after which the urea is either secreted into the GIT (the process of urea recycling) or is irreversibly lost into the environment as urinary urea. The fermented forage diets that are fed to ruminants also contribute to the inefficient use of N by ruminants by contributing high levels of nonprotein nitrogen (NPN). This is because during fermentation, the protein in the forage is degraded to NH_3-N and amino acids. Also, the excess supply (compared to requirement) of protein in ruminant diets in order to meet high targets of meat, milk and milk protein production contributes to the high loses of N.

Many studies have been conducted to find ways in which urea recycling can be made more efficient hence improving NUE and reducing N excretion. The most common suggested strategies include reducing dietary N concentrations (Olmos and Broderick, 2006a), providing adequate fermentable energy sources in diets of ruminants (Sinclair, 1993) and oscillating dietary CP (Cole, 1999; Ludden et al., 2002; Cole et al., 2003; Ludden et al., 2003; Archibeque et al., 2007 a, b, c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011; Brown, 2014; Kohler, 2016). Besides their inefficient use of dietary N, ruminants have a unique ability to convert poor-quality protein sources from the diet into microbial protein (MP), which is a major and essential contributor to metabolizable protein. Metabolizable protein is required by ruminants for their maintenance and production functions such as meat, milk and wool production.

One of the approaches that have been investigated to improve the efficiency of N utilization in ruminants is oscillating CP in diets of ruminant animals such as sheep (Cole, 1999; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) and beef cattle (Cole et al., 2003; Archibeque et al., 2007b) can be beneficial in improving N metabolism and improving NUE. Oscillating CP involves feeding high and low CP concentrations in diets of ruminant animals at specific time intervals in a cyclic manner. In the investigations that have been done previously, 48-h intervals have been shown to have the most beneficial impact on NUE (Kohler, 2016). According to Archibeque et al. (2007b) and Doranalli et al. (2011) fecal N output decreased by 11-16% when oscillating CP diets were fed to lambs compared to those fed STATIC CP diets. Nitrogen retention increased by 38% in beef cattle that were fed oscillating CP diets (Cole et al., 2003; Archibeque et al., 2007b), in sheep (Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) and by 22.5% in dairy cattle (Kohler, 2016) compared to feeding STATIC CP diets. Cole (1999) investigated the effect of

feeding oscillating CP diets (10% and 15%) at either 24-h or 48-h intervals on N retention in sheep. The results showed that N retention was not affected by oscillating dietary CP (10% and 15%) at 24-h intervals compared to those fed STATIC CP diets (12.5%) but they noted a 38% increase in N retention when the dietary CP (10% and 15%) was oscillated at 48-h intervals compared to those fed STATIC CP diets (12.5%) (Cole, 1999). The increase in N retention that was noted by other researchers (Cole et al., 2003; Archibeque et al., 2007b, c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011; Kohler, 2016) may be an indication of increased urea recycling to the GIT making more N available for MP synthesis and consequently improving productivity and reduction of the environmental impacts of livestock farming.

To my knowledge, from the perusal of available literature, there has not been any investigations on the effect of oscillating RDP content of dairy cow diets (instead of CP) in order to manipulate ruminal degradation of N and as an alternative strategy to improve NUE. Oscillating dietary RDP levels has the potential to have more pronounced effects on improving NUE as it is the component of CP that is degraded in the rumen and can directly influence ruminal ammonia concentrations. When ruminal ammonia concentrations exceed the optimal levels, the excess ammonia (which is toxic) is transported to the liver via the blood and is detoxified into urea which can then be salvaged by the animal via urea-N recycling to GIT when diets are low in N. Therefore, my research was conducted to investigate the effects of feeding oscillating dietary ruminally-degradable protein levels on production, ruminal function, and microbial protein synthesis and omasal nutrient flow in dairy cows.

2. LITERATURE REVIEW

2.1 General Environmental Impact of Excess N Excretion and the Contribution of Dairy Farming

In recent years, there has been a worldwide increase in political, economic and social interests on the impact of agriculture on the environment in terms of global warming and nutrient pollution (e.g., nitrogen [N]) from livestock production. According to various publications, the world population is expected to grow up to more than 9 billion people by 2050 (United Nations, 2004; FAO, 2006; Cleland, 2013; United Nations, 2017). With such a rapid growth rate, the need to produce high-quality food will also increase in order meet the dietary requirements of the population. According to Alexandratos and Bruinsma (2012) and Tilman et al. (2011) general food (including meat) production will increase by about 60% in order to meet the global demand. This means demand will increase linearly with population growth, hence more food will need to be produced to meet the increasing demand. However, increased food production will have some adverse impact on the environment (Capper et al, 2009). Thus, this increase in food production will come at some form of environmental cost. It is therefore critical that we formulate and put measures in place in order to improve the quality and quantity of food produced, while lowering or minimizing the environmental impact of livestock farming (Capper et al., 2009). This may be achieved by identifying and implementing production systems and practices that enable maximum utilization of available resources whilst minimizing waste and other by-products that can harm the environment (Capper, 2009).

One of the major concerns regarding livestock farming is excess N excretion from farms in the form of manure N (urine + feces). In dairy operations, the major contributor to this loss of N to the environment is the dairy cows' low N utilization efficiency of about 20-35 % (Chase et al., 2009). This means that 65-80 % is lost to the environment as waste in manure and, therefore, contributes to environmental pollution through volatilisation, denitrification, leaching and runoff (Reynal and Broderick, 2005). Most of the N is excreted in the form of urinary urea-N (Van Horn et al., 1996; Lu et al., 2019), which poses a challenge as urea-N provides the substrate material for ammonia (NH_3) production through degradation by urease enzymes that are ubiquitous in the environment. On the other hand, fecal N (found in manure) has a slower conversion to NH_3 (Varel et al., 1999) and the decomposition begins as soon as it is excreted from the cow. Manure that is applied onto croplands goes through nitrification and denitrification, two processes which result in greenhouse gas production, N_2O in this case (Whichard, 2001). Nitrification is the biological process in which ammonium (NH_4^+) is oxidised to nitrite (NO_2^-) in the soil by ammonia oxidising bacteria (AOB) then subsequently

oxidised to nitrate (NO_3^-) by nitrite oxidising bacteria (NOB) (Whichard, 2001). Denitrification is the process that follows nitrification in which nitrate is reduced to nitrite then nitrogen gases such as nitric oxide (NO), nitrous oxide (N_2O) and nitrogen gas (N_2) by anaerobic bacteria (Whichard, 2001). Of these gases (NO, N_2O, N_2), N_2O is the most notorious greenhouse gas, having 296 times a greater chance to cause global warming compared to its counterpart greenhouse gas CO_2 (Rahn and Wahlen, 1997). Ammonia that is primarily produced from urea-N degradation in manure is highly volatile, which then results in various environmental concerns such as the production of acid rain (VandeHaar and St-Pierre, 2006), compromised air quality (Burgos et al., 2007), an increase in emissions of greenhouse gases such as nitrous oxide which contribute to global warming (Reynolds and Kristensen, 2008; Vergé et al., 2009). Statistics provided by Environment Canada (2013) state that the agriculture sector contributes about 10% of greenhouse gases produced in Canada with livestock production contributing nearly half of that total (47%). Canadian dairy production contributes about 20% of the total emissions from the livestock sector (Vergé et al., 2013).

Manure N can be used on cropland as an organic fertiliser and can help increase the soil composition characteristics such as organic matter and microbial biomass (Spiehs et al., 2010; Langmeyer, 2002). Improved soil properties in turn result in improved crop yields (Khaleel et al., 1981; Araji et al., 2001). However, when there is excess N available, the surplus N results in environmental problems such N leaching, eutrophication and gaseous losses as mentioned earlier.

Canada mainly has intensive dairy production systems which has increased the pressure for the dairy producers to find ways to reduce the amount of nutrient excretion into the environment from their farms. This has necessitated the need to put in place production measures that help increase the NUE of dairy cows, to reduce N levels released into the environment and thus minimise the adverse effect of dairy production.

2.2 Nitrogen Metabolism in the Rumen

2.2.1 Dietary N Requirement for Ruminants

Ruminants require N in their diet in the form of crude protein (CP) and they have the ability to obtain high quality amino acids from poor sources of protein by means of microbial protein production. This is due to the presence of a vast microbial population which includes proteolytic (protein degrading) microbes within the reticulo-rumen (Wallace, 1996; Nagaraja, 2012). These microbes degrade the CP from the feed into N compounds such as AA, ammonia and peptides which are then used towards microbial protein synthesis (Bach et al., 2005;

Walker et al., 2005). Dietary CP can either provide N in the form of true protein or non-protein N (NPN) (Bach et al., 2005). True protein contains two components that are essential for maximum productivity in terms of protein requirements. The first one is rumen degradable protein (RDP) and rumen un-degradable protein (RUP) which escapes degradation in the rumen to be digested in the small intestines to provide amino acids (AA) (NRC, 2001). The RDP from CP is degraded by proteolytic microbes into N compounds such as AA, peptides and NH_3 (Bach et al., 2005; Doepel and Lapierre, 2006), which are then used to synthesise microbial protein (MP) (Clark et al., 1992). These rumen microbes are eventually washed out of the rumen into the small intestines where they are digested into AA. The AA from RUP digestion then complement the microbial protein AA profile produced in the rumen from RDP degradation. This combination of high-quality AA provides the animal with the required building blocks for optimal milk production as the MP from the RDP degradation is not sufficient to meet the metabolizable protein requirements of the high producing dairy cows. Amino acids that are absorbed in the small intestines are a combination of MP that flows to the rumen (thus is available for post ruminal digestion) and the protein that escapes degradation in the rumen (RUP) (Kalscheur et al., 1999; NRC, 2001). Crude protein is calculated by multiplying the N content of a feed by the value 6.25 (Siddons et al., 1985; Broderick, 2006). Non protein N is comprised of small peptides, nucleic acids, NH_3-N ad AA and is degraded to NH_3-N in the rumen by rumen microbes (Smith, 1989). Another source of N for the rumen microbes is recycled urea-N which enters the rumen via saliva secretions into the rumen or through the bloodstream via the epithelial wall by means of facilitative transporters (Stewart and Smith, 2005; Stewart et al., 2005). A third source of N for microbes is endogenous N that comes from enzymes, sloughed off epithelial cells, and glycoproteins of the mucus membranes (Bach et al., 2005). The AA derived from the MP, RUP and endogenous proteins become the metabolizable protein. The AA profile from the MP is similar to that found in meat and milk and contributes about 50-80 % of the metabolizable protein that is required for milk production in high producing dairy cows (Storm and Orskov, 1983, Jasim et al., 2015).

2.2.2 Protein Degradation and Microbial Protein Synthesis

Protein degradation occurs in the rumen due to microbial degradation. According to Prins et al. (1983), the majority of the microbes (approximately 20-40% of total biomass) within the rumen are proteolytic in nature and these proteolytic microbes are known to go up to 60% in certain circumstances (Van Gylswyk, 1990). There are 3 categories of microbes within the rumen, namely bacteria, protozoa and fungi. Bacteria have been noted to have the

main responsibility of breaking down dietary proteins, whereas ciliate protozoa generally ingest other microbes (bacteria and fungi) and feed particles and convert them to protozoal protein (Wallace, 1996). Fungi may also have proteolytic activity within the rumen but their contribution to proteolysis is relatively insignificant when compared to that of bacteria and protozoa (Wallace and Joblin, 1985; Wang et al., 2017). The main proteolytic bacteria species found in the rumen include *Prevotella* spp, *Megaspheraera elsdenii, F. succinogenes, Streptococcus bovis, Butryrivibrio fibriosolvens* (present when the protein is resistant to degradation; Wallace et al., 1987), *Ruminobacter amylophylus* and *Lachnospira–multipara* (Wallace, 1996; Walker et al., 2005).

Digestion of feed begins in the mouth where the feed is mechanically broken down and swallowed into the rumen. Upon entering the rumen, the first step in protein degradation is the attachment of the rumen microbes to the feed particles whilst soluble N adheres to bacteria. Microbes (bacteria and protozoa) can attach immediately (Cheng et al., 1983, 1984; Craig et al., 1987) to feed particles within 5 minutes of ingestion of feed by the ruminant (McAllister et al., 1994). These microbes that interact with feed particles are categorised as being associated with ruminal fluid, being loosely or tightly attached to feed particles (Czerkawski and Cheng, 1988). Microbial attachment to feed is a key step in ruminal protein degradation as it influences how quickly the microbes can act upon the feed particle (Fig 2.1) (Bach et al., 2005). Craig et al. (1987) estimated that about 70-80% of the ruminal microbes attach to feed particles that have not been digested and as mentioned before, a significant portion of these microbes are proteolytic in nature (Prins et al., 1983). Secondly, the bacteria release extracellular proteases and cell-associated proteases that breakdown feed proteins into smaller units such as peptides and amino acids (AA) (Brock et al., 1982). These proteases include aminopeptidases which are responsible for the degradation of various types of protein structure into smaller units called peptides, and also into AA and ammonia. The extent of degradation varies greatly from animal to animal (Wallace, 1996) and is affected by the protein structure and chemical composition (accessibility of the peptide bonds) and rumen conditions such as pH and the predominant group of microbes present at point of degradation (susceptibility of the peptide bonds) (Bach et al., 2005, Jasim et al., 2015).

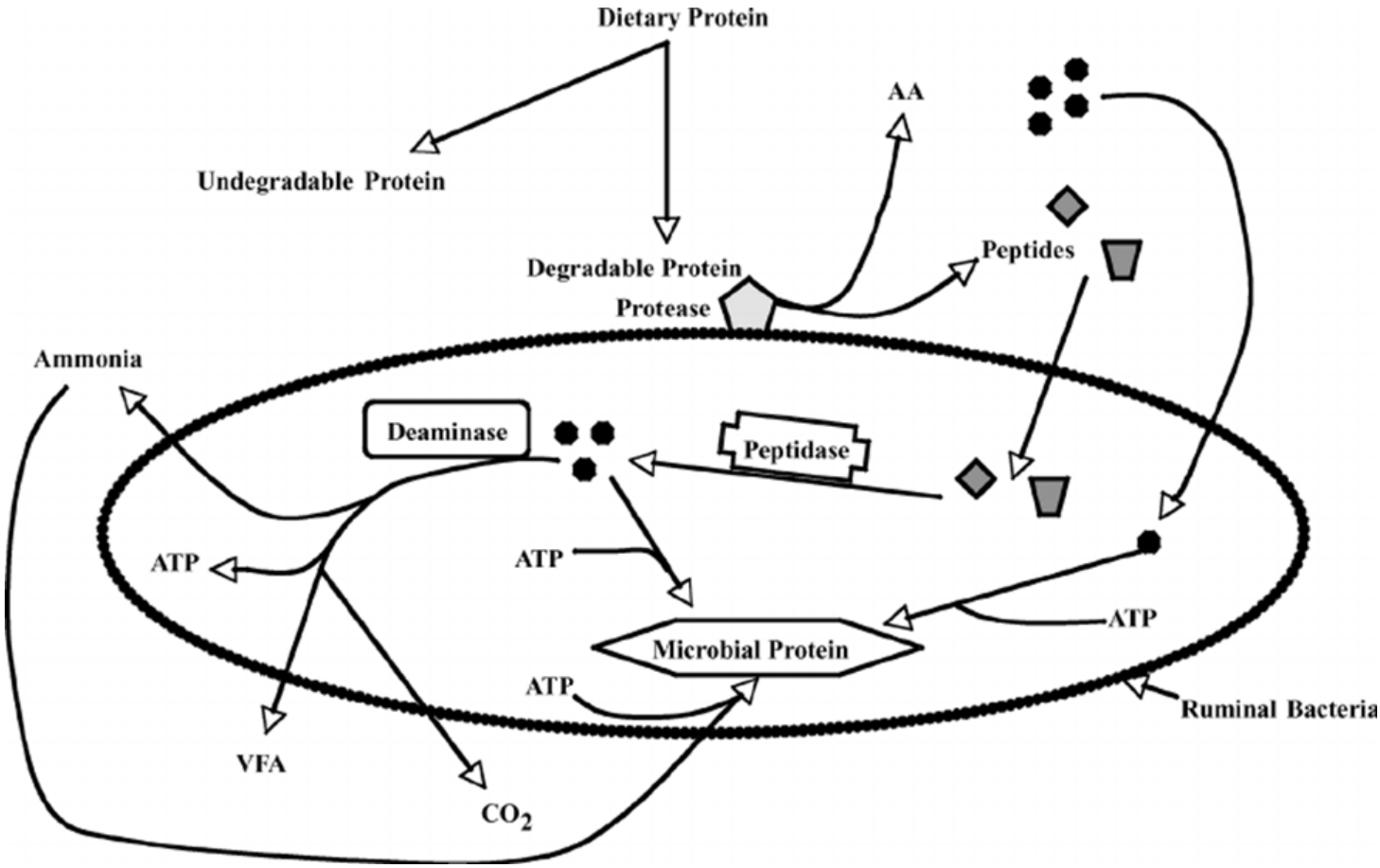

Figure 2.1. Schematic representation of ruminal protein degradation (Bach et al., 2005)

Peptide degradation by ruminal bacteria occurs in a biphasic manner beginning with peptides cleaving off from dipeptides by dipeptidyl aminopeptidases then further degraded to AA (Wallace, 1996). The dipeptidases are found in many bacterial species including the *P. ruminicola* and have been found to be high in ruminal protozoa as well (Wallace, 1996). The *P. ruminicola* species has been found to have very high levels of dipeptidyl aminopeptidase activity. According to Tamminga (1979), the AA that result from protein degradation are either incorporated into MP or they are deaminated to VFA, CO_2 and NH_3-N. The fate of the AA that enter the microbial cell is an energy dependant process. When energy (carbohydrates) is available, the AA are used directly for MP synthesis or goes through transamination (where an amine group of the AA is exchanged with a keto group of another compound) (Bach et al., 2005). However, when energy is limited, the AA are deaminated, providing a carbon skeleton for VFA production via fermentation (Bach et al., 2005). These VFA contribute to the maintenance energy of the bacteria.

2.2.3 Ammonia Production and Absorption in the Rumen

One of the by products from the degradation of nitrogenous compounds such as protein, NPN, and endogenous proteins by microbes in the rumen is ammonia (NH_3) (Abdoun et al, 2007; Reynolds and Kristensen, 2008). In addition, urea that is recycled to the rumen contributes to the ruminal NH_3 pool by action of urease bacteria that rapidly degrade any urea present in the rumen into NH_3 and CO_2 (Abdoun et al, 2007; Stewart, 2005). About 35-65% of ruminal NH_3-N leaves the rumen by being absorbed across the rumen epithelial walls, 10% by outflow of ruminal fluid into the omasum from the rumen (depends on ruminal fluid outflow rates) or it is incorporated into MP (Nolan and Stachiw, 1979; Siddons et al., 1985; Obara et al., 1991; Abdoun et al., 2007). Ammonia absorption occurs across all sections of the GI tract and according to Reynolds and Huntington (1988), 77% of ammonia is absorbed from the reticulo-rumen while 33% is absorbed from the lower GI tract (i.e. small and large intestines and caecum) depending on dietary characteristics (Huntington, 1989). Ruminal and dietary factors such as ruminal ammonia concentration, dietary rumen degradable protein and availability of rumen fermentable energy influence the quantity of ammonia that is absorbed across the rumen (Reynolds and Kristensen, 2008).

The absorption of ammonia across the rumen epithelium depends on whether it is ionized or unionized, i.e., either NH_4^+ or NH_3 respectively. When rumen pH is between 6 and 7, ammonia is present in its ionized form (NH_4^+), which is less lipid-soluble hence it requires

the aid of potassium (K^+) channels to be transported across the rumen epithelium. Ammonium (NH_4^+) is converted to ammonia (NH_3) in the rumen epithelia before being absorbed into portal blood (Doranalli et al., 2011). Ammonia (NH_3) is the lipid-soluble, unionized form which diffuses, by means of simple diffusion, into the bloodstream down a concentration gradient (Hogan, 1961). The absorption of NH_3 is therefore pH dependant and is also associated with the transport of minerals such as K, sodium (Na) and magnesium (Mg) across the apical membrane (Reynolds and Kristensen, 2008). The transportation of ammonia across the basolateral membrane is however partially understood (Abdoun et al., 2007).

2.2.4 Ureagenesis and Urea Recycling in the Rumen

Urea from the detoxification of ammonia has two fates in the animal, that is, it is either recaptured by rumen microbes and consequently becomes incorporated into MP via a process called urea recycling or it is irreversibly lost out of the body as urinary-urea. While all mammalian animals are known to exhibit urea recycling mechanisms, ruminants have the greatest rates (29 – 99%) compared to less than 39% in non ruminants (Doranalli et al., 2011). Urea recycling is an essential mechanism that enables ruminant animals to survive on diets with low N supply as it provides N for MP synthesis in the rumen. Doranalli et al. (2011) further state that urea recycling helps ruminant animals maintain a positive N balance and meet their protein requirements. The recycling of urea also helps reduce the irreversible loss of N via urine and feces by channeling N toward MP synthesis rather than excretion. This ultimately benefits the environment through reduction of N excretion.

Urea recycling can occur through direct transfer of urea from the blood into the GI tract or via salivary inflows (Reynolds, 1992; Stewart et al., 2005; Reynolds and Kristensen, 2008; Calsamiglia, 2010). This recycled urea either enters the rumen or the post ruminal sites such as the intestines and hind gut (Lapierre and Lobley, 2001). The portion that enters the rumen is the most significant one as it can be utilised within the rumen from anabolic conversions (Lapierre and Lobley, 2001; Abdoun et al., 2007), that is, to support microbial growth and subsequently contribute to the MP synthesis. This MP is then digested, absorbed and incorporated into body protein (Eisemann and Tedeschi, 2016). However, the portion that is recycled in the hind gut is not beneficial to the animal because though there is some MP synthesis, the proteins are not absorbed (Abdoun et al., 2007). The transfer of blood urea to the rumen occurs via simple diffusion down a concentration gradient, also via transporters and aquaporins (Abdoun et al., 2007; Rojen et al., 2011; Lu et al., 2014; Walpole, 2015). This concentration gradient is maintained by action of ureolytic bacteria, located in the ruminal epithelial walls, that hydrolyse the urea intraruminally (Abdoun et al., 2007). A depression in

ammonia concentration in the rumen increases the influx of urea into the rumen (Abdoun et al., 2007). However, when ammonia concentration within the rumen reaches 5 mmol/l (rumen saturation point of ammonia), it assumes an inhibitory effect on urea influx across the rumen epithelium (Lu et al., 2014). Other products of fermentation such as SCFA and CO_2 area also known to stimulate the transportation of urea across the ruminal epithelial walls (Fig 2.2.) (Abdoun et al., 2010; Lu et al., 2014). Also, the luminal pH influences urea transportation across the ruminal epithelial walls and according to Lu et al. (2014) urea influx is maximal at ~pH 6.2 and diminishes as the pH either increases or decreases beyond 6.2. There is a positive correlation between ruminal ammonia concentration and luminal pH. An increase in ammonia concentration in the rumen causes an increase in the pH of the lumen reducing the permeability of the rumen wall to urea (Lu et al., 2014). There is work that shows possible involvement of aquaporins in the transport of urea across the rumen epithelium walls particularly AQP-3, 7 and 10 which are found in the rumen epithelia (Rojen et al., 2011; Walpole, 2015).

The facilitative transporters that are responsible for urea transportation across the ruminal epithelial wall are derived from 2 variants of the UT gene namely UT-A (Slc14a2) and UT-B (Slc14a1) (Stewart et al., 2005). The UT-B transporters are specifically categorised as either UT-B1 or UT-B2 and the UT-A transporter are UT-A1- 6 (Smith et al., 1995; Fenton et al., 2000; Smith et al., 2004; Stewart et al., 2005). Facilitative transporters have been mainly identified in the kidney which is a major organ in urea regulation but some of these transports have been identified in the GI tract, brain and some reproductive organs (testis) (Stewart et al., 2005). According to Stewart et al. (2005), UT-B2 is the major urea transporter in the rumen. Various studies have been done that show that UT-B was found in cattle (Marini and Van Amburgh, 2003; Stewart et al., 2005), goats and entire GI tract in sheep. The UT-A gene was reported to exist only in sheep duodenum (Marini et al., 2004; Marini et al., 2006). Ritzhaupt et al., (1997) suggested that urea transfer into the GI tract increases due to the presence of urea transporters lined on the walls of the GI tract.

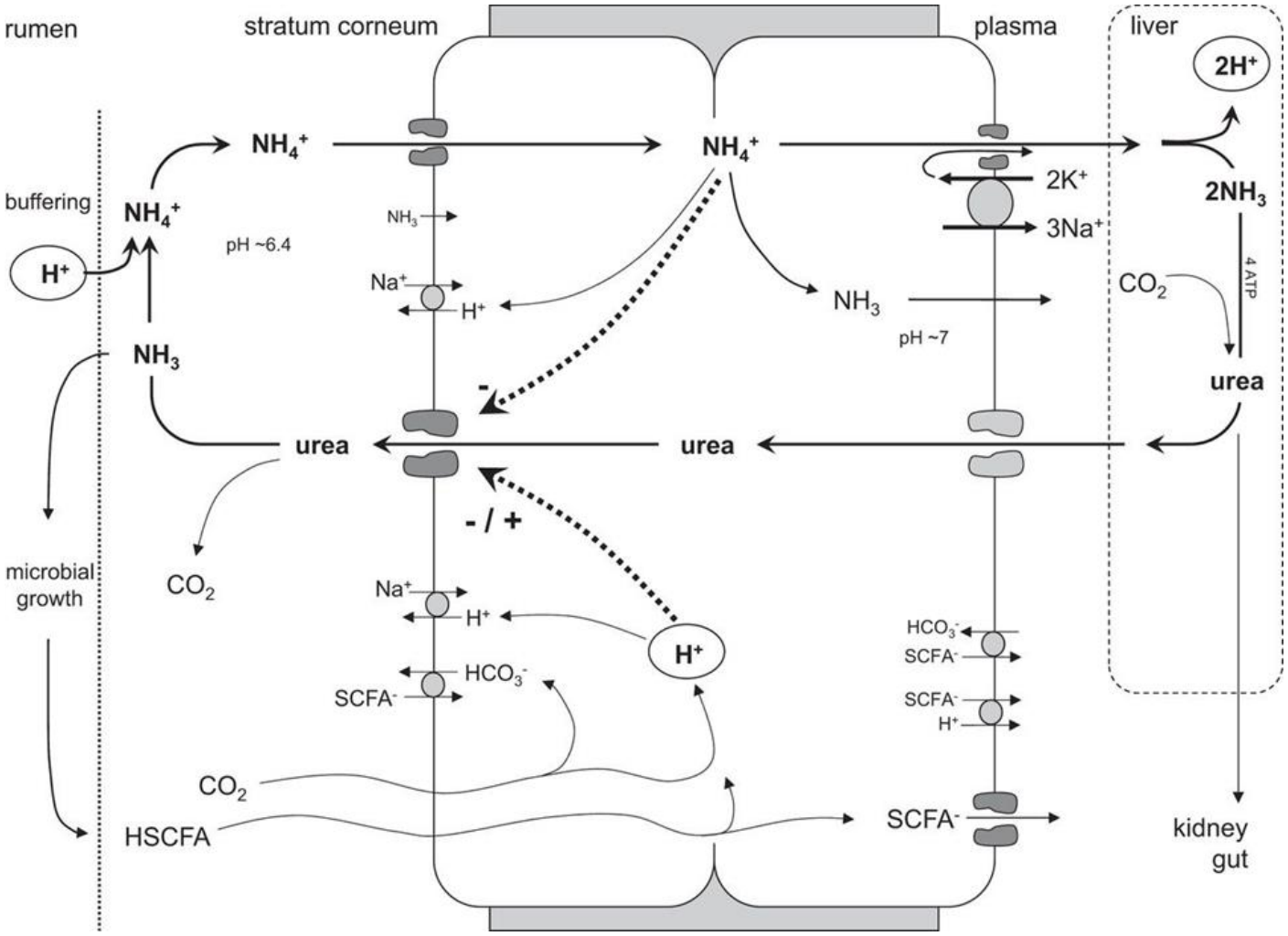

Figure 2.2. Diagrammatic illustration of urea transportation in the rumen (Lu et al., 2014)

2.3 Factors Regulating Urea Recycling in the Rumen

2.3.1 Dietary N Supply

The recycling of urea to the rumen, though not fully understood (Reynolds and Kristensen, 2008; Hristov et al., 2011) is useful in providing more N for MP synthesis when diets are low in CP content. This process is regulated by various factors such as dietary (quality and composition, N intake) and ruminal factors (ruminal NH_3-N concentration, pH) (Walker et al., 2005). These factors impact the rate of degradation, the microbial population in the rumen and consequently the amount of urea that is recycled back into the rumen (Walker et al., 2005). When diets containing low N are fed to ruminants, urea will be transferred to the GI tract from the blood (Ford and Milligan, 1970; Owens and Bergen, 1983; Leng and Nolan, 1984; Isozaki et al., 1994; Van Soest, 1994) to supplement the deficient supply of N to the microbes which eventually end up as AA for the animal in the form of MP (Walker et al., 2005; Calsamiglia, 2010). This also improves the contribution of MP to metabolizable protein. Urea recycling is therefore a mechanism used by ruminants to utilise diets with low protein content (Abdoun et al., 2007).

Low N supply to the rumen can result in some physiological changes such as reduced plasma filtration of urea by the kidneys (Leng et al., 1984), an increase in the reabsorption of urea from the initial inner collecting ducts of the kidney (Isozaki et al., 1994) and also may increases the rate at which urea is cleared from the GI tract (Ford and Milligan, 1970; Kennedy and Milligan, 1980). When dietary N supply is high, there is an increase in ruminal NH_3-N concentrations, making urea recycling less efficient (Castillo et al., 2001). Marini and Van Amburgh (2003), showed that Holstein heifers can capture up to 43% of the N that is recycled back to the rumen and use it for MP synthesis when fed diets low in N compared to the 6% that was captured when they were fed high N diets. Hence endogenous N is important source of N when dietary N supply is low.

Ruminal NH_3-N concentrations have been known to directly influence urea recycling. When the ruminal NH_3-N concentrations are low it triggers more urea recycling to the rumen, improving the NUE, however when NH_3-N concentrations are high, there is reduced recycling of urea to the rumen (Remond et al., 1993). This is because the ruminal NH_3-N concentrations directly influence the permeability of the rumen epithelium to urea-N by increasing permeability when NH_3-N concentrations are low in the rumen and vice versa (Doranalli, 2011; Lu et al., 2014) Urea-N transfer across the rumen epithelium was shown to decrease when urease activity decreased (Houpt and Houpt 1968). In another study increase in ruminal NH_3-N concentrations were shown to decrease urease activity suggesting that urea-N transfer and

ruminal NH_3-N concentration are negatively correlated (Cheng and Wallace, 1979; Kennedy and Milligan, 1980). Additionally, NH_3 alters luminal pH of the epithelia which in turn alters the functionality of urea transporter proteins (Lu et al., 2014).

The type of protein has an indirect effect on urea-N transfer as it affects ruminal NH_3-N concentrations. Researchers (Cunningham et al., 1996; Reynal and Broderick, 2005) reported that cows fed diets that had high RDP contents had an increase in ruminal NH_3-N concentrations when compared to those that received low RDP diets. This is because higher RDP diets stimulate greater intraruminal ammonia production, thereby increasing the concentration of ammonia in the rumen (Lu et al., 2014; Mutsvangwa et al., 2016). Increase in ruminal NH_3-N concentrations consequently leads to greater transfer of NH_3-N into the portal blood which means more production of urea in the liver via NH_3-N detoxification. This increases the plasma urea-N concentrations and urinary urea-N excretion. Additionally, this increase in ruminal NH_3 concentration exhibits inhibitory effects on the secretion of urea into the rumen by reducing the permeability of the rumen epithelia to urea-N as mentioned above. Reducing dietary RDP content will improve urea-N recycling by reversing the negative effects of high RDP content. Suggested measures of reducing RDP include extrusion of dietary feed ingredients and defaunation of the rumen, which eliminates protozoa (Doranalli et al., 2011). Muscher et al. (2010) reported that reducing dietary N in diets fed to goats led to an increase in urea transport across the rumen epithelium as observed from Ussing chamber experiments.

2.3.2 Dietary ruminally-fermentable Carbohydrate Supply

Carbohydrates provide energy for rumen microbes to utilise NH_3-N and synthesise MP. Supplementing ruminant diets with grain, starch or sugars (sucrose) increases energy supply to the animal. This leads to a decrease in ruminal NH_3-N concentrations thus increasing urea-N transfer to the rumen as described previously (see 2.3.1). Feeding diets that contain high levels of ruminally fermentable carbohydrates has been shown to increase urea-N transfer to the rumen (Kennedy, 1980; Kennedy and Milligan, 1980; Huntington, 1989) than to the post gastric tissues (Reynolds and Huntington, 1988; Huntington, 1996). During carbohydrate fermentation in the rumen, energy is released in the form of adenosine triphosphate (ATP). Since MP synthesis is dependant on the availability of energy, this ATP (energy) enhances the capture of both dietary and endogenous N in the rumen by rumen microbes for MP synthesis (Nocek and Russel, 1988). The use of NH_3-N by rumen microbes for MP synthesis increases with increase in the supply of ruminally fermentable carbohydrates (Nocek and Tamminga, 1991). The fermentation of carbohydrates also results in the production of SCFA and CO_2

which, as described in section 2.2.4, stimulate urea transportation across the rumen epithelial walls (Lu et al., 2014). This is because the absorption of SCFA and CO_2 alters the luminal pH which in turn alters the permeability of the rumen epithelial wall to urea (Abdoun, 2010; Lu et al., 2014; Patra and Aschenbach, 2018).

Grain processing can be used to shift the site of carbohydrate digestion from post ruminal sites to the rumen (Doranalli et al., 2011). This improves synchrony of energy and N supply to the rumen microbes for increased MP synthesis whilst reducing N losses from the rumen at the same time. (Huntington, 1996). Feeding processed grains results in an increase in the energy available from microbes (in the form of ATP) from starch fermentation. This increases NH_3-N utilization by microbes in synthesizing MP thus reducing the ruminal concentrations of NH_3-N and consequently increasing the amount of urea-N that can be recycled back to the rumen (Doranalli et al., 2011).

Feeding grain with high rumen fermentability (dry rolled barley) to dairy cows was reported to have tendency to increase urea-N recycling to the GI tract when compared to cows that were fed pelleted barley grain which is less ruminally fermentable (Gozho et al., 2007). Alio et al. (2000) demonstrated that increasing degree or extent of processing for sorghum grain that was fed to steers increased urea-N transfer to the PDV. Similarly, Theurer et al. (2002) indicated that urea-N transfer to the rumen increased by 30% when sorghum grain was steam flaked compared to feeding dry-rolled sorghum flakes. This mechanism shifts the site of carbohydrate digestion from being in the small intestines to the rumen. Thus, increasing dietary fermentable CHO supply improves urea-N recycling.

2.3.3 Efficiency of Nitrogen Utilization in Dairy Cows

Nitrogen utilisation efficiency (NUE) is a very important parameter in sustaining dairy farming and maintaining profitability. However, as mentioned earlier, dairy cows have a low NUE and this has caused a lot of concern regarding its impact on nitrogen loss to the environment. Much work has been put into improving this critical parameter in an effort to increase the productivity, profitability of dairy farming and mitigate environmental effects of dairy farming.

Milk N efficiency (MNE) is a key tool used to measure NUE in dairy cows and it is calculated as a ratio of the quantity of N excreted in milk divided by the quantity of N consumed (Chase et al., 2009). This milk N efficiency has been reported to be very low ranging from 20-35% in dairy cows, meaning that 65 to 80% of the N consumed by the dairy cow is lost in manure (Chase et al., 2009). Van Horn et al. (1996) stated that in manure N, 60-80% of the N

is from urine and this is more undesirable compared to the 20-40% that is in the faeces. On the contrary, feedlot cattle have an even lower NUE of 9-33% because unlike dairy cows that channel N towards milk production and n retention, beef cows only use feed N for retention (Bierman, 1999). Milk N efficiency is inversely related to dietary CP as demonstrated in a study by Olmos Colmonero and Broderick (2006). Their study had diets with a wide range of CP (13.5 -19.4 %) fed to lactating dairy cows and they showed that the levels of N from the diet were closely related to those found in the milk (Colmonero and Broderick, 2006). Also, manure N was directly proportional to dietary N intake with most of the N being excreted as urinary N (Colmonero and Broderick, 2006). Milk N efficiency was reported to decrease with the decrease in dietary N in the same study (Colmonero and Broderick, 2006). They then concluded that the general recommendations for CP as given by the NRC guidelines were very high. This leads to an overestimation of the cow's N requirements hence it was suggested that lowering CP to 16.5 % can improve NUE in dairy cows without altering milk production (Olmos Colmonero and Broderick 2006; Chase, 2009).

Milk urea-N (MUN) is another tool that is used in current dairy operations to measure the NUE of dairy cows and is a non-invasive and economical method of assessing and monitoring ruminant protein status (Roseler et al., 1993). This is a much easier and can be done on large herds in a short time. The amount of urea present in milk is closely related to the levels of urea in the blood (plasma urea-N, PUN) (Roseler et al., 1993; Baker, 1995; Schwab and Broderick, 2017) and is a good indicator of urinary urea-N (Jonker, 1998; Gulinski et al., 2016). This is because urea is a small water-soluble molecule that easily diffuses into other body fluids (e.g. milk, saliva, urine). Also, urea-N that is released into the bloodstream is removed via urinary excretion, hence the strong correlation between PUN and UUN. Since urea readily diffuses from the bloodstream into the milk, MUN can be used to monitor NUE.

2.4 Strategies to Increase the Efficiency of N Utilization in Ruminants

The low efficiency of dairy cows in utilizing N is a problem that results in production and environmental costs as mentioned earlier. Numerous studies have been conducted to improve the NUE of dairy cows. These strategies include reducing the dietary protein supply, providing fermentable energy in the diet and manipulating frequency of protein supply via oscillating diets. Increasing NUE will undoubtedly be beneficial to the farmers on a production and economic scale and the environment by reducing the detrimental impacts of poor NUE mentioned in section 2.1.

2.4.1 Reducing Dietary N Supply

On-farm diet formulations for dairy cows often have excess protein provided to the cows in efforts to promote an increase in production of meat and milk products. This, however, results in excess protein being lost to the environment in various ways mentioned above (section 2.1). Hence, it has long been agreed that dairy producers ought to lower the crude protein level provided in rations (Chase et al., 2007). This is proposed to have two major outcomes, the first being that it leads to improved N utilization in the cows which in turn improves profitability due to reduced feed cost. The other reason is that there will be a reduced emission of ammonia and N losses to the environment due to reducing N excretions. When done properly, this becomes a positive innovation both for the farmer and the environment (Chase et al., 2007).

Research has shown that the higher the CP content of dairy diets the higher the amount of protein available for degradation in the rumen. This tends to oversupply RDP in excess of the microbial requirements resulting in NH_3 production in excess and hence the NH_3 is converted to urea and lost in manure (Hristov et al., 2004; Olmos Colmonero and Broderick, 2006b; Chibisa and Mutsvangwa, 2013). This over supply of protein is unprofitable for producers as it is expensive to provide the protein supplements in the diet which are oversupplied to animals with low NUE, thus profit margins are reduced (Olmos Colmonero and Broderick, 2006; Chibisa and Mutsvangwa, 2013). Therefore, reducing the amount of dietary N can help improve NUE and reduce N excretions to the environment. On the contrary, underfeeding N can also cost the producers due to depressed milk yields and milk protein yields due to a limited supply of protein for MP synthesis, resulting in low profits (VandeHaar and St-Pierre, 2006). Therefore, precision feeding, where diets are formulated to provide the protein that is closer to the animal's requirements, can be beneficial. Various studies have been conducted to establish the optimum level of CP that can be fed to cows without altering productivity while minimizing N excretion.

In a study to investigate the effect of increasing CP content of the diet (15.1, 16.7 and 18.4%) with three different NDF content levels (36, 32,28), Broderick (2003) found that there was no interaction between the two variables hence the cows responded similarly to the different energy levels in the diet. An increase in milk yield and milk protein was noted when CP was increased from 15.1 to 16.7 % with no further differences when CP was increased from 16.7 to 18.4 %. They also noted that increasing CP resulted in increase in manure N excretion with urine being the main route of N excretion. This urinary N was mostly in the form of urea

N which is rapidly volatilised into ammonia. In a follow up study that had CP content ranging from 13.5 to 19.4 %, it was reported that productivity was highest when cows were fed diet containing 16.5% CP, with milk yield and milk protein yield being higher in cows fed the 16.8 and 17.1% CP diets (Olmos and Broderick, 2006a). Also, increasing CP levels resulted in increased excretion of N via urine (urinary N and urinary urea N) as a result of elevated ruminal NH_3 concentration due to the higher protein content (Olmos and Broderick, 2006a). There was no effect on fecal N excretion from the same study. In this study, high moisture corn was replaced with solvent soybean meal (SBM) which could have altered the energy value of the diet. Olmos and Broderick (2006a) also found that NUE linearly decreased as dietary CP increased.

In another study conducted by Chibisa and Mutsvangwa (2013) on the effect of reducing dietary CP from 17.3 to 15.2%, they showed that reducing CP level of the diet resulted in decreased total N excretion, however, this reduction had undesirable effects on production parameters such as milk and protein yield which were reduced by 3 kg per cow per day and 140g per cow per day respectively. Though the reduced N excretion was desirable effect, losing productivity in terms of milk and protein yield cannot be acceptable. As stated earlier in this section, the goal for improving NUE, thus reducing N excretion, is to do so without negatively affecting production.

Another study showed that feeding rumen protected methionine was an effective way of reducing dietary CP up to 13.5%without negatively affecting milk yield (Lee et al., 2012a). In this study, the effect of supplementing diet with 3 ruminally-protected AA (lysine, methionine and histidine) was investigated and results showed that DM intake and milk yield were lower by 1.5 kg/d in cows that were fed a diet that had 13.5 % CP compared to cows fed a diet with higher CP of 15.7%. It was also noted that urinary urea was lower in cows fed the 13.5% CP diet compared to those fed with the 15.7% CP diet. However, when ruminally-protected AA (lysine and methionine and histidine) were added to the 13.5% CP diet, the results showed that milk yield and DM intake were restored to the levels observed with the 15.7% CP diet. Supplementing the 13.5% CP diet with ruminally-protected AA also lowered the urinary urea and urinary N excretion compared to the 15.7% CP diet (Lee et al., 2012a). Hence it was concluded from this study that supplementing the diet with low CP level can be beneficial by maintaining production parameters whilst lowering N excretions. However, the practicality of having this done on a farm is low since ruminally-protected AA are expensive to provide, which in turn will increase production costs (Kohler, 2016).

2.4.2 Dietary Carbohydrate Supply

Energy in the form of ATP enables rumen microbes to utilize ammonia as a source of N for growth (Nocek and Russell, 1988). This ATP is harvested from the fermentation of carbohydrates (CHO) in the rumen. Another source of energy for ruminants SCFA. The supply of SCFA is influenced by the availability of fermentable CHO supply as it is a by product of CHO fermentation by microbes in the rumen.

The rate of energy released in the rumen varies according to the source of starch and processing of grains (Broderick, 2006). In order to improve NUE, ruminal carbohydrate fermentation needs to match with RDP degradation. Providing a source of readily fermentable carbohydrates when dietary energy supply is low enhances the ability of microbes to capture N (both dietary and endogenous) for MP synthesis and eventually increases the AA supply to the small intestines via MP flow from the rumen to the small intestines (Lapierre and Lobley, 2001). For rumen microbes to grow effectively and supply optimal MP for the ruminant, energy and protein need to be simultaneously available (Dijkstra et al., 1998). Increasing fermentable carbohydrate supply will improve microbial capture of N thus reducing the need for supplying expensive RUP, hence feed costs and urinary urea excretion will be reduced (Sinclair, 1993). There are studies that suggest that synchronising ruminal energy (CHO) supply and N availability results in desirable results in terms of animal productivity and most importantly, reduce N excretion from the ruminants (Castillo et al., 2001; Huntington and Archibeque, 2000). A study by Castillo et al. (2001) that had diets supplying different sources of energy to dairy cows reported that supplementing dairy cow diets with low degradable starch decreased urinary N excretion. This was likely due to the increased excretion of N in feces. This was achieved without adverse effects on DMI and milk production, which is the desirable effect of balancing energy supply to meet animal requirements for production (Castillo et al., 2001).

Energy and protein sources can be manipulated simultaneously or independently in order to achieve synchrony within the rumen. Achieving synchrony requires taking into consideration the factors such as substrate availability for microbes, rate of digestion (Hall and Huntington, 2008) N recycling to the GI tract and microbial N capture (Cole and Todd, 2008). Kolver et al. (1998) conducted a study in which they fed non-structural carbohydrates together with high quality fresh pasture to dairy cows in order to investigate effects of synchronising the degradation rate of CHO and N in dairy cows. Their results indicated that the supplemental energy source led to a 22 - 43 % decrease in ruminal NH_3-N concentrations during the 3rd and 5th hours after the morning feeding. This may suggest that there was less AA catabolism by

microbes as they utilised ruminal NH_3-N more efficiently (improved microbial N capture), however, they did not observe any effect of synchrony on N utilisation, urinary N excretion and performance of the dairy cows (Kolver et al., 1998).

2.4.3 N supply

Whilst reducing the protein content of the diet can be beneficial in reducing nitrogen waste, balancing the proportions of RUP and RDP is essential in improving NUE. This is because the type of protein fed to the ruminant influences ruminal NH_3-N concentrations which in turn influences N excretion.

Feed ingredients that provide protein in a diet can have their protein degradability modified during processing e.g. RUP fractions of feed can be increased by extracting oil from SBM using heat-generating expeller processor (Liu, 1999). Also, treating SBM with lignosulfate can be used as an alternative to increase RUP portion (Can and Yilmaz, 2002, Castro et al., 2007). When the RUP fraction is increased, there is an increase in the amount of protein supplied to the small intestine which in turn reduces N loss via manure and improves milk production (Bateman, 2005; Ipharraguerre et al., 2005). Also, as mentioned above (section 2.3.1), supplementing low CP diets with ruminally-protected AA will improve NUE. Feeding diets with high RDP results in increased ruminal NH_3-N concentrations when compared to cows fed lower RDP diets (Cunningham et al. 1996; Reynal and Broderick, 2005). In an experiment that investigated the effects of two RDP levels in diets containing similar RUP and MP concentrations on ruminal fermentation, digestibility, and transfer of ruminal NH_3-N into milk protein in dairy cows, Hristov et al. (2005) reported that high RDP diets resulted in a decrease in the milk N efficiency whilst ruminal NH_3-N concentrations and N excretion in the form of urinary urea-N increased. Hence the excess RDP was not efficiently utilised in the rumen to synthesize MP. In another study with sheep, a diet higher in RDP (raw pea) resulted in higher ruminal NH_3-N losses compared to those fed low RDP diet containing extruded pea (Rémond et al., 2009).

Feeding diets that contain high amount of fermented forages can result in high ruminal NH_3-N concentrations due to their high NPN content. The NPN content is a result of the degradation of protein into NH_3-N during fermentation and can lead to increased loss of N (Broderick, 1996). Bacteria have been known to show preference to certain sources of N such as AA, NH_3-N and peptides than others (Russell et al., 1992). Broderick et al. (1993) reported that ruminal NH_3-N, PUN and MUN were lower in cows fed diets that had alfalfa silage supplemented with AA N source compared to those that received a diet supplemented with a urea-N source.

Therefore, providing a diet supplemented with the most efficiently utilised source of N will be beneficial to the animal and the environment.

2.4.4 Oscillating Dietary Protein levels

2.4.4.1 Oscillating Dietary Crude Protein Levels

Oscillating crude protein concentrations in the diets of ruminants has been studied quite extensively as a potential means of improving NUE. Studies have been done with sheep (Cole, 1999; Ludden et al., 2002; Archibeque et al., 2007c; Doranalli and Mutsvangwa, Doranalli et al., 2011), beef cattle (Cole et al., 2003; Archibeque et al., 2007a, b, Ludden et al., 2003) and dairy cows (Brown, 2014; Kohler, 2016).

Feeding oscillating CP diets has been reported to increase total SCFA (Ludden et al., 2002a; Doranalli et al., 2011), ruminal acetate concentrations (Ludden et al., 2002a; Doranalli et al., 2011) and ruminal propionate concentrations (Doranalli et al., 2011) compared to feeding STATIC CP diets. In contrast, Kohler (2016) found no effect of feeding oscillating CP diets to dairy cows compared to feeding STATIC diets at 24-h, 48-h and 72-h intervals on total SCFA concentrations. Ruminal pH was not affected by feeding oscillating diets to ruminant animals compared to feeding STATIC diets (Ludden et al., 2002a; Doranalli et al., 2011; Kohler, 2016). Doranalli et al. (2011) reported that feeding oscillating CP diets at 48-h intervals to sheep resulted in no differences in ruminal NH3-N concentrations compared to feeding STATIC CP diets. In contrast, Ludden et al. (2002) noted that ruminal NH3-N concentrations tended to be lower in sheep that were fed high forage diets at 48-h oscillation intervals. In another study with dairy cows Kohler (2016) reported greater ruminal NH3-N concentrations in cows that were fed oscillating CP diets compared to those fed STATIC CP diets and that the oscillating frequency of 48-h intervals had greater ruminal NH3-N concentrations compared to 24-h and 72-h intervals. Various authors (Cole, 1999; Cole et al., 2003; Ludden et al., 2003; Archibeque et al., 2007b; Doranalli and Mutsvangwa 2009, Kohler, 2016) reported no effect of feeding oscillating CP diets on PUN concentrations compared to feeding STATIC CP diets. Plasma urea-N concentrations are used as an indicator of animal protein status in ruminants (Hammond, 1997) and are highly correlated to rumen NH_3-N concentrations (NH_3-N is a substrate for ureagenesis in the liver).

The frequency of oscillating is key in oscillating CP studies. Cole and Todd (2008) observed that providing supplementary protein to beef cattle that were grazing protein deficient forages at 72-h intervals had no adverse effects on animal production and performance of the

cows. This consequently resulted in the reduction of labour and feed costs. Collins and Pritchard (1992) reported that supplementing protein (SBM or corn gluten meal) to cattle and lambs that were fed low quality roughage diets (based on corn stalks) at 48-h intervals had no adverse effects on animal performance when compared to those fed a protein supplement at 24-h intervals. In this study DM and N digestion, pH and SCFA were unaffected by the diet. Also, the authors noted greater N retention when protein was supplemented every other day (48-h intervals) compared to supplementing each day (24-h intervals) (Collins and Pritchard, 1992). Similarly, Brown et al. (1995) found no effect of supplementing CP on DM intake, digestion of DM, ADF and NDF when supplement was given at 24-h, 48-h or 72-h intervals in sheep diets fed straw based diets. They also noted that the lambs had similar positive N balance and N metabolism (N retention and excretion) was unaffected by protein supplementation frequency (Brown et al., 1995). These findings prompted further investigations by other researchers on the effect of oscillating dietary CP supply on production parameters, performance and N metabolism (N retention and excretion) in ruminants (Cole, 1999; Ludden et al., 2002a,b, 2003; Cole et al., 2003; Archibeque et al., 2007a,b,c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011; Brown, 2014, Kohler, 2016).

Various researchers showed that feeding oscillating CP diets to sheep (Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009), beef cattle (Cole et al., 2003; Archibeque et al., 2007a, b) and dairy cattle (Kohler, 2016) did not affect N intake in these ruminant animals compared to when they fed STATIC CP diets. Feeding oscillating CP diets had no effect on urinary N (Cole 1999; Ludden et al., 2002b; Cole et al., 2003; Archibeque et al., 2007b, c; Kohler, 2016) and fecal N (Ludden et al., 2002b; Cole et al., 2003; Archibeque et al., 2007c; Kohler, 2016). However, Archibeque et al. (2007b) and Doranalli et al. (2011) reported that fecal N output decreased by 11-16% when oscillating CP diets were fed to lambs compared to the lambs that were fed STATIC CP diets. Nitrogen retention increased by 38% in beef cattle that were fed oscillating CP diets (Cole et al., 2003; Archibeque et al., 2007b), in sheep (Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) and by 22.5% in dairy cattle (Kohler,2016) compared to feeding STATIC CP diets. Cole (1999) investigated the effect of feeding oscillating CP diets (10% and 15%) at either 24-h or 48-h intervals on N retention in sheep. The results showed that N retention was not affected by oscillating dietary CP (10% and 15%) at 24-h intervals compared to those fed STATIC CP diets (12.5%) but they noted a 38% increase in N retention when the dietary CP (10% and 15%) was oscillated at 48-h intervals compared to those fed STATIC CP diets (12.5%) (Cole, 1999). On the contrary, Ludden et al. (2002) reported that there was no increase in N retention when lambs were fed high forage

oscillating diets that had 13% and 17% CP alternated at 48-h intervals compared to those fed a STATIC diet with 15% CP. This may have been due to the high CP concentrations in these diets which may have supplied excess metabolizable protein than was required by the lambs, hence more N was excreted. The increase in N retention that was noted by other researchers (Cole et al., 2003; Archibeque et al., 2007b, c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011; Kohler, 2016) may be an indication of increased urea recycling to the GI tract making more N available for MP synthesis and consequently improving productivity and reduction of the environmental impacts of livestock farming.

The effects of oscillating dietary CP concentration in diets of dairy cows on N utilization efficiency were investigated by Brown (2014) and Kohler (2016). The study by Brown (2014) had dietary CP (10.3 and 16.4%) oscillated at 48-h intervals and a STATIC CP diet that had 13.4% CP. In that study, oscillating CP concentrations in diets of dairy cows did not affect DM intake, milk yield and milk composition, N intake, fecal and urinary N excretion (expressed as a % of N intake) compared to feeding STATIC CP diets. The CP concentrations (10.3, 13.4 and 16.4%) were lower than the recommended CP values for high producing dairy cows according to NRC (2001) guidelines. This may have altered the responses observed in this study.

Kohler (2016) used diets that had higher CP concentrations which were closer to the values recommended by NRC (2001). The CP concentrations ranged from 14.3 – 20.3 %. Also, Kohler (2016) had 3 oscillating intervals namely, 24-, 48- and 72-h to determine the optimum frequency of oscillating dietary CP in dairy cow diets. The study by Kohler (2016) further investigated effects of feeding oscillating CP concentrations on rumen N utilization and MP synthesis. The results from these two studies have already been mentioned in the above passages. The study by Kohler (2016) concluded that 48-h intervals were the optimum interval of oscillation. The overall conclusion from this study was that oscillating dietary CP concentration at 48-h intervals improves NUE. As mentioned earlier, N retention was increased by oscillating CP content of dairy cow diets at 48-h intervals in this study. According to Cole (1999), the 48-h oscillation interval is synchronous with the retention time of digesta. The rate of passage of digesta is influenced by DM intake and dietary and roughage content hence timing of the oscillation frequency varies according to dietary composition and DM intake of the animal (Cole, 1999).

When ruminants are fed diets with an oscillating regimen, there is potential, as shown in studies mentioned above, to improve N retention and or increase NUE. This is because the depression in protein supply during the days when protein concentration is low results in

increased recycling of N to the rumen to make up for the deficiency. As explained in section (2.3.1) above, an insufficient supply of dietary protein reduces the rumen ammonia concentrations which in turn allows for greater urea-N transfer to the rumen via the blood or saliva. The recycling of urea-N to the rumen encourages MP synthesis and increases the portion of N that is used in milk secretion or absorption by the body. Ultimately, the loss of N as urinary urea-N, urinary N and fecal N is thus reduced, thus improving environmental stewardship. As a follow up study, it is logical to investigate the effects of oscillating RDP content of dairy cow diets on NUE. This is because RDP is more directly influential regarding ruminal ammonia concentrations as it is the potion that is degraded in the rumen yielding ammonia, VFA and CO_2.

2.4.4.2 Oscillating Dietary Ruminally-Degradable Protein

Oscillating dietary crude protein has been reported to result in desirable outcomes in terms of improving NUE and lowering N excretion to the environment in sheep, beef and dairy cows as mentioned above. Dietary CP is made up of two portions, RDP and RUP of which RDP is the one that mainly influences N metabolism in the rumen. It is reasonable to expect more pronounced effects of oscillating the portion of CP (RDP) that directly affects ruminal N degradation and consequently N excretion.

In the past years, there has been some research effort to investigate the effects of oscillating CP levels in order to improve NUE. As a common agricultural practice, animals fed diets with low quality forages are often given supplemental protein to maintain or improve production (wool, meat or milk). It is from this concept of infrequent supplementing of protein that the concept of oscillating dietary CP emerged. As mentioned in the previous section, oscillating CP has been shown to be beneficial to the cause of trying to improve NUE in ruminants. The benefit comes mainly from the change in the dietary protein content of the feed and allowing the animal to salvage its ability for urea-N recycling. This approach is similar when oscillating RDP level of the diet. When oscillating CP, the time when there is depression in CP cause the animal to utilise the stored ammonia in the form of urea- and recycle it back into the rumen. Since CP is RDP and RUP combined, increasing or decreasing CP level in the diet consequently alters RDP levels hence it can be surmised that oscillating RDP alone might have similar or more pronounced results. However, it is crucial to note that when oscillating CP levels in diets, the CP intake is different which mean the levels of RDP will also be different.

In oscillating RDP levels, this difference is eliminated by controlling how much RDP is provided to the rumen during each oscillation.

Again, the mechanism behind oscillating is to encourage urea recycling to the gut and help ruminant better utilise low quality feed. This creates the basis for oscillating RDP only. Rumen degradable protein directly influences rumen NH_3-N concentration levels which in turn influences the amount of urea-N. This is key in trying to improve NUE by having more of the stored urea recycled into the rumen, providing a source of rumen degradable N which is then incorporated into MPS (when dietary N supply is deficient) rather than being lost via excretion in manure

To my knowledge, there is a knowledge gap in this area as there is no published research that has investigated effects of oscillating RDP in diets of ruminants. Some studies have been conducted to investigate the effects of increasing or decreasing RDP content of diets on a STATIC diet but none, to my knowledge, have looked at the effect of oscillating RPD.

2.5 Summary

It is well appreciated that dairy farming contributes to environmental degradation through N pollution. This is because dairy cows, like all ruminants, have a low NUE which means they excrete more N into the environment than they incorporate into protein. Combined with the fact that the world population is growing and projections for an increase in demand for food especially meat and milk production, there is expedient need to reduce the detrimental impact of our intensive dairy farming operations. Various strategies have already been studied and recommended as ways to mitigate N loss from ruminants. Recently, oscillating the CP content of dairy diets has proven to improve NUE and urea-N recycling. There is a knowledge gap that needs to be filled by further investigation into the effects of oscillating RDP levels of lactating dairy cow diets. To my knowledge, there has not been any published work that has investigated the effects of oscillating RDP, hence this research study aims to come up with findings that might contribute to methods or approaches to improving NUE.

2.6 Hypothesis

Feeding oscillating dietary RDP levels (low vs. high) at 48-h intervals will improve the efficiency of N utilization (i.e., a lower N excretion, and a greater N retention and milk N secretion) in lactating dairy cows

2.7 Objectives

To determine the effects of feeding oscillating dietary RDP levels on feed intake, and milk production and composition in lactating dairy cows; and

To determine the effects of feeding oscillating dietary RDP levels on ruminal fermentation characteristics, extent of ruminal nutrient digestion, omasal outflow of nutrient, and N balance in lactating dairy cows

3. MATERIALS AND METHODS

The experiment was conducted in the Rayner Dairy Research and Teaching Facility's Metabolism Wing at the University of Saskatchewan. Cows used in this experiment were handled and cared for according to the specifications of the Canadian Council of Animal Care regulations and the University of Saskatchewan Animal Care Committee approved all experimental procedures (UCACS Protocol No. 20040048).

3.1 Animals, Experimental Design, and Dietary Treatments

To conduct this experiment, eight Holstein dairy cows (days-in-milk = 110 ± 40; average bodyweight = 734 ± 72 kg) were used in a replicated 4 x 4 Latin square design arrangement with 4 dietary treatments. Each experimental period was 28 days long with 14 days of dietary adaptation and 14 days of data and sample collection. Of the eight cows, four were ruminally-cannulated and were placed in one Latin square and were used to study dietary effects on ruminal fermentation, nutrient digestion, and N balance. All 8 cows were individually housed in tie–stalls. Three isonitrogenous (~16.5% CP) diets were formulated to contain 9.34% RDP (RDP content expressed as a % of DM; designated LRDP), 11.3% RDP (MRDP), and 12.6% RDP (HRDP). The ingredient and chemical compositions of the diets are presented in Table 3.1a. These isonitrogenous diets were then combined into 4 dietary treatments as follows: 1) feeding the LRDP diet on a continuous basis (this treatment was designated LRDP); 2) feeding the MRDP diet on a continuous basis (MRDP); 3) feeding the 9.34 and 11.3% RDP diets on an oscillating (48-h) basis (LRDP/MRDP); and 4) feeding the 9.34 and 12.6% RDP diets on an oscillating (48-h) basis (LRDP/HRDP). Experimental diets were offered to cows as total mixed rations (TMR) twice a day at 0900 and 1700 h for ad-libitum intake. The forage: concentrate ratio of the TMR was 49:51 (on a DM basis). The forage component of the TMR consisted for a mixture of barley silage (~71% on a DM basis) and alfalfa hay (~71%). Cows had free access to water.

Heat-treated soybean meal (SoyPlus; Landus Cooperative, Ames, Iowa) and untreated soybean meal were used to manipulate dietary RDP levels of the experimental diets. The in-situ technique was used to determine the RDP contents of the SoyPlus and soybean meal ingredients. Briefly, 5-g samples of soybean meal and SoyPlus meal were weighed into nylon bags with a pore size of 40 µm.

Table 3.1a. Ingredient and chemical composition of experimental diets

	Dietary treatments[1]		
	LRDP	MRDP	HRDP
Ingredient composition, % of diet DM			
Alfalfa hay	14.3	14.3	14.3
Barley silage	35.4	35.4	35.4
Barley grain	27.9	27.9	27.9
Oat hulls	3.58	4.65	6.61
Soybean hulls	2.15	0.36	0.36
Soybean meal	0.36	11.4	10.3
SoyPLUS[2]	12.2	1.79	0.36
Urea	0.00	0.00	0.64
Canola Oil	0.61	0.61	0.61
Dairy premix	1.80	1.80	1.80
Sodium bicarbonate	0.97	0.97	0.97
Salt	0.37	0.37	0.37
Limestone	0.23	0.23	0.23
Dynamite	0.17	0.17	0.17
Chemical composition			
DM, %	56.3	56.0	57.5
OM, % of DM	90.7	90.9	91.3
CP, % of DM	18.1	18.6	18.4
NDF, % of DM	32.4	31.2	31.5
ADF, % of DM	21.1	20.1	20.2
Crude fat, % of DM	3.30	3.30	3.30
RDP, % of DM[3]	9.34	11.3	12.6
Ash, % of DM	9.30	9.10	8.70

[1] LRDP = a diet containing 9.34% RDP fed on a continuous basis; MRDP = a diet containing 11.3% RDP fed on a continuous basis; LRDP/MRDP = 9.34 and 11.3% RDP diets fed on an oscillating (48-h) basis; LRDP/HRD = 9.34 and 12.6% RDP diets on an oscillating (48-h) basis

[2]SoyPlus is a treated soybean product high in RUP (Landus Cooperative., Ames, Iowa)

[3]Values are based on diet formulations using CPM Version 3.0.8.1, using actual RDP values for SoyPlus and soybean meal as determined using the in-situ nylon bag technique (see Table 3.1b) and RDP values for the rest of the ingredients based on values from the CPM database.

The samples were incubated in the rumen of lactating dairy cows (via the rumen cannula) fed the standard TMR provided at the dairy barn for 0, 2, 4, 8, 12, 24, 36, and 48 h using the gradual addition/all out schedule. After sample incubation, the nylon bags were rinsed with cold water until water was clear. The zero-hr bag was also washed at the same time with the incubated bags. After washing, the bags were dried in a forced-air oven at 50℃ for 48 h and CP content was determined using the micro-Kjeldahl method (AOAC, 1990: method 976.05). Protein degradation rates were calculated using the first order kinetics model (Tamminga et al., 1994) (Table. 3.1b).

During the 14-day data and sample collection period, individual cow feed intakes were recorded daily. Samples of the TMR and orts were collected daily during the total collection period (days 25 to 28) and stored at -20°C for later analyses. Cows were milked three times daily at 0500, 1300, and 1900 h, and the milk weights were recorded at each milking. Milk samples were collected daily on 4 consecutive days (days 25 to 28) from all three milkings and a preservative (2-bromo-2- nitropropane-1-2-diol) was added to all samples. The perseverative was dissolved into the milk by gently shaking the sample which was then kept at 4°C in the refrigerator. At the end of the 4-d milk collection period, milk samples (from all 8 cows) were then pooled by day based on milk yield and submitted to the CanWest DHI Laboratory (Edmonton, AB) for CP, fat, lactose, and milk urea-nitrogen (MUN) analysis using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerod, Denmark) according to AOAC (1990).

3.2 Data and Sample Collection

To determine the site and extent of nutrient digestion, the omasal sampling technique was used to obtain samples of digesta leaving the rumen during continuous infusions of markers (days 15 to 23). These measurements were obtained using the 4 ruminally-cannulated cows. To quantify omasal digesta flow, indigestible NDF (iNDF; Huhtanen et al., 1994), ytterbium chloride ($YbCl_3$; Siddons et al., 1985) and Cr-EDTA (Uden et al., 1980) were used as digesta markers for the large particle, small particle, and fluid phases, respectively, as described by Reynal et al. (2005). To quantify ruminal microbial protein production, ^{15}N-labelled ammonium sulphate [$(^{15}NH_4)_2SO_4$]; 10 atom percent excess ^{15}N; Cambridge Isotope Laboratories, Andover, MA) was used as a microbial marker (Reynal et al., 2005). Briefly, just before marker infusions into the rumen were initiated on day 15, samples of whole ruminal

Table 3.1b. In-situ rumen degradation characteristics of crude protein (CP) of soybean meal (SBM) and SoyPlus meal[1]

Items	SBM	SoyPlus
In-situ rumen degradation characteristics of CP[2]		
S (%)	8.33	9.76
D (%)	91.7	83.2
U (%)	0.00	7.01
T_0 (%/h)	1.09	2.47
K_d (%/h)	5.37	1.87
RUP (%)	48.6	70.7
EDCP (%)	51.4	29.3
EDCP (g/kg, DM)	236.8	127.2

[1]SoyPlus is a treated soybean product high in RUP (Landus Cooperative., Ames, Iowa)
[2]S = rapidly degradable fraction; D = potentially degradable fraction; U = undegradable fraction; T_0 = lag time (h), K_d = degradation rate; and EDCP = effective degradability of CP

contents were taken from each cow to determine background concentrations of Cr and Yb, and ^{15}N natural abundance. At the beginning of the infusions, priming doses equal to one-half of the daily dose of Yb, Cr, and ^{15}N were administered via the ruminal cannula and a 50-ml sub-sample of each marker was collected each period and stored under room temperature for later analysis of Cr an Yb concentrations. Thereafter, between days 15 and 23, marker solutions prepared in distilled water and containing Yb (2.2 g per day), Cr (2.7 g per day) and ^{15}N (182 mg per day) were continuously infused into the rumen at a constant rate of approximately 1 L/d. Sampling of ruminal contents and omasal digesta began at 0600 h on day 19 and was conducted every 8 hours until 0100 h on day 23 as follows: 0900, 1700 and 0100 h on d 19, 1300, 2100 and 0500 h on d 20, 1300, 2100 and 0500 h on d 21, and 0900, 1700 and 0100 h on d 22.

Ruminal contents were collected by hand via a ruminal cannula using a 250-mL container to collect 1,000 mL (250 mL/region) of rumen fluid from the ventral, cranial dorsal, cranial ventral and caudal ventral regions of the rumen whilst a pump was used to facilitate omasal fluid collections via the rumen cannula. To ensure that an equal number of representative samples were collected for the oscillating-RDP treatments, cows received the LRDP diet on sampling days 19 and 20 and then received either the MRDP or HRDP diets on sampling days 21 and 22 according to treatment. A 425-mL sample of omasal digesta was collected and 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 sampling period to yield 1.2- and 2.4-L composite samples, respectively. The 125-mL sub-samples was 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).

Immediately after compositing the 250-mL omasal fluid samples, each composite sample was filtered through two layers of cheesecloth and the remaining solids were washed with 250 mL of 0.85% (wt/vol) NaCl solution and then filtered again through the two layers of cheesecloth and the fluid obtained was stored on ice for use in next step. The retained solids were added to 175 mL of a chilled 0.85% (wt/vol) NaCl solution with 0.1% (wt/vol) Tween-80 were then placed in a 500-mL PAB container, mixed thoroughly and stored in an ice bath until further analysis. The fluid obtained from the first step was centrifuged (1,000 × *g* at 5°C for 5 min) to obtain a pellet which was then placed into a container labelled PAB. The PAB

contents were blended for 20 s and stored at 5°C for 24 h. The fluid obtained after the pellet was removed was centrifuged again (11,300 × g at 5°C for 30 min). After centrifugation, the fluid was discarded and the pellet was re-suspended in 50 mL of McDougall's buffer (McDougall, 1948) and re-centrifuged at 11,300 × g at 5°C for 30 min. The pellet obtained from this step was then stored at -20ºC for FAB analyses. After 24 h, the PAB contents were filtered through two layers of cheesecloth. The fluid was then processed as described previously for FAB except that, after the initial centrifugation (1,000 × g, 5°C, 5 min), the pellet obtained was discarded. The pellet obtained from this step was then stored at -20ºC for PAB analyses. All pellets were pooled by cow by period. The pooled 2.4-L composite omasal digesta samples were kept frozen at -20°C for later analyses.

Ruminal digesta samples that were collected at the same time points as for omasal sampling were strained through 4 layers of cheesecloth and ruminal fluid pH was 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% sulphuric acid H_2SO_4 and stored at -20°C for later determination of short-chain fatty acid and ammonia concentrations, respectively.

Total tract nutrient digestion and whole-body N balance were determined using 4-day (days 25 to 28) total collection of urine and faeces starting on day 25 of each experimental period. This sampling protocol was designed to capture a full oscillation cycle from the two oscillating treatments (i.e., LRDP/MRDP and LRDP/HRDP). These measurements were conducted using only the 4 ruminally-cannulated cows. Faeces were collected into large steel trays positioned over the gutter behind each tie-stall. Total daily faecal output for each cow was mixed thoroughly, quantitatively transferred into a pre-weighed plastic container, and weighed. A 2.5% sub-sample was taken daily and stored in aluminum trays at -20°C for later analyses. 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). Bladder catheter insertion was conducted as described by Crutchfield (1968) at 0900 h on day 23 to allow at least 24 h of adaptation before bladder catheters were connected to the urine collection tubing. Connection to urine collection tubing was done at 0900 h on day 24 when total collections were initiated. Urine was collected into pre-weighed 20-L Carboy polyethylene containers under acidic conditions (to achieve pH < 3) daily, with 150 mL of concentrated HCl being added to the urine collection containers to prevent microbial degradation and the loss of volatile ammonia. Total urine output was recorded daily, mixed thoroughly and then a 5% sub-sample

was collected daily (days 25 to d 28) and stored in a 5-L container at -20°C. In addition, a 2-mL sub-sample of urine was diluted with 8 mL of distilled water and stored at -20°C for the determination of urea. During total collections, blood samples were also collected daily (days 25 to 28) from the tail vein in vacutainers containing heparin just before the 0800-h feeding. Blood samples were centrifuged at 1,500 x g for 15 minutes and plasma was collected and stored at -20°C for urea and glucose analyses.

3.3 Sample Analyses

At the end of the study, frozen TMR, orts and faecal sub-samples were thawed overnight at room temperature and analyzed for DM by drying in an oven at 60°C for 48 h (AOAC, 1990). Dried TMR, orts, and faeces were ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Ground TMR, orts and fecal samples were pooled per cow for each experimental period and analyzed for organic matter (OM) by ashing at 550°C for at least 8 h, CP using the macro-Kjeldahl procedure, ether extract, acid detergent fibre (ADF; AOAC, 1990), and neutral detergent fibre (NDF; Van Soest et al., 1991). Heat-stable α-amylase and sodium sulfite were used for NDF determination.

Plasma samples were analyzed for glucose (Penner et al., 2009b) and urea using the diacetyl monoxime method of Marsh et al. (1957). Pooled urine samples that were stored frozen in the 5-L containers were thawed overnight and then total N was determined using the macro-Kjeldahl procedure (AOAC, 1990). Urinary urea-N concentration was determined using the diacetyl monoxime method (Marsh et al., 1957).

Frozen ruminal fluid samples for SCFA analysis were thawed at room temperature and then centrifuged at 18,000 x *g* for 15 min and filtered through a 0.45-µm membrane. A sub-sample (0.9 mL) of supernatant was pipetted into a clean, dry vial. Crotonic acid (1 mg/mL) was then added to each vial as an internal standard. Separation and quantification of ruminal short-chain fatty acids were done by gas chromatography as described by Erwin et al. (1961). Frozen ruminal fluid samples for the determination of ammonia concentration were thawed, centrifuged for 10 min at 18,000 x *g* to obtain a clear supernatant, and then analyzed using a phenol-hypochlorite assay (Broderick and Kang, 1980).

Omasal digesta samples (2.4-L composite sample) were thawed at room temperature and then separated into large particle (LPP), small particle (SPP), and fluid (FPP) omasal digesta phases as described by Reynal et al. (2005). To obtain these 3 particle phases, the

omasal digesta sample was filtered through one layer of cheesecloth and the solids obtained from the cheesecloth were defined as LPP. The filtrate was then centrifuged at 1,000 × *g* for 5 min at 5°C and the fluid obtained was defined as FPP and pellet was defined as SPP. The LPP, SPP and FPP samples were then freeze-dried, after which the dried samples were ground through a 1-mm screen (Christy and Norris Ltd., Chelmsford, England) and stored in plastic vials. A 1-g sample of each phase (LPP, SPP and FPP) was ashed for 8 h at 550°C in a muffle furnace (AOAC, 1990), followed by nitric acid digestion (Vicente et al. 2004) in preparation for atomic absorption spectrophotometry. Concentrations of iNDF in TMR, orts, LPP and SPP were determined using 12-day ruminal in situ incubations (Reynal et al., 2005). The TMR, orts, and LPP samples were incubated in duplicate whereas the SPP samples were incubated in triplicate. Approximately 1.5 g of the TMR, orts, and LPP phases, and approximately 3 g of the SPP phase were weighed into 5- × 10-cm nylon mesh bags (6 μm pore size; part no. 03-6/5, Sefar America Inc., Depew, NY). The bags were then incubated in the rumen of 5 ruminally-cannulated cows for 12 d after which the bags were removed and rinsed in cold water for 30 min until the water was clear. Bags were then dried in a forced-air oven at 135°C. The dried bags were weighed and analysed for NDF as previously described. The concentrations of Cr, Yb and iNDF in the LPP and SPP, and of Cr and Yb in FPP were then used to physically recombine DM from the freeze-dried LPP, SPP and FPP in the correct proportions to reconstitute the omasal true digesta (OTD) using the triple-marker method (France and Siddons, 1986). Reconstituted OTD samples were analyzed for OM, CP, ether extract, ash, NDF and ADF as already described above. For ammonia analysis, OTD samples were prepared using 10 mL of pH 2.2 Na-citrate buffer as described by (Reynal and Broderick, 2005) and ammonia determination was conducted using a phenol-hypochlorite assay (Broderick and Kang, 1980).

3.4 Calculations and Statistical Analysis

The NAN content of OTD samples was calculated as total N - ammonia N. Omasal flows of other nutrients were calculated by multiplying DM flow by their concentration in OTD. Apparent ruminal digestion of nutrients was calculated as nutrient intake – omasal flow of nutrient.

Feed intake and milk yield and composition data were analyzed as a replicated 4 x 4 Latin square using the Proc Mixed procedure of SAS (9.4, SAS Institute Inc., Cary, NC). The following model was used:

$$Y_{ijkl} = \mu + S_i + P_j + C_k (i) + T_l + ST_{il} + E_{ijkl}$$

(Y_{ijkl} = dependent variable, μ = overall mean, S_i = fixed effect of square i, P_j = fixed effect of period j, C_k (i) = random effect of cow k (within square i), T_l = fixed effect of dietary treatment l, ST_{il} = interaction between square i and treatment l, and E_{ijkl} = residual error).

Ruminal pH, ruminal concentrations of SCFA and NH^3-N, plasma urea-N, N balance, MP production, and omasal nutrient flow data were analyzed as a 4 x 4 Latin square using the Proc Mixed procedure of SAS. The following model were used:

$$Y_{ijk} = \mu + P_i + C_j + T_k + E_{ijk}$$

(Y_{ijk} = dependent variable, μ = overall mean, Pi = fixed effect of period i, C_j = random effect of cow j, T_k = fixed effect of dietary treatment k, and Eijk = residual error). Single degree of freedom contrasts were used to compare treatment effects as follows: STATIC vs OSC diets, MRDP vs LRDP/HRDP, and LRDP/MRDP vs LRDP/HRDP. Treatment effects were declared significant when $P \leq 0.05$, and tendencies when $0.05 < P \leq 0.10$.

4. RESULTS

4.1 Dietary Characteristics

The chemical compositions of experimental diets are presented in Table 3.1a. The diets were initially formulated to be isonitrogenous at 16.5% CP; however, actual dietary CP contents ranged from 18.1 to 18.6% CP. The greater CP contents based on TMR chemical analysis as compared to target CP contents could be attributed to a greater CP content of the forages (barley silage and alfalfa hay) that were used throughout the experiment. By design, diets were similar in their contents of all the major nutrients.

4.2 Dry Mater Intake, and Milk Yield and Composition

Dry matter intake was unaffected by diet ($P \geq 0.31$) (Table 4.1). Milk yield was greater ($P = 0.02$) in cows fed the LRDP/ MRDP diet compared to those fed the LRDP/HRDP diet. Energy corrected milk (ECM) was greater ($P = 0.01$) in cows fed the LRDP/MRDP diet compared to those fed the LRDP/HRDP diet. Feed efficiency and energy corrected milk (ECM) were unaffected by diet ($P \geq 0.13$). Milk fat content was unaffected by diet ($P \geq 0.14$). Milk fat yield tended greater ($P = 0.10$) in cows fed the OSC RDP diets compared to those fed the MRDP diet. Milk protein content was greater ($P < 0.01$) in cows fed the OSC RDP diets compared to those fed the STATIC diets. Also, milk protein content was greater ($P < 0.01$) in cows fed the OSC RDP diets compared to those fed the MRDP diet. Milk protein content tended to be greater ($P = 0.10$) in cows fed the LRDP/ MRDP diet compared to those fed the LRDP/HRDP diet. Milk protein yield was greater ($P = 0.02$) in cows fed the OSC RDP diets compared to those fed the STATIC diets. Milk protein yield was greater ($P = 0.02$) in cows fed the LRDP/ MRDP diet compared to those fed the LRDP/HRDP diet. Milk lactose content was greater ($P = 0.04$) in cows fed the LRDP/MRDP diet than those fed the LRDP/HRDP diet. Milk lactose yield was greater ($P = 0.05$) in cows fed the LRDP/MRDP diet than those fed the LRDP/HRDP diet. Milk urea-N content was greater ($P < 0.01$) in cows fed the MRDP diet compared to those fed the OSC RDP diets. Milk urea-N content was greater ($P < 0.01$) in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Plasma urea-N concentration tended to be greater ($P = 0.10$) in cows fed the OSC RDP diets compared to those fed the STATIC diets.

Table 4.1. The influence of feeding oscillating dietary ruminally-degradable protein (RDP) levels on dry matter intake (DM intake), milk yield and composition, and plasma urea-N in dairy cows[1]

	Diet[2]					Contrasts: *P* value[3]		
Item	LRDP	MRDP	LRDP/MRDP	LRDP/HRDP	SEM	STATIC vs. OSC	MRDP vs. OSC	LRDP/MRDP vs. LRDP/HRDP
DM intake, kg/d	29.1	29.3	28.9	28.8	0.85	0.27	0.31	0.87
Milk yield, kg/d	42.0	42.8	43.1	41.0	1.72	0.54	0.30	0.02
ECM[4], kg/d	42.3	41.9	43.5	41.9	1.68	0.41	0.38	0.13
Feed efficiency[5]	1.47	1.43	1.49	1.49	0.05	0.33	0.29	0.96
Milk fat, %	3.49	3.36	3.51	3.51	0.11	0.27	0.14	0.99
Milk fat yield, kg/d	1.49	1.42	1.52	1.47	0.07	0.23	0.10	0.35
Milk protein, %	3.08	3.12	3.19	3.17	0.05	<0.01	<0.01	0.01
Milk protein yield, kg/d	1.27	1.31	1.40	1.31	0.05	0.02	0.15	0.02
Milk lactose, %	4.57	4.51	4.59	4.52	0.05	0.76	0.27	0.04
Milk lactose yield, kg/d	1.92	1.93	1.96	1.87	0.1	0.73	0.70	0.05
Milk urea-N, mg/dL	14.2	16.1	14.6	15.6	0.45	0.61	<0.01	<0.01
Plasma urea-N, mg/dL	12.7	14.2	14.2	14.9	0.58	0.10	0.64	0.46

[1]Values are least squares means obtained from 8 cows. Means were calculated based on data collected during the 14-d data and sample collection period.

[2] LRDP = diet containing 9.34% RDP fed on a continuous basis; MRDP = diet containing 11.3% RDP fed on a continuous basis; LRDP/MRDP = diets containing 9.34 and 11.3% RDP fed on an oscillating (48-h) basis; and LRDP/HRDP = diets containing 9.34 and 12.6% RDP fed on an oscillating (48-h) basis.

[3]STATIC = LRDP + MRDP, and OSC = LRDP/MRDP + LRDP/HRDP.

[4] Energy-corrected milk = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)] (Orth, 1992).

[5]Feed efficiency = ECM/DM intake.

4.3 Apparent Nitrogen Balance

Data on the influence of feeding oscillating dietary RDP levels on urinary N excretion, fecal excretion and apparent N balance are presented in Table 4.2. DM intake was greater (P = 0.01) in cows fed the LRDP/MRDP diet compared to those fed the LRDP/HRDP diet. Nitrogen intake was not affected by diet ($P \geq 0.63$). Total urinary excretion was unaffected by diet ($P \geq$ 0.15). Urinary N excretion (expressed as g/d) was greater (P = 0.04) in cows fed the MRDP diet compared to those fed the OSC RDP diets. Also, urinary N excretion (expressed as g/d) was greater (P = 0.03) in cows fed the LRDP/MRDP diet compared to those fed the LRDP/HRDP diet. Urinary N excretion (expressed as % of N intake) was unaffected by diet ($P \geq 0.27$). Urinary urea-N excretion (expressed as g/d) was unaffected by diet ($P \geq 0.11$); however, urinary urea-N excretion (expressed as % of N intake) was greater (P = 0.05) in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Total fecal N excretion (expressed as DM, kg/d) and fecal N excretion (expressed as g/d or % of N intake) was greater (P = 0.05) in cows fed OSC RDP diets compared to those fed the MRDP diet. Total N excretion (expressed as g/d) was greater (P = 0.03) in cows fed the MRDP diet compared to those fed the OSC RDP diets. Total N excretion (expressed as % of N intake) was unaffected by diet ($P \geq 0.78$). Milk N secretion (expressed as g/d or % of N intake) was unaffected by diet ($P \geq 0.21$). Apparent N balance (expressed as g/d) was unaffected by diet ($P \geq 0.23$). Productive N (i.e., apparent N balance + milk N) was unaffected by diet ($P \geq 0.33$).

4.4 Apparent Total-Tract Nutrient Digestibility

Apparent total-tract digestibility of DM tended to be greater (P = 0.06) in cows fed the STATIC RDP diets compared to those fed the OSC RDP diets. Also, cows fed the STATIC MRDP diet had greater DM digestibility (P = 0.03) compared to cows fed the OSC RDP diets. Also, DM digestibility was greater (P = 0.02) in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Apparent total-tract digestibility of OM tended to be greater (P = 0.07) in cows fed the STATIC RDP diets compared to those fed the OSC RDP diets. Also, cows fed the STATIC MRDP diet had greater OM digestibility (P = 0.03) compared to cows fed the OSC RDP diets. Also, OM digestibility was greater (P = 0.02) in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Apparent total-tract digestibility of CP tended to be greater (P = 0.10) in cows fed the OSC RDP diets compared to those fed the STATIC diets. Apparent total-tract digestibilities of ether extract, ADF and NDF were not affected ($P \geq 0.14$) by dietary treatments (Table 4.3).

4.5 Ruminal Fermentation Characteristics

Data on the influence of feeding oscillating dietary RDP levels on ruminal fermentation characteristics are presented in Table 4.4. Ruminal acetate concentration was greater ($P < 0.01$) in cows fed the OSC RDP diets compared to those fed the STATIC diets. Also, cows fed the OSC RDP diets had a greater ($P < 0.01$) ruminal acetate concentration compared to cows fed the MRDP diet. Ruminal acetate concentration was greater ($P < 0.01$) in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Ruminal concentrations of propionate were greater ($P = 0.03$) in cows fed the OSC RDP diets compared to those fed the STATIC diets. Ruminal butyrate concentrations were unaffected by diet ($P \geq 0.24$). Isobutyrate concentration tended to be greater ($P = 0.08$) in cows fed the STATIC RDP diets than those fed the OSC RDP diets. Isobutyrate concentration was ($P < 0.01$) in cows fed the MRDP diet than those fed the OSC RDP diets. Also, isobutyrate concentration was greater ($P = 0.01$) in cows fed the LRDP/MRDP diet than those fed the LRDP/HRDP diet. Ruminal valerate concentration was greater ($P = 0.03$) in cows fed the LRDP/MRDP diet compared to those fed the LRDP/HRDP diet. Ruminal isovalerate concentration was greater ($P < 0.01$) in cows fed the MRDP diet than those fed the OSC RDP diets. Also, ruminal isovalerate concentration was greater ($P < 0.01$) in cows fed the LRDP/MRDP diet than those fed the LRDP/HRDP diet. Ruminal concentrations of total SCFA were greater ($P = 0.01$) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Ruminal ammonia-N concentration, acetate: propionate ratio and ruminal pH were not affected by diet ($P \geq 0.20$).

4.6 Ruminal Digestion and Omasal Nutrient Flow

Data on the influence of feeding oscillating dietary RDP levels on ruminal digestion and nutrient flow are presented in Table 4.5. Intake, omasal flow and apparent digestion of DM were not affected by diet ($P \geq 0.14$). Apparent digestion of DM when expressed as a percentage of DM intake tended to be greater ($P = 0.10$) in cows fed the OSC RDP diets compared to those fed the STATIC RDP diets. Intake, ruminal digestion and nutrient flow of OM were not affected by diet ($P \geq 0.11$). Apparent digestion of OM when expressed as a percentage of OM intake not affected by diet ($P \geq 0.11$). Nitrogen intake (expressed as g/d) and omasal flow of N (expressed as g/d) were unaffected by diet ($P \geq 0.11$). Apparent digestion of N (expressed as g/d) tended to be greater ($P = 0.06$) in cows fed the OSC RDP diets compared to those fed the

MRDP diet. Apparent digestion of N (expressed as g/d) was greater (P = 0.02*)* in cows fed the OSC diets compared to those fed the STATIC diets. Apparent digestion of N (expressed as % of N intake) tended to be greater (P = 0.06*)* in cows fed the OSC RDP diets compared to those fed the MRDP diet. Apparent digestion of N (expressed as % of N intake) was greater in (P = 0.02*)* in cows fed the OSC diets compared to those fed the STATIC diets. Omasal flow of NH_3-N (expressed as g/d) was unaffected by diet ($P \geq 0.52$). Omasal flow of NAN tended to be greater (P = 0.10*)* in cows fed the STATIC diets than those fed the OSC diets. Omasal flow of NAN (expressed as % of N intake) was greater (P = 0.02*)* in cows fed the STATIC diets than those fed the OSC diets. Also, omasal flow of NAN (expressed as % of N intake) tended to be greater (P = 0.06*)* in cows fed the MRDP diet compared to those fed the OSC RDP diets. Intake and omasal flow of NDF were not affected by diet ($P \geq 0.22$). Apparent digestion of NDF when expressed as a percentage of NDF intake was greater (P = 0.03*)* in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. Apparent digestion of NDF (expressed as % of NDF intake) was greater in ($P < 0.01$) in cows fed the LRDP/HRDP diet than those fed the LRDP/MRDP diet.

Apparent digestion of NDF (expressed as kg/d) tended to be greater (P = 0.10*)* in cows fed the LRDP/HRDP diet than those fed the LRDP/MRDP diet. Cows fed the LRDP/MRDP diet tended to have greater ADF intake (P = 0.07) than those fed the LRDP/HRDP diet. Apparent digestion of ADF when expressed as a percentage of ADF intake tended to be greater (P = 0.06) in cows fed the OSC diets compared to those fed the STATIC diets. Apparent digestion of ADF when expressed as a percentage of ADF intake was greater ($P < 0.01$*)* in cows fed the OSC RDP diets than those fed the MRDP diet. Apparent digestion of ADF when expressed as a percentage of ADF intake was greater ($P < 0.01$*)* in cows fed the LRDP/HRDP diet than those fed the LRDP/MRDP diet. Intake and ruminal digestion of EE were unaffected by diet (≥ 0.26). Omasal flow of EE was greater (P = 0.05) in cows fed the LRDP/MRDP diet compared to those fed the LRDP/HRDP diet. Also, omasal flow of EE tended to be greater (P = 0.09) in cows fed the OSC RDP diets compared to those fed the MRDP diet.

Table 4.2. The influence of feeding oscillating dietary ruminally-degradable protein (RDP) levels on urinary N excretion, faecal N excretion and apparent N balance in dairy cows [1]

	Diet[2]					Contrasts: *P* value[3]		
Item	LRDP	MRDP	LRDP/MRDP	LRDP/HRDP	SEM	STATIC vs. OSC	MRDP vs. OSC	LRDP/MRDP vs. LRDP/HRDP
DM Intake, kg/d	26.9	29.4	30.2	25.8	0.82	0.83	0.13	0.01
N intake, g/d	773	802	821	798	43.9	0.63	0.90	0.72
Urinary excretion								
Total, kg/d	40.4	44.8	45.3	43.2	1.75	0.15	0.46	0.18
Total N, g/d	199	243	232	200	19.4	0.58	0.04	0.03
Total N, % of N intake	25.2	28.6	27.9	27.8	1.93	0.27	0.48	0.92
Urea-N								
g/d	153	176	149	187	17.5	0.84	0.63	0.11
% of urinary N	79.3	82.3	57.3	89.7	9.47	0.17	0.18	0.05
Fecal excretion								
DM, kg/d	8.65	9.60	9.05	8.43	0.65	0.56	0.30	0.51
N, g/d	197	205	180	191	22.5	0.18	0.18	0.50
N, % of N intake	25.6	22.8	23.8	24.2	1.90	0.60	0.04	0.49
Total N excretion								
g/d	397	449	412	391	37.5	0.16	0.03	0.30
% of N intake	50.8	51.6	51.9	51.8	3.61	0.78	0.93	0.96
Milk N								
g/d	191	195	193	181	22.5	0.53	0.50	0.33
% of N intake	24.9	24.4	23.6	22.7	1.12	0.21	0.37	0.60
Apparent N balance, g/d	187	162	213	224	35.6	0.25	0.23	0.84
Productive N[4], g/d	376	356	406	405	38.9	0.35	0.33	0.98

[1]Values are least squares means obtained from 4 ruminally-cannulated cows. Means were calculated from data collected during the 4 d of total collections (d 25-28).

[2] LRDP = diet containing 9.34% RDP fed on a continuous basis; MRDP = diet containing 11.3% RDP fed on a continuous basis; LRDP/MRDP = diets containing 9.34 and 11.3% RDP fed on an oscillating (48-h) basis; and LRDP/HRDP = diets containing 9.34 and 12.6% RDP fed on an oscillating (48-h) basis.

[3]STATIC = LRDP + MRDP, and OSC = LRDP/MRDP + LRDP/HRDP.

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

Table 4.3. The influence of feeding oscillating dietary ruminally-degradable protein (RDP) levels on apparent total-tract digestibilities in dairy cows[1]

	Diet[2]					Contrasts: *P* value[3]		
Item	LRDP	MRDP	LRDP/MRDP	LRDP/HRDP	SEM	STATIC vs. OSC	MRDP vs. OSC	LRDP/MRDP vs. LRDP/HRDP
Apparent total-tract digestibility, %								
DM	75.0	74.1	71.0	73.1	1.09	0.06	0.03	0.02
OM	75.6	75.1	72.2	74.2	0.47	0.07	0.03	0.02
CP	73.4	76.0	75.5	75.4	1.08	0.10	0.28	0.81
NDF	64.2	58.6	57.6	60.8	2.32	0.43	0.85	0.27
ADF	63.5	57.4	52.8	56.5	2.98	0.18	0.53	0.35
EE	79.7	79.9	79.1	77.2	1.99	0.14	0.20	0.27

[1]Values are least squares means obtained from 4 ruminally-cannulated cows. Means were calculated from data collected during the 4 d of total collections (d 25-28).

[2] LRDP = diet containing 9.34% RDP fed on a continuous basis; MRDP = diet containing 11.3% RDP fed on a continuous basis; LRDP/MRDP = diets containing 9.34 and 11.3% RDP fed on an oscillating (48-h) basis; and LRDP/HRDP = diets containing 9.34 and 12.6% RDP fed on an oscillating (48-h) basis.

[3]STATIC = LRDP + MRDP, and OSC = LRDP/MRDP + LRDP/HRDP.

Table 4.4. The influence of feeding oscillating dietary ruminally-degradable protein (RDP) levels on ruminal fermentation characteristics in dairy cows[1]

	Diet[2]					Contrasts: *P* value[3]		
Item	LRDP	MRDP	LRDP/MRDP	LRDP/HRDP	SEM	STATIC vs. OSC	MRDP vs. OSC	LRDP/MRDP vs. LRDP/HRDP
Ruminal SCFA, m*M*								
Acetate	72.4	73.3	73.9	77.5	2.28	<0.01	<0.01	<0.01
Propionate	21.8	23.7	24.5	23.9	1.16	0.03	0.41	0.46
Butyrate	13.6	13.8	14.2	13.6	0.87	0.56	0.82	0.24
Isobutyrate	0.90	1.07	0.97	0.92	0.05	0.08	<0.01	0.01
Valerate	1.44	1.59	1.59	1.46	0.07	0.74	0.16	0.03
Isovalerate	1.32	1.61	1.55	1.33	0.12	0.44	<0.01	<0.01
Total SCFA	114	118	120	121	3.79	0.01	0.15	0.56
Acetate: propionate ratio	3.20	3.17	3.15	3.30	0.12	0.75	0.73	0.37
Ruminal NH_3-N, mg/dL	14.2	19.8	17.9	18.8	1.58	0.20	0.26	0.54
Ruminal pH	6.19	6.19	6.20	6.20	0.08	0.76	0.77	0.96

[1]Values are least squares means obtained from 4 ruminally-cannulated cows.
[2] LRDP = diet containing 9.34% RDP fed on a continuous basis; MRDP = diet containing 11.3% RDP fed on a continuous basis; LRDP/MRDP = diets containing 9.34 and 11.3% RDP fed on an oscillating (48-h) basis; and LRDP/HRDP = diets containing 9.34 and 12.6% RDP fed on an oscillating (48-h) basis.
[3]STATIC = LRDP + MRDP, and OSC = LRDP/MRDP + LRDP/HRDP.

Table 4.5. The influence of feeding oscillating dietary ruminally-degradable protein (RDP) levels on nutrient flow and ruminal digestion in dairy cows[1]

	Diet[2]					Contrasts: *P* value[3]		
Item	LRDP	MRDP	LRDP/MRDP	LRDP/HRDP	SEM	STATIC vs. OSC	MRDP vs. OSC	LRDP/MRDP vs. LRDP/HRDP
DM								
Intake, kg/d	27.3	27.9	30.1	26.9	0.90	0.53	0.71	0.14
Omasal flow, kg/d	21.6	20.1	21.3	20.0	0.95	0.75	0.49	0.15
Apparent digestion, kg/d	7.34	6.02	7.36	8.48	1.08	0.33	0.23	0.52
Apparent digestion, % of DM intake	21.3	23.2	27.2	30.9	3.67	0.10	0.23	0.49
OM								
Intake, kg/d	24.8	25.5	27.3	24.4	0.73	0.48	0.74	0.11
Omasal flow, kg/d	17.5	16.7	17.2	15.9	0.80	0.59	0.93	0.19
Apparent digestion, kg/d	8.68	7.20	8.93	9.91	0.96	0.20	0.13	0.52
Apparent digestion, % of OM intake	34.1	28.6	34.0	37.7	2.90	0.21	0.11	0.44
N								
Intake, g/d	775	784	836	814	33.5	0.21	0.35	0.66
Omasal flow, g/d	784	779	721	672	47.7	0.11	0.19	0.49
Apparent digestion, g/d	-9.32	5.90	115	142	47.5	0.02	0.06	0.69
Apparent digestion, % of N intake	-1.51	0.03	13.3	17.4	5.57	0.02	0.05	0.62
NH_3-N, g/d	35.5	45.6	40.9	44.5	3.79	0.59	0.55	0.52
NAN[4]								
g/d	749	733	680	627	46.8	0.10	0.20	0.45
% of N intake	96.9	94.2	81.8	77.1	5.55	0.02	0.06	0.57
NDF								
Intake, kg/d	10.0	9.37	10.8	9.39	0.47	0.59	0.44	0.22
Omasal flow, kg/d	6.58	6.15	6.16	5.22	0.39	0.32	0.49	0.32
Apparent digestion, kg/d	4.61	3.15	3.24	4.35	0.37	0.83	0.24	0.10
Apparent digestion, % of NDF intake	41.1	38.4	30.1	45.4	1.56	0.26	0.76	0.03

Table 4.5. (cont…) The influence of feeding oscillating dietary ruminally-degradable protein (RDP) levels on nutrient flow and ruminal digestion in dairy cows[1]

	Diet[2]					Contrasts: *P* value[3]		
Item	LRDP	MRDP	LRDP/MRDP	LRDP/HRDP	SEM	STATIC vs. OSC	MRDP vs. OSC	LRDP/MRDP vs. LRDP/HRDP
ADF								
Intake, kg/d	6.21	6.11	7.13	5.97	0.25	0.21	0.21	0.07
Omasal flow, kg/d	4.00	3.75	4.40	3.70	0.33	0.63	0.51	0.14
Apparent digestion, kg/d	2.53	2.10	2.23	2.78	0.43	0.67	0.47	0.39
Apparent digestion, % of ADF intake	43.2	35.2	23.3	48.2	1.62	0.06	<0.01	<0.01
Ether Extract								
Intake, kg/d	0.58	0.58	0.65	0.60	0.04	0.26	0.35	0.42
Omasal flow, kg/d	0.50	0.42	0.67	0.42	0.05	0.12	0.09	0.05
Apparent digestion, kg/d	0.10	0.15	0.05	0.10	0.08	0.53	0.44	0.65
Apparent digestion, % of EE intake	15.4	23.5	4.78	14.2	9.43	0.32	0.26	0.50

[1]Values are least squares means obtained from 4 ruminally-cannulated cows. Means were calculated from data collected during the 4 d of ruminal and omasal sampling (d 19 – 22).
[2] LRDP = diet containing 9.34% RDP fed on a continuous basis; MRDP = diet containing 11.3% RDP fed on a continuous basis; LRDP/MRDP = diets containing 9.34 and 11.3% RDP fed on an oscillating (48-h) basis; and LRDP/HRDP = diets containing 9.34 and 12.6% RDP fed on an oscillating (48-h) basis.
[3]STATIC = LRDP + MRDP, and OSC = LRDP/MRDP + LRDP/HRDP.
[4]NAN = Non- NH_3-N

5. DISCUSSION

In dairy cows, the efficiency of N utilization is poor, with only 20 to 35% of dietary N intake being captured as milk N; conversely, 65 to 80% of dietary N intake is excreted through urine and faeces (Tamminga, 1992; Chase et al., 2009; Reynolds et al., 2013). The excretion of excessive amounts of N causes environmental pollution (Van Horn et al., 1996), thus considerable research effort has been directed towards improving the efficiency of N utilization in dairy cows. Various feeding strategies have been investigated with the objective of reducing N excretion without negatively affecting production in ruminants, and these include reducing dietary protein supply (Broderick, 2003; Chase et al., 2007; Chibisa and Mutsvangwa, 2013), balancing fermentable carbohydrate supply with protein supply (Castillo et al., 2000; Huntington and Archibeque, 2000), and oscillating CP content of diets (Cole, 1999; Ludden et al., 2002; Cole et al., 2003; Ludden et al., 2003; Archibeque et al., 2007a,b,c; Doranalli and Mutsvangwa, Doranalli et al., 2011; Brown, 2014; Kohler, 2016). It has been reported that feeding oscillating CP at 48-hr intervals (i.e., feeding a low CP diet for 2 d followed by a high CP diet for the following 2 d, and then repeated in a cyclic manner) can improve the efficiency of N utilization in sheep (Cole, 1999; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009), beef steers (Cole et al., 2003; Archibeque et al., 2007b), and dairy cows (Kohler, 2016). With this approach, depressed ruminal ammonia-N concentrations during part of the oscillation cycle when animals are fed low CP diets stimulates urea secretion from the bloodstream into the rumen where it can support microbial protein synthesis (Cole, 1999). The repartitioning of urea from urinary excretion to secretion into the rumen is partly responsible for the improvement in N utilization that is observed when feeding oscillating CP diets. Because dietary RDP level can also influence ruminal ammonia-N concentrations which, in turn, can influence urea secretion into the rumen (Lu et al., 2014), the current study was conducted to investigate the effects of feeding oscillating dietary rumen degradable protein (RDP) levels on production, ruminal function, omasal nutrient flow and N utilization in dairy cows.

To my knowledge, there are no published studies that have investigated the effects of feeding oscillating dietary RDP levels on production performance in dairy cows, so direct comparisons of observations from the present study with those from previous studies are not possible. A limited number of studies have investigated the effects of feeding oscillating dietary CP levels on various parameters in dairy cows, so comparisons of observations from the present study will mostly be with those studies. In this current study, DM intake was not affected by oscillating dietary RDP levels compared to feeding STATIC RDP levels in dairy cow diets. In a study conducted at the University of Saskatchewan, Kohler (2016) examined the influence of

oscillating dietary CP concentrations on production in dairy cows and reported that oscillating CP concentrations at 48-h intervals in diets of dairy cows resulted in no effect on DM intake compared to feeding STATIC CP concentrations. Also, Brown (2014) investigated the effects of oscillating CP levels of dairy cow diets and reported that oscillating dietary CP levels at 48-h intervals had no effect on DM intake compared to feeding STATIC CP levels. The lack of effect of feeding oscillating CP levels on DM intake in these previous studies agrees with results from the current study. Also, previous studies investigating the effects of feeding oscillating dietary CP levels in sheep (Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009) and beef cattle (Cole et al., 2003; Archibeque et al., 2007) failed to detect any dietary effects on DM intake. Contrary to these findings, Doranalli et al., (2011) reported that oscillating CP levels at concentrations of 10.3 and 16.1 % at 48-h intervals tended to lower DM intake compared to feeding STATIC CP diets in sheep. According to NRC (2001), an increase in dietary RDP content leads to an increase in dry matter intake. An increase in dietary RDP content provides for greater ruminal N supply in the form of NH_3, AA and peptides which, in turn, can potentially enhance microbial protein synthesis and overall microbial activity (Lazzarini et al., 2009).

Milk production was not affected by diet when comparing STATIC RDP diets to oscillating RDP diets. In general, milk yield is positively correlated with DM intake, that is, an increased DM intake results in increased milk production (NRC, 2001); therefore, it was not surprising that milk yields were similar between cows fed STATIC RDP and oscillating RDP diets because their DM intakes were similar. The similar milk yields when comparing oscillating RDP diets to STATIC RDP diets concur with the findings from other researchers who investigated effects of oscillating CP in diets of dairy cows (Brown, 2014; Kohler, 2016). However, milk yield was greater in cows fed the LRDP/MRDP diet compared to those fed the LRDP/HRDP diet, even though there was no difference in DM intake between the 2 treatment groups. Besides DM intake, changes in ruminal or total-tract nutrient digestion can alter post-absorptive nutrient supply which, consequently, can result in changes in milk production. In the current study, total-tract nutrient digestion of DM, OM and CP was greater in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diet. The noted differences can not be used to explain the higher milk yield in cows fed the LRDP/MRDP diet since they had lower total tract nutrient digestibility of DM, OM and CP, meaning they had less post-absorptive nutrient supply for milk production compared to cows fed the LRDP/MRDP diet. Therefore, the reason(s) why cows fed the LRDP/MRDP diet had a greater milk yield than those fed the LRDP/HRDP diet is unclear.

Milk fat content was not affected by dietary treatment. This concurs with findings from other studies that reported no effects of oscillating dietary CP on milk fat content in dairy cows (Brown, 2014; Kohler, 2016). However, in contrast to previous research conducted with dairy cows which reported no effect of oscillating CP in dairy diets on milk fat yield (Brown, 2014; Kohler, 2016), results from the current study showed that milk fat yield tended to increase when cows were fed oscillating RDP diets compared to the STATIC MRDP diet. This may be because cows fed the oscillating RDP diets had a greater acetate concentration compared to those fed the STATIC RDP diets. Acetate is the precursor for milk fat synthesis (Linn, 1988). This would suggest that the higher acetate concentrations observed consequently provided a greater substrate supply for milk fat synthesis which would influence milk fat content. Considering that milk fat yield is a function of milk yield and milk fat content, the lack of effect of dietary treatment on milk fat content and yield amongst the oscillating RDP and STATIC MRDP treatments, the tendency for higher milk fat yield observed is unclear.

Milk protein content was altered by dietary treatment, with all 3 contrasts that were tested being significant. Firstly, milk protein content was higher in cows fed oscillating RDP diets compared to those fed STATIC RDP diets. The analytical procedures at the DHI Laboratories (Edmonton, AB) where milk compositional analysis was conducted measure total (crude) protein content of the milk, which is composed of true protein (primarily casein) and non-protein nitrogen (NPN) components (primarily milk urea-N; MUN) (Cerbulis and Farrell, 1975; DePeters and Cant, 1992; DePeters and Ferguson, 1992; Murphy and O'Mara, 1993; Baker et al., 1995; Ruska and Jonkus, 2014). Because MUN concentration was similar in cows fed STATIC and oscillating RDP diets, it can be surmised that the difference in milk protein content between the 2 treatment groups can be attributed to differences in milk true protein content. Metabolizable protein that is digested in the small intestine is the major source of amino acids that are used for mammary synthesis of milk true protein (NRC, 2001). Omasal NAN flow, which can be used as an indicator of metabolizable protein flow from the rumen, tended to be greater in cows fed the STATIC RDP diets compared to those fed the oscillating RDP diets. These results would then suggest that substrate (i.e., amino acids) availability for milk protein synthesis was greater in cows fed the STATIC RDP diets compared to those fed the oscillating RDP diets; however, this is incongruent with the current observations in which cows fed the oscillating diets had greater milk protein content. The reasons for these discrepant observations are unclear. Besides amino acid supply, other factors can also influence milk protein content. For example, Thomas and Martin (1988) reported that an increase in propionate concentration resulted in an increase in milk protein content in dairy cows. Because

ruminal absorption of propionate is a concentration-dependent process (Dijkstra et al., 1993; Lopez et al., 2003; Bannik et al., 2008), it can be surmised that greater ruminal concentrations of propionate resulted in greater portal uptake of propionate. Propionate is the major substrate for hepatic gluconeogenesis, so a greater post-absorptive availability of propionate would potentially spare amino acid use for gluconeogenesis (NRC, 2001), thus providing more substrate for milk protein synthesis. In the present study, cows fed the oscillating RDP diets had a greater ruminal propionate concentration compared to those fed the STATIC RDP diets, which could have made more amino acids available for milk protein synthesis. Also, milk protein yield was greater in cows fed the oscillating RDP diets compared to those fed the STATIC RDP diets. Because milk protein yield is calculated as milk yield × milk protein content and there was no difference in milk yield between the 2 treatment groups, the difference in milk protein yield reflects the observed difference in milk protein content.

Milk protein content was greater in cows fed oscillating RDP diets compared to those fed STATIC MRDP diet. This is contrary to the findings from studies conducted previously with dairy cows which showed that feeding oscillating CP content in diets of dairy cows had no effect on milk protein content (Brown, 2014; Kohler, 2016). Milk protein yield was higher in cows fed oscillating RDP diets compared to those fed STATIC RDP diets. This is contrary to the findings from a study conducted previously with dairy cows which showed that feeding STATIC CP diets resulted in a higher protein yield compared to feeding oscillating CP diets (Kohler, 2016). Brown (2014) reported that feeding oscillating CP diets had no effect on milk protein yield compared to feeding STATIC CP diets. In this present study, higher protein yield results are not surprising as the cows fed the oscillating RDP diets had higher milk protein content compared to those fed STATIC RDP diets. This is because milk protein yield is a function of milk yield and milk protein content. Although milk yield was not affected by dietary treatment, the higher protein content in cows fed oscillating RDP diets explains the higher milk protein yield observed in this present study. According to Thomas (1980) and Oltner et al. (1985), diets high in RDP contents are most likely to increase milk urea levels which influence milk protein content. Also, milk protein content was higher in cows fed LRDP/MRDP diet compared to those fed the LRDP/HRDP diet. This is inconsistent with the above reasoning that the higher the RDP level, the greater the milk protein content.

Feeding oscillating RDP diets had no effect on milk lactose yield and content compared to feeding STATIC RDP diets. This concurs with the findings from other researchers who found no effect of oscillating CP levels in dairy cow diets on lactose yield and content (Brown, 2014; Kohler, 2016). However, feeding the LRDP/MRDP diet resulted in a higher lactose

content and yield compared to feeding LRDP/HRDP diet in cows. Lactose is generally a constant constituent of milk thus the cause for the difference noted in lactose yield and content between LRDP/MRDP and LRDP/HRDP diets is unknown.

Milk urea-N concentration was similar between cows fed oscillating RDP diets compared to those fed STATIC RDP diets; however, it was greater in cows fed the STATIC MRDP diet compared to those fed the oscillating RDP diets. This conflicts with results from other researchers (Brown, 2014; Kohler, 2016) who found no effect of oscillating dietary CP content on MUN concentration. When considering the level of RDP in the MRDP diet compared to that of the oscillating RDP diets, the MRDP diet had a higher level of RDP (MRDP = 11.3%; as % of dietary DM) being supplied constantly throughout the feeding period whereas the oscillating diets have a lower RDP level on average (LRDP/MRDP = 10.3%; LRDP/HRDP = 10.97%). This would suggest that the STATIC MRDP diet had a higher RDP concentration which led to more MUN concentration. This might also prove that oscillating RDP content was efficient in improving N utilization. On dairy farms, MUN is used as a parameter to assess the nutrient status of lactating dairy cows (Jonker et al., 1999). This is because high levels of MUN indicate an excess supply of dietary protein or inefficient use of N.

Also, MUN concentration was greater in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diets. This may have been due to the higher concentration of RDP in the LRDP/HRDP diet (10.97%) compared to 10.3% in the LRDP/MRDP diet.

Although PUN concentration is positively correlated to MUN concentration, in this current study, PUN concentration tended to increase when cows were fed the oscillating RDP diets, particularly the LRDP/HRDP diet. This is contrary to the MUN concentration results which showed no effect of oscillating RDP diet on MUN concentration. The relationship between these two parameters (PUN and MUN) is due to the passive diffusion of urea between the bloodstream and milk glands (i.e., urea moves from the bloodstream into the milk until an equilibrium is reached) (Clark et al., 1978; Roseler et al., 1993; Baker, 1995). Another measurement that is highly correlated to PUN is ruminal NH_3-N concentration and, surprisingly, diet had no effect on ruminal NH_3-N concentrations in the present study. Other researchers reported no effect of oscillating CP in diets of ruminants compared to feeding STATIC CP diets on PUN concentration (Cole, 1999; Cole et al., 2003, Ludden et al., 2003; Archibeque et al., 2007b; Doranalli and Mutsvangwa, 2009; Kohler, 2016).

Apparent total-tract nutrient digestibility of DM and OM tended to be greater in cows fed STATIC RDP diets compared to cows fed OSC RDP diets. This may be attributed to the higher RDP content of the STATIC RDP diets compared to the OSC RDP diets. Generally, an

increase in dietary RDP content encourages greater digestibility of nutrients. Also, Apparent total-tract nutrient digestibility of DM and OM tended to be greater in cows fed the LRDP/HRDP diet compared to those fed the LRDP/MRDP diets. This agrees with the fact mentioned earlier that the higher the RDP level of a diet the greater the digestibility. This is because the LRDP/HRDP had a greater RDP level than the LRDP/MRDP diet. On the contrary, other researchers reported no effect of oscillating dietary protein content of ruminant diets on apparent digestibilities of DM (Cole, 1999; Ludden et al., 2002b; Archibeque et al., 2007a,b; Doranalli and Mutsvangwa, 2009; Kohler, 2016), and OM (Ludden et al., 2002a; Archibeque et al., 2007a,b; Doranalli and Mutsvangwa, 2009; Kohler, 2016).The Apparent total-tract nutrient digestibility of NDF, ADF and EE were unaffected by dietary treatments in the current study. This concurs with findings from various studies that fed oscillating CP diets at 48-h intervals, similar to the regimen used in the current study. These various studies reported that oscillating CP in diets fed to ruminants (i.e., sheep and dairy cattle) had no effects on total tract digestibilities of NDF and ADF (Ludden et al., 2002a, b; Kohler, 2016). However, on the contrary, Doranalli and Mutsvangwa (2009) reported that total-tract digestibilities of ADF and NDF were greater in sheep that were fed oscillating CP diets at 48-h intervals when compared to feeding STATIC CP diets. In ruminants, dietary fibre is mostly digested in the rumen due to the fermentative activities of ruminal microorganisms primarily the cellulolytic bacteria. The positive response in fibre digestibility that has been reported previously when feeding oscillating CP diets was partly attributed to an improvement in the proliferation of ruminal cellulolytic microorganisms that are mainly responsible for fibre digestion as a result of an increase in ruminal N supply from recycled urea-N (Doranalli and Mutsvangwa, 2009). Doranalli and Mutsvangwa (2009) also reported a greater bacterial NAN flow in sheep fed oscillating CP diets compared to those fed STATIC CP diets, which would be indicative of greater microbial proliferation within the rumen with oscillating CP diets. In the present study, the impacts of feeding oscillating compared to STATIC dietary CP on whole-body urea kinetics and bacterial NAN flow were not assessed, so no definitive conclusions can be made regarding dietary impacts on microbial growth in the rumen. However, in the current study, ruminal NH_3-N concentration was unaltered by dietary treatment; therefore, it can be surmised that ruminal N supply might not have been influenced by dietary treatment and, presumably, this could partly explain why feeding oscillating dietary CP did not alter total-tract fibre digestion when compared to feeding STATIC CP diets.

One other objective of this study was to determine the effects of oscillating RDP levels in diets of dairy cows on ruminal fermentation characteristics (SCFA, pH, and NH_3). Ruminal

concentrations of acetate were greater in cows fed the oscillating RDP diets compared to those fed the STATIC RDP diets. This is consistent with results from other researchers (Ludden et al., 2002a; Doranalli et al., 2011), who reported an increase in ruminal concentrations of acetate when lambs were fed oscillating CP diets. Acetate production in the rumen indicates fibre fermentation rate and the higher concentrations observed in this current study might be an indication of increased recycling of N into the rumen when cows received low RDP levels in their diet, which in turn might have altered the pattern of fibre fermentation (Ludden et al., 2002a). Also, feeding oscillating RDP diets resulted in greater acetate concentration compared to feeding STATIC MRDP diet and this may be due to the same reason as mentioned previously. The LRDP/HRDP diet resulted in greater acetate concentration compared to the LRDP/MRDP diet and this is a logical response considering that the LRDP/HRDP diet provided a greater supply of RDP which may have elevated the amount of ammonia converted to urea. This urea-N would be recycled back into the rumen thus changing fermentation patterns in the rumen in favor of acetate (Ludden et al., 2002a). However, results from this present study indicate that there were no differences in the NH_3 concentration levels across treatments. The reasons for this misnomer are not clear.

Propionate was greater in cows fed oscillating RDP diets compared to those fed the STATIC RDP diets. This is consistent with results from a study conducted with sheep (Doranalli et al., 2011) that reported an increase in ruminal concentrations of propionate when lambs were fed oscillating CP diets. In that study, the sheep were fed high concentrate diets which may have contributed to the increase noted in propionate concentration. On the contrary, Kohler (2016) found no effect of oscillating CP in diets of dairy cows on acetate and propionate concentrations. Branched-chain fatty acid (BCFA) concentrations were affected by dietary treatments. These BCFA, namely isobutyrate, valerate and isovalerate are by-products of protein degradation (valine, proline and leucine respectively) in the rumen (Andries et al., 1987; Becht, 1987; Liu et al., 2018; Zhao et al., 2019) and are used for the biosynthesis of the aforementioned amino acids and some higher BCFA. Isobutyrate concentration tended to be greater in cows fed STATIC RDP diets compared to those fed oscillating RDP diets. Also, cows fed STATIC MRDP diets had greater isobutyrate concentration than cows fed oscillating RDP diets. Isovalerate concentration was greater in cows fed STATIC RDP diets compared to those fed oscillating RDP diets. The greater ruminal concentration of the BCFA in STATIC RDP diets compared to oscillating RDP diets is probably a result of a higher and continuous supply of RDP (11.3 vs. 10.3 or 10.9 from oscillating diets) to the rumen thus supplying more substrate for protein degradation which yields BCFA. Total short-chain fatty acid (SCFA)

concentrations were greater in cows fed the oscillating RDP diets compared to those fed the STATIC RDP diets. Similarly, when sheep were fed diets with oscillating CP, other researchers reported an increase in SCFA concentrations (Doranalli et al., 2011; Ludden et al., 2002a). However, in a recent study with dairy cows, oscillating dietary CP was found to have no effect on SCFA concentrations (Kohler, 2016). Since acetate and propionate are part of the major SCFA and were both higher in oscillating RDP treatments, this would consequently result in an increase in the SCFA concentrations observed in this current study. Finally, acetate to propionate ratio was unaffected by dietary treatments. The lack of effect observed in this present study concurs with findings from a study done recently on dairy cows fed oscillating CP diets by Kohler (2016). Contrary to these results, Ludden et al., (2002) reported that oscillating dietary CP resulted in an increase in the acetate to propionate ratio of lambs. Ludden et al., (2002) alluded that their findings were a result of a shift in rumen fermentation patterns in the favor of more acetate production. In this present study however, both acetate and propionate increased proportionately across the diets hence the lack of effect observed.

Ruminal concentrations of NH_3-N were not affected by dietary treatments in this current study. The mean ruminal concentrations of NH_3-N were well above the minimum threshold (5 mg/dL) for optimum MP synthesis (Satter and Slyter, 1974). Similar results were observed in a study with oscillating CP in sheep that were fed high concentrate diets (Doranalli et al., 2011). On the contrary, results from a recent study with dairy cows showed an increase in ruminal NH_3-N concentrations in cows that were fed oscillating CP diets compared to those fed STATIC CP diets (Kohler, 2016). Ruminal NH_3-N concentrations are affected by the amount of degradable protein and NPN available in the diets (Seal and Reynolds, 1993; Abdoun et al.,2007; Reynolds and Kristensen, 2008). When diets have a higher supply of RDP than is required by the animal, a surplus of ruminal NH_3-N is then produced (Bach et al., 2005; Olmoneros and Broderick 2006). Excess NH_3-N is removed from the rumen and transported to the liver where it is converted to urea-N (Bach et al., 2005; Abdoun et al.,2007). The lack of effect of diet on ruminal NH_3-N concentrations observed in the current study may be an indication that the differences in dietary RDP content were not sufficiently large to result in measurable differences in ruminal NH_3-N concentrations. Also, the reported dietary RDP contents of 9.34, 11.3 and 12.6% (as % of DM) were calculated based on in situ determinations of the RDP content of SoyPlus and "book values" for RDP contents of the rest of the dietary ingredients. Although the calculated differences in dietary RDP contents were deemed large, dietary processing (e.g., pelleting) could have influenced dietary characteristics (e.g., particle size and extent of ruminal degradation) and, in turn, actual ruminal protein degradation in vivo.

Unfortunately, in vivo determinations of dietary RDP contents were not conducted, so no definitive conclusions can be drawn.

Ruminal pH was not affected by dietary treatments as was found by other researchers (Ludden et al., 2002a; Doranalli et al., 2011; Kohler, 2016). The similarities observed in pH values across dietary treatments may be an indication that DM intake was unaffected as explained by Kohler (2016).

Another objective of this study was to determine effects of oscillating dietary RDP levels on N balance. As discussed, earlier, the efficiency of N utilization in dairy cows is low. As a result, most of the N that dairy cows consume is lost in manure, which results in environmental pollution. Also, the loss of dietary N to the environment is economically costly for dairy producers as protein-containing feed ingredients are expensive. In the present study, N intake in g/d was unaffected by dietary treatment. Nitrogen intake is a function of dietary N content and DM intake. In this current study, DM intake was similar across dietary treatments and there were marginal differences in dietary N content amongst dietary treatments, hence the lack of effect of diet on N intake was somewhat expected. In other studies, there was no effect of feeding oscillating CP diets to dairy cows (Brown, 2014; Kohler, 2016), sheep (Cole, 1999; Ludden et al., 2002a,b; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) and beef cattle (Cole et al., 2003; Archibeque et al., 2007a,b) on N intake, concurring with the findings from this current study.

Fecal N excretion was unaffected by diet, similar to the findings of other researchers (Ludden et al., 2002 b; Cole et al., 2003; Archibeque et al., 2007c; Kohler, 2016). Perusal of literature shows that N intake largely does not affect fecal N excretion (Xia et al., 2018). Urinary N excretion (g/d) was higher in cows that received the STATIC MRDP diet compared to those that received the oscillating RDP diets. According to other researchers, urinary N excretion is positively correlated to MUN (Jonker et al., 1998, Kauffman and St-Pierre, 2001; Kohn et al., 2002; Nennich et al., 2006). In this current study, MUN levels were higher in cows fed STATIC MRDP diets compared to those fed oscillating RDP diets, which agrees with the observed dietary effects on urinary N excretion. Also, urinary N excretion was higher in cows that received oscillating LRDP/MRDP diet compared to those that received the oscillating LRDP/HRDP diet. This is unusual as, normally, the diet with the higher RDP levels would consequently have higher urinary excretion. Also, as explained above, MUN is positively correlated to urinary N excretion. However, the reverse is observed here as cows fed LRDP/HRDP diet had had higher MUN concentrations compared to those fed LRDP/MRDP diet but had lower urinary N excretion. Another factor that influences urinary N excretion is

urea-N which arises mostly from rumen NH_3-N that is absorbed into the portal blood to be detoxified in the liver into urea. However, in this present study, ruminal NH_3-N was unaffected by dietary treatment. On the contrary, other researchers found no effect of oscillating CP in the diets of ruminants compared to those fed STATIC diets on urinary N excretion (Cole, 1999; Ludden et al., 2002b; Cole et al., 2003; Archibeque et al., 2007 b, c; Kohler, 2016). Overall, when expressed as a percentage on N intake, urinary N excretion was not affected by dietary treatment.

Urinary urea-N expressed as a percentage on urinary N was higher in cows fed the oscillating LRDP/HRDP diet compared to those fed the oscillating LRDP/MRDP diet. This is logical as the LRDP/HRDP diet would have a higher level of RDP than the LRDP/MRDP diet. Urinary urea-N is known to be strongly correlated to ruminal NH_3 concentration as an elevation in the ruminal NH_3 results in excess NH_3 being converted to urea which is either recycled to GI tract or lost via urinary excretion (Burgos et al., 2007; 2010). Since Ruminal NH_3 concentration is positively correlated to dietary RDP levels (Seal and Reynolds, 1993; Reynolds and Kristensen, 2008; Mutsvangwa et al., 2016). Thus, the expectation would be that the slightly higher RDP levels of the LRDP/HRDP diet would explain the difference observed when compared to the LRDP/MRDP diet as it would influence greater ruminal NH_3 concentration. However, as already stated earlier, ruminal NH_3 concentration was unaffected by dietary treatment and can not be used to explain differences observed in urinary urea-N expressed as a percentage on urinary N. Also, UUN is positively correlated to MUN concentration (Burgos et al., 2007) though results have been highly varied (Spek et al.,2013). In this current study, cows fed the LRDP/HRDP diet had greater MUN concentration compared to those fed the LRDP/MRDP diet which is consistent with fact that MUN and UUN are strongly corelated.

Total N excretion (g/d) was greater in cows fed the STATIC MRDP diet compared to those fed the oscillating RDP diets, however, when expressed as a percentage of N intake, total N excretion was unaffected by dietary treatment. Milk N was not affected by diet and ranged between 23-25%, consistent with the low range of milk N (20-35%) stated in literature (Chase et al., 2009). This was consistent with the findings from Kohler (2016) who found no effect of oscillating CP in diets of dairy cows compared to feeding STATIC CP diets on milk N. Furthermore, this indicates that oscillating RDP in diets of dairy cows does not improve NUE as milk N is used to measure a cow's efficiency to utilize N by dividing the quantity of N excreted in milk by the quantity of N consumed by the cow (Kennedy and Milligan, 1980; Chase, 2009).

Apparent N balance was unaffected by oscillating RDP in diets of cows compared to feeding STATIC RDP diets. This is contrary to the findings of other researchers who reported increases in apparent N balance when oscillating CP in diets of ruminants. Kohler (2016) reported that feeding oscillating CP diet to dairy cows resulted in a 22.5% increase in apparent N balance compared to feeding STATIC CP diets. Cole (1999) reported that oscillating CP (10 and 15%) in diets of lamb at 48-h intervals increased N retention in comparison to lambs fed a STATIC CP diet (12%). Also, Cole (1999), reported in another trial that N retention improved by 38% when lambs were fed oscillating CP diets compared to lambs that were fed STATIC CP diets. Similarly, other researchers reported an increase in N retention when ruminants were fed oscillating diets, indicating the possibility of increased utilisation of dietary N (Cole et al., 2003; Archibeque et al., 2007b, c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011). In contrast to these findings, Ludden et al. (2002) reported that feeding high forage oscillating diets to sheep at 48-h intervals (13 and 17%) had no effects on N retention compared to feeding sheep with a STATIC CP diet (15%). The lack of effect of oscillating CP noted in the study by Ludden et al. (2002) may have been caused by the high CP levels in the diets which may have resulted in excess N being excreted due to excess supply of metabolizable protein. However, the lack of effect observed in this present study may be due to the marginal differences in the dietary N content of the treatments.

Another objective of this study was to investigate the effects of oscillating dietary RDP levels in dairy diets on ruminal nutrient digestion and omasal nutrient flow. Apparent digestion of DM in the rumen was not affected by dietary treatment. On the contrary, Kohler (2016) reported that oscillating CP in diets of dairy cows resulted in higher apparent ruminal digestion of DM compared to feeding STATIC CP diets. However, when expressed as a percentage of DM intake, apparent DM digestion tended to be greater in cows fed the RDP oscillating diets compared to those fed the STATIC RDP diets. This agrees with the results from another researcher who reported that oscillating CP in diets of dairy cows resulted in higher apparent DM digestion compared to feeding STATIC CP diets (Kohler, 2016). However, in another study with sheep, the researchers found no effect of oscillating dietary CP on apparent DM digestion (Ludden et al., 2002). However, in this current study the DM intake results were unaffected by dietary treatment as already mentioned above. Ruminal digestion of OM was not affected by oscillating RDP in diets of dairy cows. Similarly, Ludden (2002a) reported that feeding oscillating CP (13.2 and 16.7% CP) diets to sheep at 48-h intervals had no effect on OM digestion when compared to STATIC diets that had 15% CP. On the contrary, Kohler

(2016) found that dairy cows that received oscillating CP diets had higher apparent ruminal digestion of OM compared to those that were fed STATIC CP diets.

Nitrogen intake was similar across dietary treatments and consequently the flow of N at the omasal canal was also unaffected by dietary treatment. Perusal of literature shows that N intake directly influences omasal N flow (Brito et al., 2007a, b; Brito et al.,2009; Chibisa and Mutsvangwa, 2013); hence, it was not surprising that omasal flow of N was not influenced by dietary treatment since N intake was also unaffected. Similarly, a research conducted at the university of Saskatchewan with dairy cows reported no effect of oscillating dietary CP content compared to feeding STATIC CP diets (Kohler, 2016). Kohler (2016), however, reported that there was a tendency to increase the flow of N at the omasal canal when dairy cows were fed STATIC diets compared to those fed oscillating CP diets. Nitrogen that was apparently digested in the rumen was higher in cows fed the oscillating RDP diets compared to those fed the STATIC RDP diets. Also, N that was apparently digested in the rumen was higher in cows fed the oscillating RDP diet compared to those fed the STATIC MRDP diets. In the present study, cows that were fed STATIC RDP diets, had greater omasal N flow than N intake. This indicates possible urea recycling into the rumen, meaning STATIC diets increased urea-N recycling to the rumen compared to the oscillating RDP diets. This is contrary to research done by other researchers which indicated that feeding oscillating CP diets resulted in increased urea recycling (Archibeque et al., 2007c; Doranalli et al., 2011). According to Broderick et al. (2010) as the dietary CP and RDP levels increase, ruminal N balance becomes more positive. However, a study from our lab conducted by Chibisa et al. (2013) that had diets containing high dietary CP and RDP levels of 18.9 and 12.1 % respectively reported negative ruminal N balance from their study, contrary to the sentiments from the meta-analytical study by Broderick et al. (2010) mentioned above. In agreement with the results from Chibisa et al. (2013), other researchers reported a negative apparent ruminal N digestion in cows that were fed diets with CP levels that were higher than 18% (Reynal et al., 2003; Olmos Colmonero and Broderick, 2006a). In the current study, diets contained high CP levels ranging from 18.1–18.6% and RDP levels ranging from 9–12.3% hence the positive N balance reported agrees with the suggestion that the higher the CP and RDP levels of the diet, the more positive the ruminal N balance becomes (Broderick et al., 2010).

Non-ammonia nitrogen flow tended to be greater in cows fed STATIC RDP diets compared to those fed oscillating RDP diets with an average of 95.5% of the N intake compared to 79.5% when cows were fed oscillating RDP diets. Similarly, Kohler (2016) reported that cows that received STATIC CP diets tended to have a higher NAN flow compared to cows fed

oscillating CP diets. In that study, they also measured total bacterial NAN as a percentage of NAN flow which tended to be higher in cows fed STATIC CP diets compared to oscillating CP diet. However, in the current study, bacterial NAN was not measured hence definitive conclusions cannot be made from these results.

Apparent digestion of NDF and ADF were unaffected by diet. Similarly, when oscillating CP diets were fed to sheep, there was no effect on NDF and ADF compared to feeding STATIC CP diet (Ludden et al., 2002a). Kohler (2016) observed no effect of feeding oscillating CP diets to dairy cows on ADF apparent digestion in the rumen when compared to feeding STATIC CP diets but noted that NDF digestion was higher in cows fed the oscillating CP diets compared to the STATIC diet.

6. GENERAL DISCUSSION

The low N utilization efficiency of dairy cows has resulted in concerns from various sectors regarding N excretion into the environment. Though manure N can be used a source of good fertilizer (Spiehs et al., 2010; Langmeyer, 2002), excess amounts N form the manure causes environmental pollution in the form air, water and ground pollution (VanderHaar and St-Pierre, 2006; Burgos et al., 2007).

Various feeding strategies have been investigated to try and improve N utilization and reduce the loss of N to the environment and thus reduce the negative impact thereof. The most recent work in dairy cows has involved oscillating dietary CP to try and improve NUE and increase urea-N recycling (Brown, 2014; Kohler, 2016). This current study is a continuation/extension of a previous study in our lab (Kohler, 2016) which reported that feeding oscillating CP diets to dairy cows at 48-h intervals resulted in improved N efficiency and N retention. The purpose of this current study was to investigate the effects of feeding oscillating dietary rumen degradable protein (RDP) levels on production, ruminal function, omasal nutrient flow and N utilization in dairy cows.

This thesis had two main objectives and these were; 1) To determine the effects of feeding oscillating dietary RDP levels on feed intake, and milk production and composition in lactating dairy cows; and 2) to determine the effects of feeding oscillating dietary RDP levels on ruminal fermentation characteristics, extent of ruminal nutrient digestion, omasal outflow of nutrient, and N balance in lactating dairy cows.

Feed intake was not affected by dietary treatment in this study and this concurs with other researchers who found no effect of oscillating dietary CP in dairy cow diets (Brown, 2014; Kohler, 2016). Milk production was also unaffected by dietary treatment and this was expected since DM intake is highly correlated to milk production (NRC, 2001). Milk composition had varied results. Milk fat content was unaffected by diet, however feeding oscillating RDP diet tended to increase milk yield and this tendency was attributed to the greater concentration of acetate in oscillating RDP treatments compared to STATIC RDP treatments. All 3 contrasts for milk protein content were affected by dietary treatment as explained in the discussion. Milk protein yield was higher in cows fed oscillating RDP diets contrary to findings from another study with dairy cows (Kohler, 2016) but consistent with the fact that milk protein content was also greater in cows fed oscillating RDP diets compared to STATIC RDP diets. This is because milk protein yield is a function of milk yield and milk protein content.

Moreover, total nutrient digestibilities were unaffected by dietary treatment, a result that is consistent with other researchers' findings in (Cole, 1999; Ludden et al., 2002a, b; Archibeque et al., 2007a, b; Doranalli and Mutsvangwa, 2009; Kohler, 2016).

Regarding ruminal fermentation characteristics, ruminal concentrations of acetate and propionate were greater in cows fed oscillating RDP diets compared to those fed STATIC RDP diets. Concentrations of BCFA tended to be or were greater in cows fed STATIC RDP diets compared to those fed oscillating RDP diets. Acetate to propionate concentration was unaffected by dietary treatment. Due to the greater concentrations of acetate and propionate in the rumen of cows fed oscillating RDP diets compared to cows fed STATIC RDP, total SCFA concentrations were greater in cows fed oscillating RDP diet compared to those fed STATIC RDP diets. One of the expected outcomes of this study was an increase in ruminal concentrations of NH_3-N in cows fed higher levels of dietary RDP. This is because greater supply of RDP in the rumen results in more production of ammonia and when produced in excess, the surplus ammonia is transported to the liver where it is converted to urea. This urea then serves as a source of N for the animal in times of deficient supply of N in the diet, hence the idea of oscillating high and low RDP level diets. However, in this study, oscillating dietary RDP levels had no effect on ruminal concentrations of NH_3-N. This may indicate that though there were differences reported in the RDP levels of the diets, the differences were not significant enough to cause notable changes in the concentrations of NH_3-N in the rumen.in agreement with other researchers, ruminal pH was not affected by dietary treatments (Ludden et al., 2002a; Doranalli et al., 2011; Kohler, 2016).

Another objective of tis study was to determine effects of oscillating dietary RDP levels in dairy cow diets on N balance in lactating dairy cows. Nitrogen intake tended to be greater in cows fed oscillating diets compared to those fed STATIC RDP however results on N excretion show no effect of diet on N excretion indicating that the tendency for increased N intake did not cause high N excretion. Fecal N excretion was unaffected by dietary treatment whilst urinary N excretion was higher in cows fed STATIC MRDP diets compared to those fed oscillating diets. Because MUN concentration was higher in cows fed STATIC MRDP diets, this was expected since there is a positive correlation between urinary N and MUN (Jonker et al., 1998, Kauffman and St-Pierre, 2001; Kohn et al., 2002; Nennich et al., 2006). Urinary urea-N expressed as a percentage on urinary N was higher in cows fed the oscillating LRDP/HRDP diet compared to those fed oscillating LRDP/MRDP diet. Total excretion of N was greater in cows fed STATIC MRDP diet compared to those fed oscillating RDP diets but when expressed as a percentage of the N intake, it was unaffected by dietary treatment. Apparent N balance

was not affected by dietary treatment contrary to findings from other researchers who noted a improvement in apparent N balance when dairy cows (Kohler, 2016) and other ruminants were fed oscillating CP diets (Cole, 1999; Cole et al., 2003; Archibeque et al., 2007b, c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011).

Regarding the extent of ruminal nutrient digestion and omasal outflow of nutrient, DM and OM intake, flow at the omasal canal and apparent digestion were all unaffected by diet. DM apparent digestion expressed as a percentage of N intake tended to be greater in cows fed the oscillating RDP diets compared to those fed STATIC RDP diets. Cows fed STATIC RDP diets had greater apparent digestion of N, signifying a possible recycling of urea into the rumen in cows fed STATIC MRDP diet. This is contrary to our expectation that urea recycling would be increased by oscillating RDP levels in diets of dairy cows. The apparent digestion of ADF and NDF were not affected by dietary treatment. However, apparent digestion of ADF expressed a s a percentage of N intake tended to be higher in cows fed STATIC RDP diets compared to those fed oscillating RDP diets. Also, apparent digestion of ADF expressed a s a percentage of N intake was greater in cows fed oscillating RDP diets compared to those fed STATIC MRDP diets. Feeding the oscillating LRDP/HRDP diet resulted in greater apparent digestion of ADF expressed a s a percentage of N intake compared to feeding the oscillating LRDP/MRDP diet.

7. GENERAL CONCLUSIONS

While oscillating dietary CP levels has been shown to improve NUE of dairy cows, oscillating dietary RDP levels of dairy cow diets did not result in the expected similar results. Though oscillating RDP diets showed greater milk protein yield and content, ruminal acetate and propionate concentrations and total SCFA, it generally did not improve NUE. Therefore, inline with our objectives, oscillating RDP in diets of lactating dairy cows did not have meaningful impact on reducing the influence of dairy farming on the environment in terms of N pollution.

LITERATURE CITED

Abdoun, K., Stumpff, F., and Martens, H. 2007. Ammonia and urea transport across the rumen epithelium: a review. Animal Health Research Reviews / Conference of Research Workers in Animal Diseases, 7(1–2), 43–59. https://doi.org/10.1017/S1466252307001156

Abdoun, K., Stumpff, F., Rabbani, I., and Martens, H. 2010. Modulation of urea transport across sheep rumen epithelium in vitro by SCFA and CO2. Am. J. Physiol. Gastrointest. Liver Physiol. 298, G190–G202. doi: 10.1152/ajpgi.00216. 2009

Agle, M., Hristov, A. N., Zaman, S., Schneider, C., Ndegwa, P. M., and Vaddella, V. K. 2010. Effect of dietary concentrate on rumen fermentation, digestibility, and nitrogen losses in dairy cows. Journal of Dairy Science, 93(9), 4211–4222. https://doi.org/10.3168/jds.2009-2977.

Alexandratos, N. and J. Bruinsma. 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, FAO.

Alio, A., C. B. Theurer, O. Lozano, J. T. Huber, R. S. Swingle, A. Delgado-Elorduy, P. Cuneo, D. De Young, and K. E. Webb, Jr. 2000. Splanchnic nitrogen metabolism by growing beef steers fed diets containing sorghum grain flaked at different densities. J. Anim. Sci. 77:1355-1363.

Andries, J. I., Buysse, F. X., De Brabander, D. L., Cottyn, B. G. 1987. Isoacids in ruminant nutrition: Their role in ruminal and intermediary metabolism and possible influences on performances - A review. Animal Feed Science and Technology. vol 18, Issue 3, October 1987, Pages 169-180. https://doi.org/10.1016/0377-8401(87)90069-1

Araji, A. A., Z. O. Abdo, and P. Joyce, 2001. Efficient use of animal manure on cropland – economic analysis. Biores. Technol. 79:179-191.

Archibeque, S. L., D. N. Miller, H. C. Freetly, E. D. Berry, and C. L. Ferrell. 2007c. The influence of oscillating dietary protein concentrations on finishing cattle. 1. Feedlot performance and odorous compound production. J. Anim. Sci. 85:1487-1495.

Archibeque, S. L., H. C. Freetly, and C. L. Ferrell. 2007b. Net portal and hepatic flux of nutrients in growing wethers fed high-concentrate diets with oscillating protein concentrations. J. Anim. Sci. 85:997-1005.

Archibeque, S. L., H. C. Freetly, N. A. Cole, and C. L. Ferrell. 2007a. The influence of oscillating dietary protein concentrations on finishing cattle. II. Nutrient retention and ammonia emissions. J. Anim. Sci. 85:1496-1503.

Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th Ed. AOAC, Arlington, VA.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88:(E. Suppl.):E9-E21.

Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. J. Dairy Sci. 78:2424-2434.

Bannink, A., France, J., Lopez, S., Gerrits, W.J.J., Kebreab, E., Tamminga, S., Dijkstra, J.

2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Animal Feed Science and Technology Volume 143, Issues 1–4, 22 May 2008, Pages 3-26. https://doi.org/10.1016/j.anifeedsci.2007.05.002

Bateman, H. G., II. 2005. Response to increased rumen undegradable protein intake by lactating dairy cows. Prof. Anim. Sci. 21:263–271.

Becht, Richard R. 1987. Effects of Isoacids on Ruminal Metabolism and Milk Production. Iowa State University Veterinarian: Vol. 49: Iss. 1, Article 3. Available at: https://lib.dr.iastate.edu/iowastate_veterinarian/vol49/iss1/3

Belanche, A., G. de la Fuente, J. M. Moorby, and C. J. Newbold. 2012. Bacterial protein degradation by different rumen protozoal groups. J. Anim. Sci. 90:4495-4505.

Bierman, S., G. E. Erickson, T. J. Klopfenstein, R. A. Stock, and D. H. Shain. 1999. Evaluation of nitrogen and organic matter balance in the feedlot as affected by level and source of dietary fiber. J. Anim. Sci. 77:1645-1653.

Brito, A. F., and G. A. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. Journal of Dairy Science 90: 1816-1827.

Brito, A. F., G. F. Tremblay, H. Lapierre, A. Bertrand, Y. Castonguay, G. Bélanger, R. Michaund, C. Benchaar, D. R. Oullet, and R. Berthiaume. 2009. Alfalfa cut at sundown and harvested as baleage increases bacterial protein synthesis in late-lactation dairy cows. J. Dairy Sci. 92:1092-1107.

Brock, F. M., C. W. Forsberg, and J. G. Buchanan Smith. 1982. Proteolytic activity of rumen microorganisms and effects of proteinase inhibitors. Appl. Environ. Microbiol. 44:561-569.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64-75.

Broderick, G. A. 2003. Effects of Varying Dietary Protein and Energy Levels on the Production of Lactating Dairy Cows. J. Dairy Sci, 86, 1370–1381.

Broderick, G. A., J. H. Yang, and R. G. Koegel. 1993. Effect of steam heating alfalfa hay on utilization by lactating dairy cows. J. Dairy Sci. 76:165.

Broderick, G. A. "Forage protein utilization." Informational Conference with Dairy and Forage Industries. US Dairy Forage Research Center. Madison, WI. Vol. 65. 1996.

Broderick, G. A. Nutritional Strategies to Reduce Crude Protein in Dairy Diets. 2006. 21st Annual Southwest Nutrition & Management Conference , February 23-24, 2006 , Tempe, AZ

Broderick, G. A., P. Huhtanen, S. Ahvenjärvi, S. M. Reynal, and K. J. Shingfield. 2010. Quantifying ruminal N metabolism using the omasal sampling technique in cattle - A meta-analysis. J. Dairy Sci. 93:3216-3230.

Brown, A. N., Weiss, W. P., Firkins, J. L., and Daniels, K. M. 2014. Effects of Oscillating Crude Protein Content of Dairy Cow Diets. MSc (Animal Science) thesis, The Ohio State University.

Brown, D. R., F. C. Hinds, and R. M. Collins. 1995. Effect of supplementation frequency on diet digestion and nitrogen metabolism of growing lambs fed low-quality forage. Prof.Anim. Sci. 12:24-27.

Burgos, S. A., J. G. Fadel, and E. J. DePeters. 2007. Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: Relation of milk urea nitrogen to urine urea nitrogen excretion. J. Dairy Sci. 90:5499-5508.

Calsamiglia, S., Ferret, A., Reynolds, C. K., Kristensen, N. B., and van Vuuren, A. M. 2010. Strategies for optimizing nitrogen use by ruminants. Animal The Animal Consortium, 4(7), 1184–1196. https://doi.org/10.1017/S1751731110000911

Can, A., and A. Yilmaz. 2002. Usage of xylose or glucose as non-enzymatic browning agent for reducing ruminal protein degradation of soybean meal. Small Rumin. Res. 46:173-178.

Capper, J. L., Cady, R. A., and Bauman, D. E. 2009. The environmental impact of dairy production: 1944 compared with 2007. Journal of Animal Science. https://doi.org/10.2527/jas.2009-1781

Castillo, A. R., Kebreab, E., Beever, D. E., Barbi, J. H., Sutton, J. D., Kirby, H. C., and France, J. 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. Journal of Animal Science.

Castillo, A. R., Kebreab, E., Beever, D. E., and France, J. 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution . BioI. and Environ. Sci., BioI. Abstr., Chern. Abstr. Rev., Anim. Breed. Abstr., Dairy Sci. Abstr., Pig News Inform. Poultry Abstr. Vet. Bull. Ind. Vet., Field Crop Abstr., Herb. Abstr., Maize Abstr., Soya Bean Abstr, 9. Retrieved from http://ciuw.warman.net.pllalf/psjc.

Castro, Borucki S. I., Phillip, L. E., Lapierre, H., Jardon, P. W., and Berthiaume, R. 2007. Ruminal Degradability and Intestinal Digestibility of Protein and Amino Acids in Treated Soybean Meal Products. J. Dairy Sci. 90:810–822.

Cerbulis, J., Farrell, H.M., 1975. Composition of milks of dairy cattle. 1. Protein, lactose, and fat contents and distribution of protein fraction. J. Dairy Sci. 58, 817–827.

Chase, L. E., R. J. Higgs, and M. E. Van Amburgh. 2009. Feeding low crude protein rations to dairy cows-opportunities and challenges. Proceedings of the 2009 Cornell Nutr. Conf. for Feed Manufacturers. Syracuse, NY. Pp 220-226.

Chase, L. E. 2007. “How efficiently are your cows using ration nitrogen?” National Feed Management for Livestock and Poultry. Washington State University. https://puyallup.wsu.edu/lnm/publications.

Cheng, K.J., and R.J. Wallace. 1979. The Mechanism of Passage of Endogenous Urea Through the Rumen Wall and the Role of Ureolytic Epithelial Bacteria in the Urea Flux. Br. J. Nutr. 42:553–557. doi:10.1079/BJN19790147.

Cheng, K.-J., C. S. Stewart, D. Dinsdale, and J. W. Costerton. 1983/84. Electron microscopy of the bacteria involved in the digestion of plant cell walls. Anim. Feed Sci. Technol. 10:93.

Chibisa, G. E., and T. Mutsvangwa. 2013. Effects of feeding wheat or corn-wheat dried distillers grains with solubles in low-or high-crude protein diets on ruminal function, omasal nutrient flows, urea-N recycling, and performance in cows. J. Dairy Sci. 96:6550-6563.

Clark, J. H., H. R. Spires, and C. L Davis. 1978. Uptake and metabolism ofnitrogenous components by the lactating mammary gland. Fed. Proc. 37:1233–1238.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304–2323

Cleland, J. 2013. World Population Growth; Past, Present and Future. Environmental and Resource Economics, 55(4), 543–554. https://doi.org/10.1007/s10640-013-9675-6

Cole, N. A. 1999. Nitrogen retention by lambs fed oscillating dietary protein concentrations. Journal of Animal Science, 77(1), 215–222. https://doi.org/10.2527/1999.771215x

Cole, N. A. and R. W. Todd. 2008. Opportunities to enhance performance and efficiency through nutrient synchrony in concentrate-fed ruminants. J. Anim. Sci. 86: E318-E333.

Cole, N.A., L.W. Greene, F.T. McCollum, T. Montgomery, and K. McBride. 2003. Influence of oscillating dietary crude protein concentration on performance, acid-base balance, and nitrogen excretion of steers. J. Anim. Sci. 81:2660-2668.

Collins, R. M., and R. H. Pritchard. 1992. Alternate day supplementation of corn stalk diets with soybean meal or corn gluten meal fed to ruminants. J. Anim. Sci. 70:3899-3908.

Craig, W. M., G. A. Broderick, and D. B. Ricker. 1987. Quantitation of microorganisms associated with the particulate phase of ruminal ingesta. J. Nutr. 117:56.

Crutchfield, W. O. 1968. A technique for placement of an in-dwelling catheter in the cow. Vet. Med. Small Anim. Clin. 63:1141-1144.

Cunningham, K. D., M. J. Cecava, T. R. Johnson, and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79:620–630.

Czerkawski, J. W., and K.-J. Cheng. 1988. Compartmentation in the rumen. In: P. N. Hobson (Ed.) The Rumen Microbial Ecosystem. p 361. Elsevier Science Publishing, New York.

DePeters, E.J., and Cant, J.P., 1992. Nutritional factors influencing the nitrogen composition ofbovine milk: a review. J. Dairy Sci. 75, 2043–2070.

DePeters, E. J., and J. D. Ferguson. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. J. Dairy Sci. 75: 3192.

Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of Volatile Fatty Acids from the Rumen of Lactating Dairy Cows as Influenced by Volatile Fatty Acid Concentration, pH and Rumen Liquid Volume. Br. J. Nutr. 69:385. doi:10.1079/BJN19930041.

Dijkstra, J., J. France, and D. R. Davies. 1998a. Different mathemati- cal approaches to estimating microbial protein supply in rumi- nants. J. Dairy Sci. 81:3370–3384.

Doepel, L., and Lapierre, H. 2006. Challenges in Protein Nutrition for Dairy Cows Take Home Messages. WCDS Advances in Dairy Technology, 18, 57–67.

Doranalli, K., and T. Mutsvangwa. 2009. Nitrogen utilization in growing lambs fed oscillating dietary protein concentrations. Anim. Feed Sci. Technol. 152:33-41.

Doranalli, K., Penner, G. B., and Mutsvangwa, T. 2011. The Journal of Nutrition Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions. J. Nutr, 141, 560–567. https://doi.org/10.3945/jn.110.133876

Eisemann, J. H., and Tedeschi,O. L. 2016. Predicting the amount of urea nitrogen recycled and used for anabolism in growing cattle. Journal of Agricultural Science, 154, 1118-1129. doi:10.1017/S00218596616000228

Environment Canada. 2013. Canada's Emissions Trends. http://www.ec.gc.ca/gesghg/985F05FB-4744-4269-8C1A-D443F8A86814/1001Canada's%20Emissions%20Trends%202013_e.pdf. (Accessed March 29, 2016).

Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acids analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1776.

Fenton RA, A. Howorth, G. J. Cooper, R. Meccariello, I. D. Morris, and C. P. Smith. 2000. Molecular characterization of a novel UT-A urea transporter isoform (UT-A5) in testis. Am J Physiol Cell Physiol 279:C1425-1431

Food and Agriculture Organization of the United Nations (FAO). 2006. Livestock's Long Shadow. Environmental effects and options. Rome. http://www.virtualcentre.org. (Accessed Mar 21, 2016).

Ford, A. L., and L.P. Milligan. 1970. Tracer studies of urea recycling in sheep. Canadian J. Anim. Sci. 50:129-135.

France, J., and R. C. Siddons. 1986. Determination of digesta flow by continous marker infusion. J. Theor. Biol. 121:105–120.

Gozho, G. N., Hobin, M. R., and Mutsvangwa, T. 2007. Interactions Between Barley Grain Processing and Source of Supplemental Dietary Fat on Nitrogen Metabolism and Urea-Nitrogen Recycling in Dairy Cows. Journal of Dairy Science, 91(1), 247–259. https://doi.org/10.3168/jds.2007-0407

Guliński, P., Salamończyk, E., and Młynek, K. 2016. Improving nitrogen use efficiency of dairy cows in relation to urea in milk - A review. Animal Science Papers and Reports.

Hall, M. B., and G. B. Huntington. 2008. Nutrient synchrony: Sound in theory, elusive in practice. J. Anim. Sci. 86:E287-E292.

Hammond, A. C. 1997. Update on BUN and MUN as a guide for protein supplementation in cattle. In: Proc. Florida Ruminant Nutr. Symp., Univ. Florida, Gainesville. p. 43-52.

Harmeyer, J., and H. Martens. 1980. Aspects of urea metabolism in ruminants with reference to the goat. J. Dairy Sci. 63:1707-1728.

Hogan, J. P. 1961. The absorption of ammonia through the rumen of the sheep. Aust. J. Biol.

Sci. 14:448-460.

Houpt, T. R., and Houpt, K. A. 1968. Transfer of urea nitrogen across the rumen wall. Am. J. Physiol. 214, 1296–1303. doi: 10.1152/ajplegacy.1968.214.6.1296

Hristov, A. N., R. P. Etter, J. K. Ropp, and K. L. Grandeen. 2005. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Anim. Sci. 82:3219-3229.

Hristov, A. N., Hanigan, M., Cole, A., Todd, R., McAllister, T. A., Ndegwa, P. M. and Rotz, A. 2011. Review: Ammonia emissions from dairy farms and beef feedlots. Can. J. Anim. Sci. 91:1-35.

Huhtanen, P., Kaustell, K., Jaakkola S. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol., 48 (1994), pp. 211-227

Huntington, G. B. 1989. Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. Can. J. Anim. Sci. 69:215-223.

Huntington, G. B., and S. L. Archibeque. 2000. Practical aspects of urea and ammonia metabolism in ruminants. J. Anim. Sci. 78:742-749.

Huntington, G. B., E. J. Zetina, J. M. Whitt, and W. Potts. 1996. Effects of dietary concentrate level on nutrient absorption, liver metabolism, and urea kinetics of beef steers fed isonitrogenous and isoenergetic diets. J. Anim. Sci. 74:908-916.

Ipharraguerre, I. R., Clark, J. H., and Freeman, D. E. 2005. Varying Protein and Starch in the Diet of Dairy Cows. I. Effects on Ruminal Fermentation and Intestinal Supply of Nutrients. J. Dairy Sci, 88, 2537–2555. https://doi.org/10.3168/jds.S0022-0302(05)72931-3

Isozaki, T., A. G. Gillin, C. E. Swanson, and J. M. Sands. 1994. Protein restriction sequentially induces new urea transport processes in rat initial IMCD. Am. J. Physiol. Ren. Physiol. 266:F756-F761.

Jasim, U., K.Z. Haque, K. Jasimuddin, and K. Mohammad. 2015. Dynamics of Microbial Protein Synthesis in the Rumen - A Review.

Jonker, J. S., Kohn, R. A., and Erdman, R. A. 1999. Milk Urea Nitrogen Target Concentrations for Lactating Dairy Cows Fed According to National Research Council Recommendations 1. Journal of Dairy Science, 82(6), 1261–1273. https://doi.org/10.3168/jds.S0022-0302(99)75349-X

Kalscheur, K. F., Vandersall, J. H., Erdman, R. A., Kohn, R. A., and Russek-Cohen, E. (1999). Effects of Dietary Crude Protein Concentration and Degradability on Milk Production Responses of Early, Mid, and Late Lactation Dairy Cows. J Dairy Sci, 82, 545–554.

Kauffman, A.J., and N.R. St-Pierre. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. J. Dairy Sci. 84:2284-2294.

Kennedy, P. M. 1980.The effects of dietary sucrose and the concentration of plasma urea and rumen ammonia on the degradation of urea in the gastrointestinal tract of cattle. Br. J. Nutr. 43:125-140.

Kennedy, P. M., and L. P. Milligan. 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. Can. J. Anim. Sci. 60:205-221.

Khaleel, R., K. R. Reddy, and M. R. Overcash. 1981. Changes in soil physical properties due to organic waste applications: a review. J. Environ. Qual. 10:133-141.

Kim, K. H., Y. G. Oh, J. J. Choung, and D. G. Chamberlain. 1999. Effects of varying degrees of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in cattle consuming grass silage. J. Sci. Food Agric. 79:833-838.

Köhler, J. 2016. The influence of oscillating dietary crude protein concentrations on milk production and nitrogen utilization in lactating dairy cows. http://hdl.handle.net/10388/7292

Kohn, R. A., K. F. Kalscheur, and E. Russek-Cohen. 2002. Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen. J. Dairy Sci. 85:227–233.

Kolver, E., L. D. Muller, G. A. Varga, and T. J. Cassidy.1998. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. J Dairy Sci.81:2017-2028.

Langmeier, M., E. Frossard, M. Kreuzer, P. Mäder, D. Dubois, A. Oberson, S. Recous, and B. Nicolardot. 2002. Nitrogen fertilizer value of cattle manure applied on soils originating from organic and conventional farming systems. Agronomie 22:789-800.

Lapierre, H., and Lobley, G. E. 2001. Nitrogen Recycling in the Ruminant: A Review. Journal of Dairy Science, 84, E223–E236. https://doi.org/10.3168/jds.s0022-0302(01)70222-6

Lazzarini, I., Detmann, Edenio, Sampaio, Cláudia Batista, Paulino, Mário Fonseca, Valadares Filho, Sebastião de Campos, Souza, Marjorrie Augusto de, & Oliveira, Fabrício Albani. 2009. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. Revista Brasileira de Zootecnia, 38(10), 2021-2030. https://dx.doi.org/10.1590/S1516-35982009001000024

Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012a. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. J. Dairy Sci. 95:6042–6056.

Leng, R. A., and Nolan, J. V. 1984. Nitrogen Metabolism in the Rumen. Journal of Dairy Science, 67(5), 1072–1089. https://doi.org/10.3168/jds.s0022-0302(84)81409-5

Liu, K. 1999. Chemistry and nutritional value of soybean components. Pages 25–114 in Soybeans: Chemistry, Technology and Utiliza- tion. Monsanto, ed. Aspen Publishers, Gaithersburg, MD.

Liu, Q., C. Wang, G. Guo, W.J. Huo, Y.L. Zhang, C.X. Pei, S.L. Zhang, and H. Wang. 2018. Effects of Branched-Chain Volatile Fatty Acids Supplementation on Growth Performance, Ruminal Fermentation, Nutrient Digestibility, Hepatic Lipid Content and Gene Expression of Dairy Calves. Anim. Feed Sci. Technol. 237:27-34. Doi:10.1016/j.anifeedsci.2018.01.006.

López, S., F.D. Hovell, J. Dijkstra, and F. J. 2004. Effects of Volatile Fatty Acid Supply on

their Absorption and on Water Kinetics in the Rumen of Sheep Sustained by Intragastric Infusions. J Anim Sci.

Lu, Z., F. Stumpff, C. Deiner, J. Rosendahl, H. Braun, K. Abdoun, J. R. Aschenbach, and H. Martens. 2014. Modulation of sheep ruminal urea transport by ammonia and pH. Am. J. Regul. Integr. Comp. Physiol. 307:R558-R570.

Lu Z, Shen H and Shen Z. 2019. Effects of Dietary-SCFA on Microbial Protein Synthesis and Urinal Urea-N Excretion Are Related to Microbiota Diversity in Rumen. Front. Physiol. 10:1079. doi: 10.3389/fphys.2019.01079

Ludden, P. A., Wechter, T. L., and Hess, B. W. 2002b. Effects of oscillating dietary protein on ruminal fermentation and site and extent of nutrient digestion in sheep. Journal of Animal Science.

Ludden, P. A., T. L. Wechter, E. J. Scholljegerdes, and B. W. Hess. 2003. Effects of oscillating dietary protein on growth, efficiency, and serum metabolites in growing beef steers. Prof. Anim. Sci. 19:30-34.

Ludden, P.A., T.L. Wechter, and B.W. Hess. 2002. Effects of oscillating dietary protein on nutrient digestibility, nitrogen metabolism, and gastrointestinal organ mass in sheep. J. Anim. Sci. 80:3021-3026.

Marini, J. C., and M. S. Attene-Ramos. 2006. An improved analytical method for the determination of urea nitrogen isotopomers in biological samples utilizing continuous flow isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 20(24):3736-3740.

Marini, J. C., and M. E. Van Amburgh. 2003.Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81: 545-552.

Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. J. Anim. Sci. 82:1157-1164.

Martens, H., J. Kudritzki, K. Wolf, and M. Schweigel. 2001. No evidence for active peptide transport in forestomach epithelia of sheep. J Anim Physiol Anim Nutr (Berl) 85:314-324.

Marsh W. H., B. Fingerhut, and E. Kirsch. 1957. Determination of urea nitrogen with the diacetyl method and an automatic dialyzing apparatus. Am. J. Clin. Pathol. 28, 681-688.

McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. 43:99-109.

Murphy, J. J., O'Mara, F. 1993: Nutritional manipulation of milk protein concentration and its impact on the dairy industry. Livestock Production Science 35: 117-134.

Muscher, A. S., B. Schroder, G. Breves, and K. Huber 2010. Dietary nitrogen reduction enhances urea transport across goat rumen epithelium. J. animal Sci. 88:3390-3398.

Mutsvangwa, T., Davies, K. L., McKinnon, J. J., and Christensen, D. A. 2016. Effects of dietary crude protein and rumen-degradable protein concentrations on urea recycling, nitrogen balance, omasal nutrient flow, and milk production in dairy cows. Journal of Dairy Science, 99(8), 6298–6310. https://doi.org/10.3168/jds.2016-10917

Nagaraja, T. G. 2012. A Microbiologist ' s View on Improving Nutrient Utilization in Ruminants. Proc 23rd Annual Florida Ruminant Nutrition Symposium, 135–161. Retrieved from http://www.mendeley.com/research/microbiologist-s-view-improving-nutrient-utilization-ruminants-1/

Nennich, T. D. et al. 2006. Prediction and Evaluation of Urine and Urinary Nitrogen and Mineral Excretion from Dairy Cattle. Journal of dairy science 89: 353-364

Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship ruminal protein and carbohydrate availability to microbial protein synthesis and milk production. J. Dairy Sci. 71:2070.

Nocek, J. E., and S. Tamminga. 1991. Site of Digestion of Starch in the Gastrointestinal Tract of Dairy Cows and Its Effects on Milk Yield and Composition. J. Dairy Sci.

Nolan, J. V., and Stachiw, S. 1979. Fermentation and nitrogen dynamics in Merino sheep given a low-quality-roughage diet. British Journal of Nutrition, 42(1), 63–80. https://doi.org/10.1079/bjn19790090.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press. Washington, DC.

Obara, Y., D. Dellow, and J. Nolan. 1991. Effects of energy-rich supplements on nitrogen kinetics in ruminants. Page 551 in Physiological Aspects of Digestion and Metabolism in Rumi- nants. T. Tsuda, Y. Sasaki, and R. Kawashima, ed. Academic Press, San Diego, CA.

Olmos Colmenero, J. J., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704–1712.

Oltner, R., M. Emanuelson, and H. Wiktorsson. 1985. Urea concentrations in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. Livestock Prod. Sci. 12:47.

Owens, F.N., and W.G. Bergen. 1983. Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications. J. Anim. Sci. 57 Suppl 2:498-518.

Patra, A. K., and Aschenbach, J. R. 2018. Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. Journal of Advanced Research, 13, 39–50. https://doi.org/10.1016/j.jare.2018.02.005.

Penner, G.B., L.L. Guan, and M. Oba. 2009b. Effects of Feeding Fermenten on Ruminal Fermentation in Lactating Holstein Cows Fed Two Dietary Sugar Concentrations. J. Dairy Sci. 92:1725-1733. Doi:10.3168/jds.2008-1706.

Prins, R. A., D. L. vanRheenen, and A. T. van't Klooster. 1983. Characterization of microbial proteolytic enzymes in the rumen. J. Microbiol. Serol. 49: 585-595.

Rahn, T., and Wahlen, M. 1997. Stable isotope enrichment in stratospheric nitrous oxide. Science, 278(5344), 1776–1778. https://doi.org/10.1126/science.278.5344.1776.

Rémond, D., J.P. Chaise, E. Delval, and C. Poncet. 1993. Net transfer of urea and ammonia

across the ruminal wall of sheep. J. Anim. Sci. 71: 2785-2792.

Rémond, D., L. Bernard, I. Savary-Auzeloux, and P. Noziere. 2009. Partitioning of nutrient net fluxes across the portal-drained viscera in sheep fed twice daily: effect of dietary protein degradability. Br. J. Nutr. 102:370-381. Doi:10.1017/S0007114508199470.

Reynal, S. M., and Broderick, G. A. 2005. Effect of Dietary Level of Rumen-Degraded Protein on Production and Nitrogen Metabolism in Lactating Dairy Cows. Journal of Dairy Science, 88(11), 4045–4064. https://doi.org/10.3168/jds.s0022-0302(05)73090-3.

Reynolds, C. K. 1992. Metabolism of nitrogenous compounds by ruminant liver. J. Nutr. 122:850.

Reynolds, C. K., Crompton, L. A., Mills, J. A. N., Barratt, C. E. S., and Barber, D. G. 2013. " Feed efficiency in Dairy Cattle " Improving the Efficiency of Feed Nitrogen Utilization. International Dairy Nutrition Symposium Wageningen, 21.

Reynolds, C. K., and Kristensen, N. B. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. Journal of Animal Science, 86(14 Suppl), 293–305. https://doi.org/10.2527/jas.2007-0475.

Ritzhaupt, A., G. Breves, B. Schroder, C. G. Winckler, and S. P. Shirazi-Beechey. 1997. Urea transport in gastrointestinal tract of ruminants: effect of dietary nitrogen. Biochem. Soc. Trans. 25:S490 (Abstr.).

Rojen, B. A., Poulsen, S. B., Theil, P. K., Fenton, R. A., and Kristensen, N. B. 2011. Short communication: Effects of dietary nitrogen concentration on messenger RNA expression and protein abundance of urea transporter-B and aquaporins in ruminal papillae from lactating Holstein cows. J. Dairy Sci. 94, 2587-2591.

Roseler, D. K., J. D. Ferguson, C. J. Sniffen, an J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525-534.

Ruska, D., and D. Jonkus. 2014. Crude Protein and Non-Protein Nitrogen Content in Dairy Cow Milk. Proc. Latv. Univ. Agric. 32:4–8. doi:10.2478/plua-2014-0011.

Russell, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effect of carbohy- drate limitation on degradation and utilization ofcasein by mixed rumen bacteria. J. Dairy Sci. 66:763–775.

Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70:3551-3561.

Sairanen, A., H. Khalili, J. I. Nousiainen, S. Ahvenjarvi, and P. Huhtanen. 2005. The effect of concentrate supplementation on nutrient flow to the omasum in dairy cows receiving freshly cut grass. J. Dairy Sci. 88(4):1443-1453.

SAS Institute. 2004. SAS/STAT 9.4 User’s Guide. SAS Institute Inc., Cary, NC

Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr. 32: 199-208.

Schwab, Charles G., Broderick, Glen A. 2017. A 100-Year Review: Protein and amino acid

nutrition in dairy cows. J. Dairy Sci. 100:10094–10112 https://doi.org/10.3168/jds.2017-13320

Siddons, R. C., Paradine, J., Gale, D. L., and Evans, R. T. 1985. Estimation of the degradability of dietary protein in the sheep rumen by in vivo and in vitro procedures. British Journal of Nutrition, 54(2), 545–561. https://doi.org/10.1079/bjn19850139

Sinclair, L. A., Garnsworth, P. C., Newbold, J. R., and Buttery, P. J. 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 120, 251–263. doi: 10.1017/s002185960007430x

Smith, R. H. 1989. Nitrogen metabolism in the ruminant stomach. In: H.D. Block, B.O. Eggum, A.G. Low, O. Simon, and T. Zebrowska (ed.) Protein Metabolism in Farm Animals: Evaluation, Digestion, Absorption, and Metabolism. p.165. Oxford University Press, New York, NY.

Smith CP, W. S. Lee, S. Martial, M. A. Knepper, G. You, J. M. Sands, and M. A. Hediger. 1995. Cloning and regulation of expression of the rat kidney urea transporter (rUT2). J Clin Invest 96:1556-1563.

Smith, C.P., E. A. Potter, R. A. Fenton, and G. S. Stewart. 2004. Characterization of a human colonic cDNA encoding a structurally novel urea transporter, hUT-A6. Am J Physiol Cell Physiol 287:C1087-1093.

Spek, J. W., J. Dijkstra, G. van Duinkerken, W. H. Hendriks, and A. Bannink. 2013. Prediction of urinary nitrogen and urinary urea ni- trogen excretion by lactating dairy cattle in northwestern Europe and North America: A meta-analysis. J. Dairy Sci. 96:4310–4322.

Spiehs, M. J., B. L. Woodbury, D. D. Tarkalson, B. J. Wienhold, and R. A. Eigenberg. 2010. Long term effects of annual additions of animal manure on soil chemical, physical, and biological properties in the Great Plains. International Symposium on Air Quality and Manure Management for Agriculture, CD-Rom Proceedings of the 13-16 September 2010 Conference. ASABE Publication Number 711P0510cd

Stewart, G. S. 2005. UT-B is expressed in bovine rumen: potential role in ruminal urea transport. AJP: Regulatory, Integrative and Comparative Physiology, 289(2), R605–R612. https://doi.org/10.1152/ajpregu.00127.2005

Stewart, Gavin S., and Smith, C. P. 2005. Urea nitrogen salvage mechanisms and their relevance to ruminants, non-ruminants and man. Nutrition Research Reviews, 18(1), 49–62. https://doi.org/10.1079/nrr200498

Storm, E., and E.R. Ørskov. 1983. The Nutritive Value of Rumen Micro-Organisms in Ruminants. Br. J. Nutr. 50:463. Doi:10.1079/BJN19830114.

T. A. McAllister, H. D. Bae", G. A. Jones., and K.-J. C. 1994. Microbial attachment and feed digestion in the rumen. Journal of Animal Science, 72(11), 3004–3018. https://doi.org/10.2527/1994.72113004x

Tamminga, S. 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49:1615 -1630.

Tamminga, S. 1992. Nutrition Management of Dairy Cows as a Contribution to Pollution

Control. Journal of Dairy Science, 75, 345–357. https://doi.org/10.3168/jds.S0022-0302(92)77770-4

Theurer, C. B., G. B. Huntington, J. T. Huber, R. S. Swingle, and J. A. Moore. 2002. Net absorption and utilization of nitrogenous compounds across ruminal, intestinal, and hepatic tissues of growing beef steers fed dry-rolled or steamflaked sorghum grain. J. Anim. Sci. 80(2):525-532.

Thomas, P. C. 1980. Influence of nutrition on the yield and content of protein in milk. Dietary protein and energy supply. Int. Dairy Fed. Bull. Doc. 125:142.

Thomas, P.C. and P.A. Martin. 1988. The influence of nutrient balance on milk yield and composition. In: Nutrition and Lactation in the Dairy Cow. Ed. P.C. Garnsworthy. Butterworths, London, p. 97.

Tilman, D., Balzer, C., Hill, J., Befort, B. L. 2011. Global food demand and the sustainable intensification of agriculture. PNAS. Vol 108. 50:20260-20264.

Udén, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta: Rate of passage studies. J. Sci. Food Agric. 31:625-632.

United Nations. 2004World population to 2300. New York, NY: United Nations, Population Division.

United Nations, Department of Economic and Social Affairs, Population Division 2017. World Population Prospects: The 2017 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP/248.

United Nations, Department of Economic and Social Affairs, Population Division 2019. World Population Prospects 2019: Highlights (ST/ESA/SER.A/423).

Van Gylswyk, N. O. 1990. Enumeration and presumptive identification of some functional groups of bacteria in the rumen of dairy cows fed silage-based diets. FEMS Microbiol. Ecol. 73:243–254

Van Horn, H. H., G. L. Newton, and W. E. Kunkle. 1996. Ruminant nutrition from an environmental perspective: factors affecting whole-farm nutrient balance. J. Anim. Sci. 74:3082-3102.

VandeHaar, M. J., and N. St-Pierre. 2006. Major advances in nutrition: Relevance to the sustainability of the dairy industry. J. Dairy Sci. 89:1280-1291.

VanSoest, P.J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca, NY. 496 pp.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3593-3597.

Varel, V.H., J. a Nienaber, and H.C. Freetly. 1999. Conservation of Nitrogen in Cattle Feedlot Waste with Urease Inhibitors. J. Anim. Sci. 77:1162–1168.

Vergé, X. P. C., Dyer, J. A., Desjardins, R. L., and Worth, D. 2009. Long-term trends in greenhouse gas emissions from the Canadian poultry industry. Journal of Applied Poultry

Research, 18(2), 210–222. https://doi.org/10.3382/japr.2008-00091

Vergé, X. P. C., Maxime, D., Dyer, J. A., Desjardins, R. L., Arcand, Y, and Vanderzaag, A. 2013. Carbon footprint of Canadian dairy products: Calculations and issues. J. Dairy Sci. 96 :6091–6104. http://dx.doi.org/ 10.3168/jds.2013-6563

Vicente, F., A. Sarraseca, A. de Vega, and J. A. Guada. 2004. Performance of several Cr and Yb analytical techniques applied to samples of different biological origin (digesta or faeces). J. Sci. Food Agric. 84:2035-2040.

Wang S, Giller K, Kreuzer M, Ulbrich SE, Braun U and Schwarm A 2017 Contribution of Ruminal Fungi, Archaea, Protozoa, and Bacteria to the Methane Suppression Caused by Oilseed Supplemented Diets. Front. Microbiol. 8:1864. doi: 10.3389/fmicb.2017.01864.

Walker, N.D., Newbold, C.J., Wallace, R.J. 2005. Nitrogen metabolism in the rumen. A.N. Hristov, E. Pfeffer (Eds.), Nitrogen and Phosphorus Nutrition of Cattle and Environment, CAB International, Wallingford, UK (2005), pp. 71-115

Wallace, R. 1996. The proteolytic systems of ruminal microorganisms. CrossRef Listing Of Deleted DOIs, 45(Suppl. 1), 301–308. https://doi.org/10.1051/rnd:19960653

Wallace, R. J., and Joblin, K. N. 1985. Proteolytic activity of a rumen anaerobic fungus. FEMS Microbiology Letters, 29(1–2), 19–25. https://doi.org/10.1111/j.1574-6968.1985.tb00828.x

Wallace, R. J. 1997. Peptide metabolism and its efficiency in ruminant production. In Rumen Microbes and Digestive Physiology in Rumi- nants (Onodera, R. et al., eds.), pp 95–105. Japan Sci. Soc. Press, Tokyo/S Karger, Basel

Walpole, M., B. Schurmann, P. Górka, G. Penner, M. Loewen, and T. Mutsvangwa. 2015. Serosalto-Mucosal Urea Flux across the Isolated Ruminal Epithelium is Mediated via Urea Transporter-B and Aquaporins when Holstein Calves are Abruptly Changed to a Moderately Fermentable Diet. J. Dairy Sci. 98:1204-1213. Doi:10.3168/Jds.2014-8757.

Whichard, D. 2001. Nitrogen Removal From Dairy Manure Wastewater Using Sequencing Batch Reactors. Blacksburg, VA. PDF-Masters Thesis. http://hdl.handle.net/10919/34412.

Zhao, K., Liu, W., Lin, X.Y., Hu, Z.Y., Yan, Z.G., Wang, Y., Shi, K.R., Liu, G.M., Wang, Z.H. 2019. Effects of rumen-protected methionine and other essential amino acid supplementation on milk and milk component yields in lactating Holstein cows. 102(9):7936-7947. doi: 10.3168/jds.2018-15703. Epub 2019.