Impact of Cold Acclimatization on Nutrient Utilization and Enteric Methane Emissions of Beef Cows Overwintered on Low-Quality Forage Diets Supplemented with Dried Distillers Grain with Solubles

By

Jennilee Nicole Bernier

A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Animal Science

University of Manitoba

Winnipeg, Manitoba, Canada

ABSTRACT

This study was conducted to determine if nutrient utilization and enteric methane (CH_4) emissions could be improved in overwintering beef cows consuming low-quality forage supplemented with protein in the form of dried distillers grain with solubles (DDGS) in thermal-neutral and cold-stressed environments. Thirty mature, dry and non-pregnant beef cows were divided into three treatment groups and fed diets consisting of low-quality (6.0% crude protein; CP) forage with no DDGS (control, CON), 10% DDGS (borderline sufficient CP, 8.7% CP), or 20% DDGS (excess CP, 11.6% CP). Cold acclimatization did not appear to affect nutrient intake and digestibility by beef cows, but increased N and P excretion by 1.2x and 2.5x, respectively. Cold acclimatized cows reduced energy excretion by 26.8% (7.1 vs. 5.2 ± 0.30% GEI in fall and winter, respectively; $P < 0.0001$) in accordance with a 33.8% increase in rumen fluid rate of passage (ROP). Supplementation with DDGS improved digestibility of N and P (40.6 vs. 61.2 ± 2.45% N and -23.9 vs. 5.7 ± 5.95% P for CON and 20%DDGS, respectively; $P < 0.0001$) by increasing digestible substrate in the diet. Protein supplementation increased rumen NH_3-N concentrations (1.5, 2.1 and 3.1 ± 0.15 mg 100 mL^{-1}; $P < 0.0001$) enough to increase rumen fermentation efficiency, resulting in 18.5% lower enteric CH_4 emissions when CP was fed in excess of animal requirements. Total excretion of N and P were increased two- and 45-fold, respectively, when excess CP was fed. Reduced enteric CH_4 emissions as a result of cold acclimatization suggest an advantage for the Canadian beef herd in terms of environmental sustainability. Supplementing CP in excess of cow requirements may improve nutrient utilization and rumen fermentation efficiency, and

mitigate enteric CH_4 emissions in beef cows fed low-quality forage diets, but may also contribute to greater N and P loading of soil and ground water.

Keywords: dried distillers grain with solubles, low-quality forage, nutrient utilization, protein, digestibility, fermentation efficiency, cold acclimatization, methane, beef cow

ACKNOWLEDGEMENTS

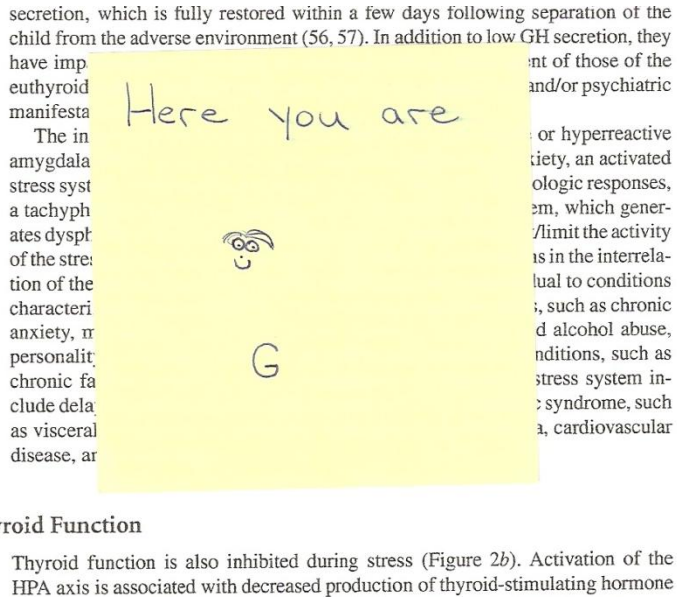
I extend a warm thank you to my mentor, Dr. Kim Ominski, for her endless advice, support, encouragement and guidance throughout the course of my graduate program. I thank Dr. Karin Wittenberg for her advice and guidance throughout, and Dr. Kees Plaizier for his advice in research design, sampling and mathematical calculations, and for serving on my committee. Dr. Kevin Campbell is gratefully acknowledged for his expertise in cold acclimatization, helpful comments and for sitting on my committee.

This research would not have been possible without the financial support from: Manitoba Beef Producers; Beef Cattle Research Council; Manitoba Rural Adaptation Council; GreenCover; Manitoba Sustainable Agriculture Practices Program; Agri-Food Research and Development Initiative.

I am indebted to technician Janice Haines for her support, encouragement, dedication and expertise in every aspect of laboratory analysis and repair of methane collection systems. I thank technicians Terri Garner, Jason Neufeld, Karen Lim and Prakash Sharma for their assistance with field and lab work. I am grateful to Colleen Wilson, M.Sc. for her advice in methane collection research and assistance with field work. Thank you to Drs. Loreen Onischuk and Gary Crow for their statistical expertise.

I thank my boyfriend, Sebastian, whose patience and support has aided the completion of this thesis in countless ways. And last, but certainly not least, a special thank you goes to my parents for their endless support and encouragement throughout the years of my education, for inspiring my love for beef cattle and agriculture, and for teaching me that with hard work and determination anything can be accomplished.

This thesis is dedicated in loving memory to my dear friend, Gary Rent, who was unable to complete his own thesis as his life was taken far too soon. His passion for the topic of cold stress was enlightening and inspiring, and his friendship and smile will be forever cherished and missed.



Thyroid Function

Thyroid function is also inhibited during stress (Figure 2*b*). Activation of the HPA axis is associated with decreased production of thyroid-stimulating hormone

TABLE OF CONTENTS

LIST OF TABLES.....xi

LIST OF FIGURES.....xiii

ABBREVIATIONS.....xv

1. INTRODUCTION.....1

2. LITERATURE REVIEW.....3

2.1. THE CURRENT STATE OF CANADIAN CATTLE PRODUCTION SYSTEMS.....3

2.1.1. Forage-based overwintering strategies in Western Canada.....3

2.1.2. Wheat-based DDGS as a protein supplement for beef cow diets.....4

2.2. ENVIRONMENTAL IMPACT OF OVERWINTERING BEEF CATTLE IN WESTERN CANADA.....5

2.2.1. Greenhouse gas emissions and global warming.....5

2.2.2. Livestock enteric methane production.....5

2.2.2.1. Global emission estimates.....5

2.2.2.2. Canadian emission estimates.....6

2.2.2.3. Canadian emission estimates for mature beef cows.....7

2.2.3. Methane production from rumen microbial fermentation.....9

2.2.3.1. Rumen methanogenic bacteria.....9

2.2.3.2. Substrates and energetics of methanogenesis.....10

2.2.3.3. Factors which impact methanogenesis.....13

2.2.3.3.1. Type and quality of feed.....13

2.2.3.3.2. Level of feed intake and feeding frequency.....14

2.2.3.3.3. Rate of passage of rumen digesta.....16

2.2.3.3.4. Effect of protein (nitrogen) supplementation.....18

2.2.4. Nutrient excretion.....19

2.3. PROTEIN NUTRITION AND METABOLISM IN CATTLE.....21

2.3.1. Definition of protein deficiency.....21

2.3.2. Rumen ammonia nitrogen as an indicator of nitrogen status.....23

2.3.3. Serum urea nitrogen as an indicator of nitrogen status.....24

2.3.3.1. Relationship between serum urea nitrogen and urine and fecal nitrogen excretion.....25

2.3.4. Effect of protein deficiency on microbial fermentation.....28

2.3.4.1. Volatile fatty acids as energetic indicators of microbial protein synthesis.....29

2.3.4.2. Purine derivatives as indicators of microbial protein synthesis....30

2.3.5. Effect of protein supplementation on dry matter intake.....33

2.3.6. Effect of protein supplementation on nutrient digestibility.....34

2.4. CATTLE RESPONSE TO COLD ACCLIMATIZATION.....36

2.4.1. Effect of cold on thyroid hormone concentrations.....36

2.4.2. Effect of cold on dry matter intake.....38

2.4.3. Effect of cold on microbial fermentation.....39

2.4.4. Effect of cold on the rate of passage of rumen digesta.....40

2.4.5. Effect of cold on nutrient utilization.....41

2.4.5.1. Effect of cold on nutrient digestibility.....42

2.4.5.2. Effect of cold on nutrient excretion and balance.....45

2.4.5.3. Effect of cold on blood urea nitrogen concentrations.....46

2.5. SUMMARY.....47

3. RESEARCH HYPOTHESES AND OBJECTIVES.....49

3.1. Hypotheses.....49

3.2. Objectives.....51

4. MANUSCRIPT.....52

4.1. INTRODUCTION.....53

4.2. MATERIALS AND METHODS.....56

4.2.1. Feeding Strategy and Animal Management.....56

4.2.2. Feed Samples and Analysis.....60

4.2.3. Blood Sampling and Analysis.....61

4.2.4. Enteric Methane Emissions.....62

4.2.4.1. Methane and SF_6 Analysis.....62

4.2.5. Rumen Fluid Sampling and Analyses.....64

4.2.6. Particulate and Fluid Rate of Passage.....65

4.2.7. Fecal and Urine Sampling and Analyses.....67

4.2.8. Statistical Analysis.....68

4.3. RESULTS AND DISCUSSION.....71

4.3.1. Nutrient Utilization.....71

4.3.1.1. Impact of Cold Acclimatization on Nutrient Intake.....71

4.3.1.2. Impact of Protein Supplementation on Nutrient Intake.....83

4.3.1.3. Influence of Cold Acclimatization on N and P Recycling and Digestibility.....92

4.3.1.4. Influence of Protein Supplementation on N and P Recycling and Digestibility.....106

4.3.2. Rumen Fermentation Efficiency.....113

4.3.2.1. Influence of Cold Acclimatization on Rumen Fermentation Efficiency.....113

4.3.2.2. Influence of Protein Supplementation on Rumen Fermentation Efficiency.....118

4.3.3. Nutrient Excretion.....121

4.3.3.1. Influence of Cold Acclimatization on Nutrient Excretion.....121

4.3.3.2. Influence of Protein Supplementation on Nutrient Excretion.....134

4.4. CONCLUSIONS.....141

5. GENERAL DISCUSSION.....143

5.1. Cold Acclimatization and DMI.....143

5.2. Protein Supplementation and DMI.....144

5.3. Cold Acclimatization and N and P Recycling and Digestibility.....145

5.4. Protein Supplementation and N and P Recycling and Digestibility.....147

5.5. Cold Acclimatization and Fermentation Efficiency.....148

5.6. Protein Supplementation and Fermentation Efficiency.....150

5.7. Cold Acclimatization and Nutrient Excretion.....153

5.8. Protein Supplementation and Nutrient Excretion.....154

5.9. Wheat-based DDGS in Overwintering Beef Cow Nutrition.....157

5.10. Future Research.....158

6. CONCLUSIONS AND RECOMMENDATIONS.....160

7. REFERENCES.....165

8. APPENDIX.....203

Appendix 4. Calculations for Determination of CO_2 Equivalents and Net GHG Emissions from Total Nitrogen Excretion and Enteric CH_4 Emissions.....206

LIST OF TABLES

Table 1. Methanogen species present in ruminant livestock.....11

Table 2. Actual ingredient and nutrient composition (DM basis) of TMR fed to cows in thermal-neutral and cold-acclimatized periods.....57

Table 3. Dry matter intake, available CP intake, energy intake (DM basis), serum urea nitrogen and enteric methane production values of feedlot cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....73

Table 4. Nitrogen parameters of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20%DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....74

Table 5. Phosphorus parameters and body weight loss of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....75

Table 6. Particulate and fluid rate of passage of cows consuming low-quality forage (CON), and low-quality forage supplemented with 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....80

Table 7. Rumen pH, ammonia-N and volatile fatty acid concentrations of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....87

Table 8. Comparison of thyroid hormone concentrations between outdoor (feedlot) and indoor (metabolism unit) housing of cows in thermal-neutral (fall) and cold-stressed (winter) environments.....101

Table 9. Comparison of thyroid hormone concentrations between seasons in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in outdoor (feedlot) or indoor (metabolism unit) housing.....111

Table 10. Urine excretion and purine derivatives of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....116

Appendix 1. Trace Mineralized Stock Salt Guaranteed Analysis.....203

Appendix 2. Biofos/Dicalcium Erco Phosphate (Fine) Guaranteed Analysis.....204

Appendix 3. Forage Analysis Energy Calculations, DM Basis.....205

LIST OF FIGURES

Figure 1. Timeline of study.....59

Figure 2. Diet by season interaction for restricted-fed available nitrogen intake in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....76

Figure 3. Diet by season interaction for restricted-fed phosphorus intake in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....77

Figure 4. Diet by season interaction for serum urea nitrogen in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....93

Figure 5. Diet by season interaction for urine excretion (% BW) in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....94

Figure 6. Diet by season interaction for apparent nitrogen digestibility in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....98

Figure 7. Diet by season interaction for urine nitrogen concentration in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....123

Figure 8. Diet by season interaction for urine nitrogen excretion in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....124

Figure 9. Diet by season interaction for nitrogen retention in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....126

Figure 10. Diet by season interaction for phosphorus retention in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.....129

ABBREVIATIONS

ACP	=	Available crude protein
ACPI	=	Available crude protein intake
ADF	=	Acid detergent fibre
ADI-CP	=	Acid detergent insoluble crude protein
AOAC	=	Association of Official Analytical Chemists
A:P	=	Acetate:propionate
BCS	=	Body condition score
BW	=	Body weight
BUN	=	Blood urea nitrogen
Ca	=	Calcium
CH_4	=	Methane
Co	=	Cobalt
CO_2	=	Carbon dioxide
CP	=	Crude protein
Cr	=	Chromium
Cr_2O_3	=	Chromic oxide
d	=	day
DDGS	=	Dried distillers grain with solubles
DE	=	Digestible energy
DIP	=	Digestible intake protein
DM	=	Dry matter
DMI	=	Dry matter intake
EDTA	=	Ethylenediaminetetraacetic acid
GE	=	Gross energy
GEI	=	Gross energy intake
GIT	=	Gastrointestinal tract
GWP	=	Global warming potential
h	=	hour
hd	=	head
IPCC	=	International Panel on Climate Change
K	=	Potassium
ME	=	Metabolizable energy
Mcal	=	Megacalorie
Mg	=	Magnesium
mmHg	=	Millimetre of mercury (Torr)
MP	=	Microbial protein
N	=	Nitrogen

NDF	=	Neutral detergent fibre
NH_3-N	=	Ammonia nitrogen
NE_m	=	Net energy for maintenance
P	=	Phosphorus
PD	=	Purine derivatives
PUN	=	Plasma urea nitrogen
RDP	=	Rumen degradable protein
RIA	=	Radioimmunoassay
ROP	=	Rate of passage
SEM	=	Standard error of the mean
SF_6	=	Sulphur hexafluoride
SUN	=	Serum urea nitrogen
T_3	=	Triiodothyronine
T_4	=	Thyroxine
TDN	=	Total digestible nutrients
TH	=	Thyroid hormone
TMR	=	Total mixed ration
VFA	=	Volatile fatty acids
wk	=	week
yr	=	year

1. INTRODUCTION

The Canadian beef cow herd is exposed to long periods of cold temperatures and a range of forage qualities in the winter months. Cold-acclimatized beef cattle are believed to improve dry matter intake (DMI), nutrient utilization and microbial fermentation, thereby reducing enteric methane (CH_4) production, as compared to thermal-neutral cattle. Further, overwintering diets for beef cows commonly consist of low-quality forages with an inadequate nutrient profile to meet animal nutrient requirements. If dietary CP is the most limiting factor in beef cow diets, a CP deficiency can cause reductions in rumen microbial growth and function, which reduces DMI, dietary digestion and microbial fermentation, and ultimately results in reduced nutrient utilization. Protein supplements can be included in low-quality overwintering diets to ensure CP requirements are met and avoid BW, body condition and/or reproductive losses. Protein supplementation of low-quality forages requires examination, however, as potential improvements in nutrient utilization may be offset by increased nutrient excretion and a greater risk of nutrient loading in the soil and ground water.

Dried distiller's grain with solubles is an inexpensive, high protein by-product of ethanol processing. Across western Canada, wheat-based DDGS is a relatively new protein supplement that is accessible to beef cattle producers. However, little is known about the role of wheat-based DDGS as a protein supplement with low-quality forages, and as such, its effectiveness as a protein supplement with low-quality forage-based overwintering diets requires evaluation.

In recent years, more stringent nutrient management legislation has made it necessary for the Canadian beef cattle sector to explore low-cost strategies to improve the environmental sustainability of beef cattle production, including methods to reduce nutrient loading of soil and ground water, and mitigate enteric CH_4 production. However, few studies have been conducted, particularly with beef cows, to evaluate the impact of cold acclimatization and protein supplementation on nutrient utilization and CH_4 emissions from Canadian beef cattle, which spend a large portion of each year in sub-zero temperatures.

This study was conducted to evaluate nutrient utilization and enteric CH_4 production in beef cows overwintered on low-quality forage supplemented with protein (DDGS). Parameters examined included DMI and nutrient intake, N and P digestibility, fermentation efficiency, and nutrient excretion, including N, P and enteric CH_4 emissions.

2. LITERATURE REVIEW

2.1. THE CURRENT STATE OF CANADIAN CATTLE PRODUCTION SYSTEMS

2.1.1. Forage-based overwintering strategies in Western Canada

During the winter months, beef cows in Western Canada are exposed to long periods of cold, which may be defined as temperatures lower than -7.8°C (Marston et al., 1998). As a consequence of the use of extended grazing strategies, forage quality may not be adequate (i.e. $\leq$ 6% CP) to meet animal CP requirements which range from 6.8 to 8.9% CP (DM basis) in thermal-neutral, mature, dry beef cows weighing 636 kg from 8 to 12 months post-partum (National Research Council (NRC), 1996). Cattle that are CP deficient can experience inefficient rumen fermentation and, in turn, inefficient utilization of dietary nutrients if CP is the most limiting factor for rumen microbes (Maeng et al., 1976; Köster et al., 1996). Exposure to prolonged cold or cold acclimatization, defined as the return of the animal to normal (thermal-neutral) physiological function during prolonged cold exposure, or as a reduced susceptibility to cold exposure after being previously exposed to cold temperatures (Sykes et al., 1969), has been shown to decrease the efficiency of nutrient utilization (Kennedy et al., 1982). Further, it has been suggested to decrease enteric methane (CH_4) emissions (Christopherson, 1976; Kennedy et al., 1977). It is possible that supplementing typical Western Canadian forage-based diets with protein supplements may serve to improve performance (Funston et al., 2008; Larson et al., 2009) and reduce enteric CH_4 emissions (Johnson and Johnson, 1995). Identification of environmentally sustainable, low-cost production practices in

overwintering cattle is critical to the future of the cattle industry. Therefore, further investigation is required before protein supplementation of low-quality forage diets in cold-acclimatized cattle can be identified as beneficial in terms of the economic and environmental sustainability of the cattle industry.

2.1.2. Wheat-based DDGS as a protein supplement for beef cow diets

Expansion of a wheat-based ethanol industry in Canada has led to the production of dried distillers grain with solubles (DDGS), a by-product of ethanol production. This feedstuff may play an important role as a protein supplement in beef cow diets. Dried distillers grain with solubles contains an appealing nutrient profile, as it has high CP and energy content comparable to or higher than other popular protein and energy supplements such as soybean meal, cereal grains or corn, which allows it to replace these traditional supplements in cattle diets (Leupp et al., 2009). Further, it is a great source of readily digestible, non-forage fiber (Ham et al., 1994). Typically with feedlot cattle, DDGS acts as a protein source when supplemented at 6 to 15% of diet DM, and as an energy source when fed at greater proportions (Klopfenstein, 2001). Past research has proven that fermentation of the relatively available fibre in distillers co-products, such as DDGS, results in dramatically (33%) lower methane emissions than feedstuffs with similar digestibility (Johnson and Johnson, 1995). Similarly, Wainman et al. (1984) reported that fermentation of the highly available fibre in co-products like DDGS results in 33 to 50% lower CH_4 production than normally generated by other feedstuffs with similar digestibility. However, little is known about net greenhouse gas (GHG)

emissions and the efficiency of nutrient utilization in overwintered beef cattle that are exposed to cold temperatures and supplemented with wheat-based DDGS.

2.2. ENVIRONMENTAL IMPACT OF OVERWINTERING BEEF CATTLE IN WESTERN CANADA

2.2.1. Greenhouse gas emissions and global warming

Global warming occurs from the increase in atmospheric temperature as a result of increasing concentrations of GHGs, which trap infrared radiation in the Earth's atmosphere. From 1906 to 2005, the Earth's surface temperature increased by 0.74 ± 0.18 °C and is predicted to climb by an additional 1.1 to 6.4 °C during the 21st century (Intergovernmental Panel on Climate Change (IPCC), 2007c). The major GHGs include carbon dioxide (CO_2), CH_4 and nitrous oxide (N_2O). Carbon dioxide is the largest source of GHGs and is therefore assigned a global warming potential (GWP) of one. Methane, which is produced by both natural and anthropogenic sources, is the second most significant GHG with a GWP of 25, indicating that CH_4 has a heat absorbing ability 25 times greater than CO_2 (IPCC, 2007b). Further, CH_4 has an atmospheric lifespan of 12 years before it is converted to CO_2 (IPCC, 2007b).

2.2.2. Livestock enteric methane production

2.2.2.1. Global emission estimates

Global greenhouse gas emissions increased by 70% from 1970 to 2004, and by 24% from 1990 to 2004, with 14% of global GHG emissions originating from agriculture (IPCC, 2007d). Global CH_4 emissions alone have risen by 40% since 1970, mainly due

to an 85% increase in the use of fossil fuels (IPCC, 2007d). Agriculture is considered the greatest source of CH_4 emissions, generating about 50% of global anthropogenic CH_4 emissions in 2005 (IPCC, 2007d). Agricultural CH_4 emissions are projected to increase by approximately 60% until 2030; potentially doubling by the end of the 21st century due to increased global consumption of meat and dairy products driving expansion of livestock herds worldwide (IPCC, 2007a). Global livestock enteric CH_4 emissions alone are projected to increase by 21% between 2005 and 2020 (IPCC, 2007a).

2.2.2.2. Canadian emission estimates

The National Greenhouse Gas Inventory Report by Environment Canada (2010) indicates that agricultural emissions accounted for 62 Mt CO_2 eq or 8.5% of total 2008 national GHG emissions, of which 13% were attributed to CH_4. In 2008, national enteric CH_4 emissions contributed 56%, equivalent to 22 Mt CO_2 eq, of total animal GHG emissions in Canada. Further, enteric fermentation accounted for 3.0% of Canadian GHG emissions, 35% of agricultural GHG emissions, 22% of total national CH_4 emissions and 88% of CH_4 emissions from agriculture (Environment Canada, 2010). Enteric CH_4 emissions from all ruminant livestock in Canada increased by 29% between 1990 and 2008, with enteric emissions from beef cattle increasing by 36% (from 14 to 19 Mt CO_2 eq) during that time frame. This increase was attributed primarily to a growth in cattle population, but also to a 26% increase in animal body weight since 1990, resulting in higher DMI per head and corresponding greater enteric fermentation (Environment Canada, 2010). However, from 2007 to 2008 enteric CH_4 emissions from all ruminant

livestock decreased by 4.3% or 1 Mt CO_2 eq, with no change in emissions from beef cattle.

2.2.2.3. Canadian emission estimates for mature beef cows

As of January 2011, Canada's total cattle population was approximately 12.5 million head, with 4.3 million beef cows; representing a decrease of approximately 18,800 beef cows since July 2010 (Statistics Canada, 2011a). These 2011 inventories represent a national decrease of 1.5 million head (-10.8%) of cattle between July 2010 and January 2011, or a decrease of 445,000 head (-3.4%) of cattle from January 2010 to January 2011 (Statistics Canada, 2011b). Due to the large, fluctuating population of beef cows in Canada, accurate estimation of enteric CH_4 emissions from cattle is paramount if the industry is to understand and mitigate the environmental impact of beef cattle production. With accurate estimates for each category of beef cattle, total enteric CH_4 emissions can be determined as long as the national herd inventory is known and in turn, reductions or increases in emissions can be accurately identified and monitored.

Intergovernmental Panel on Climate Change (2006) Tier-1 methodology, which estimates CH_4 emissions by applying an emission factor for specific animal categories, provides an estimate of 53 kg CH_4 hd^{-1} yr^{-1} for non-lactating, mature beef cows grazing forage and receiving seasonal supplements. Tier-2 methodology generates more accurate estimates of enteric fermentation than Tier-1 methodology by considering several factors; including animal categories, productivity and management, as well as diet quality and DMI for determination of gross energy intake (GEI). Using this methodology, a conversion factor is applied to these parameters to obtain an estimate of 76 kg CH_4 hd^{-1}

yr^{-1} for mature beef cows in North America fed low-quality forages; a value which is 43.4% higher than that of Tier-1 (IPCC, 2006). Canadian research studies predict emission factors of 126 kg CH_4 hd^{-1} yr^{-1} for beef cows (Ominski et al., 2007), which is 65.8% higher than estimates determined with Tier-2 methodology. However, it should be noted that these estimates were generated using best estimates for beef cows grazing grass pasture (McCaughey et al., 1999) as data for overwintering cows was not available.

Large variations in estimated CH_4 losses within and between ruminant livestock classes can be associated with such factors as the quantity, quality and type of consumed feedstuffs, animal genetics and environment. However, IPCC Tier-2 methodology generates uncertainty with estimates of enteric CH_4 emissions from ruminant livestock as reduced feed digestibility due to higher DMI, differences in diet chemical composition, and genotype or breed variations that may affect feed requirements and consumption are not considered in this methodology (IPCC, 2006). Further, Tier-2 methodology recognizes that cold temperatures during Canadian winters may increase net energy for maintenance (NE_m) requirements by 30% and thereby increase enteric CH_4 production, however, it makes no further consideration for the impact of cold stress on the variation in ruminal microbial population, or ruminal particulate passage and digestion kinetics (IPCC, 2006). As described above, these limitations are particularly relevant to enteric CH_4 emission predictions from Canadian beef cattle, as herds in this region are exposed to feedstuffs of varying qualities and extreme environmental temperatures each year.

2.2.3. Methane production from rumen microbial fermentation

Methane is a by-product of microbial fermentation of feedstuffs in the rumen. Production of CH_4 is less energetically efficient than production of the volatile fatty acids acetate, propionate and butyrate, which ruminants utilize for meat and milk production (Boadi et al., 2004). Livestock CH_4 production includes emissions from enteric fermentation and manure (Environment Canada, 2010). One third of global anthropogenic CH_4 emissions are generated by enteric fermentation. Approximately 87% of enteric CH_4 emissions are produced via enteric fermentation in the rumen; 95% of which are eructated into the atmosphere (Murray et al., 1976). The remaining 13% of CH_4 emissions are produced via hind gut fermentation; 89% of these emissions are reabsorbed and emitted through the lungs with only 11% released by flatulence (Murray et al., 1976), but estimates of enteric CH_4 emissions generally do not account for this latter proportion (Environment Canada, 2010).

2.2.3.1. Rumen methanogenic bacteria

Methane is produced by methanogenic bacteria which constitute most of the Archaea domain of microorganisms (Janssen and Kirs, 2008; Kumar et al., 2009). Methanogens can be found in many environments with redox potentials lower than -300 mV (Stewart and Bryant, 1988) and pH ranges between 6.0 and 8.0, such as wetlands, landfills, marshes, peat bogs, soil, intestinal tracts and rumens (Kumar et al., 2009). Of all the environments inhabited by methanogens, the rumen generates the largest source of CH_4 through its process of enteric fermentation (Kumar et al., 2009). Rumen methanogens are unique as they are strictly anaerobic, and undergo different growth and

rumen removal rates depending on whether they are suspended in rumen fluid or attached to particulate material, protozoa or the rumen wall (Janssen and Kirs, 2008). Although 28 genera and 113 species of methanogens have been classified, only nine species belonging to five genera (Table 1) have been identified in the rumen. Further, only seven species from four of the five genera are known to inhabit the rumen of cattle (Joblin, 2005; Janssen and Kirs, 2008; Kumar et al., 2009). Of these five genera, the *Methanobrevibacter* and *Methanomicrobium* are the most abundant archaea in the rumen, constituting 61.6 and 14.9% of total rumen archaea populations, respectively (Janssen and Kirs, 2008). However, many research studies have found that the presence and proportion of methanogen species in a rumen can differ depending on the presence of several factors (see section 2.2.3.3.) which impact methanogenesis (Wright et al., 2004; McSweeney et al., 2007; Wright et al., 2007; Janssen and Kirs, 2008; Kumar et al., 2009).

2.2.3.2. Substrates and energetics of methanogenesis

Methanogenesis involves conversion of organic matter to CO_2 and CH_4. Proteins, starches and cell walls of consumed feedstuffs are fermented to hydrogen, volatile fatty acids (VFA), CO_2 and ammonium (NH_4) by anaerobic rumen microbes, including bacteria, fungi and ciliate protozoa. Hydrogen, and often formate ($CHOO^-$), are the main substrates utilized by rumen methanogens to generate CH_4 during methanogenesis (Kumar et al., 2009). Acetate and methyl-containing compounds, such as methanol and methylamines, are utilized in methanogenesis to a lesser degree (Patterson and Hespell,

Table 1: Methanogen species present in ruminant livestock (Joblin, 2005; Kumar et al., 2009)

Genus	Species	Host Ruminant	Morphology and Gram Reaction
Methanobacterium	*formicium*	Bovine, ovine	Filamentous long rods; gram variable
	bryantii	Bovine	
Methanobrevibacter	*ruminantium*	Bovine, ovine, cervine	Coccobacilli; gram positive
	smithii	Ovine	
	olleyae	Bovine, ovine	
	millerae	Bovine, ovine	
Methanomicrobium	*mobile*	Bovine	Motile curved rods; gram negative
Methanosarcina	*barkeri*	Bovine, caprine	Pseudosarcina; gram negative
Methanoculleus	*olentangyi*	Cervine	Irregular cocci; gram negative

1979; Janssen and Kirs, 2008). As a result, methanogens and hydrogen-producing microbes are often present in clusters or attached to one another in the rumen, particularly in the fluid fraction (Kumar et al., 2009). Methanogens and ciliate protozoa most commonly demonstrate symbiosis and their interaction is believed to be responsible for up to 37% of ruminal methane emissions (Finlay et al., 1994). This symbiotic relationship allows methanogens to carry out their critical role of preventing hydrogen accumulation, thereby maintaining a low partial pressure of hydrogen in the rumen to ensure and promote an efficient fermentation process (Joblin, 2005; Janssen and Kirs, 2008).

Methanogenesis results in an energetic loss to ruminants as methanogens in the rumen break down available substrates to obtain their own source of energy and nutrients while producing an end-product that has no nutritional value to the animal (Boadi et al., 2004). It is estimated that ruminants can lose 2 to 15% of ingested energy as enteric CH_4 emissions alone (Moss et al., 2000). Shifting rumen fermentation towards production of the VFA propionate in lieu of acetate, butyrate and CH_4 results in more efficient use of dietary energy by the animal and reduces the wasteful production of enteric CH_4 as an end-product (Johnson and Johnson, 1995). This is because propionate production competes for hydrogen in the rumen (Boadi et al., 2004b), thereby reducing the hydrogen available to rumen methanogens for methanogenesis.

2.2.3.3. Factors which impact methanogenesis

2.2.3.3.1. Type and quality of feed

Beef cattle diets are comprised of different feedstuffs that impact the VFA profile and amount of enteric CH_4 generated during ruminal fermentation. Forages are high in fibre and increase enteric CH_4 production, whereas concentrates (grains) result in less fermentable substrate and reduce enteric CH_4 production (Kumar et al., 2009). Acetate and propionate are two VFAs that are directly correlated with ruminal CH_4 emissions, where propionate is inversely related to CH_4 production (Kumar et al., 2009). Feeding concentrate-based diets promotes propionate formation through a shift in microbial population towards amylolytic bacteria (Van Kessel and Russell, 1996). Unlike the process of acetate and butyrate synthesis which involves production of H_2, propionate production requires a net uptake of H_2 which decreases rumen pH and reduces the H_2 available for methanogenesis (Wolin, 1960; Hungate, 1966; Whitelaw et al., 1984; Van Kessel and Russell, 1996). It is believed that lower enteric CH_4 emissions in grain-fed cattle are a result of a pH and microbial population interaction (Johnson and Johnson, 1995), as the high starch or soluble carbohydrate concentrations cause rumen pH to drop lower than that which occurs in forage-fed cattle and below the range which permits survival of methanogenic bacteria (Boadi et al., 2004b). Therefore, a large acetate:propionate (A:P) ratio corresponds with increased CH_4 production, and vice versa. In general, feeding high-forage diets will result in a larger A:P ratio than when feeding high-concentrate diets (Whitelaw et al., 1984). Enteric CH_4 losses as a percent of gross energy intake (% GEI) range from 4 – 12% in forage-fed cattle to 2 – 3% in grain-fed ($\geq$ 90% concentrate) cattle (Johnson and Johnson, 1995). Canadian studies have

supported these estimates with reports of CH_4 emissions as high as 11% in backgrounding steers grazing low-quality grass pastures (Ominski et al., 2006) and as low as 2 – 3% in feedlot steers consuming grain-based diets (Boadi et al., 2004a).

Methane emissions for beef cattle fed forage-based diets vary according to differences in forage quality. In general, CH_4 losses as a % GEI are lower when cattle have access to higher quality forage (Boadi et al., 2004b). Forage quality is greater in the spring when plants are immature and less fibrous, versus the summer and fall when plants are more mature, have increasing fibre content and, therefore, have lower digestibility (DeRamus et al., 2003). As indicated in a review by Boadi et al. (2004b), grazing cattle on pastures with high-quality, immature forage stands results in higher DMI, as well as a potential reduction of enteric CH_4 emissions (% GEI) by 25% or more. Likewise, the quality of dry hay influences the production of CH_4, with greater emissions resulting from consumption of lower quality, lower digestibility hay. A study which grazed beef cows on higher-quality ryegrass or fed lower-quality (< 7% CP) grass hay with various protein supplements reported significantly higher (11 to 16%) CH_4 emissions in supplemented treatments than in the non-supplemented ryegrass treatment, and attributed these higher CH_4 emissions to the low-digestibility of the lower-quality hay (DeRamus et al., 2003).

2.2.3.3.2. Level of feed intake and feeding frequency

Feed intake is a function of feed quality, where higher DMI is associated with higher quality feed. Dry matter intake of forage-based diets may be restricted before nutrient requirements are met because rumen fill becomes a limiting factor with forage

intake (Baile and Forbes, 1974). High forage diets have a high fibre content which causes slow digestion and rumen passage rates of consumed feed, and results in the rumen remaining full for a prolonged period (Allen, 1996). Rumen fill also contributes to differences in DMI between high and low-quality forages, as reduced intakes are associated with lower-quality forages that have lower digestibility, and vice versa (DeRamus et al., 2003). Dairy and beef heifers ad libitum fed low, medium and high quality legume and grass forage (in-vitro organic matter digestibilities of 38.5, 50.7 and 61.5%, respectively) demonstrated significant increases in DMI of 6.3, 8.9 and 9.7 kg d^{-1} with increasing forage quality (Boadi and Wittenberg, 2002). Using a mathematical model of rumen digestion and considering two different diets (100% alfalfa and 30 alfalfa: 70 concentrate), Benchaar et al. (2001) reported that CH_4 production increased with increasing DMI for both diets, but when expressed as a % GEI, CH_4 emissions actually decreased with increasing DMI. Beauchemin and McGinn (2006) have also demonstrated that increasing DM intake from maintenance to 2.5x maintenance results in emission reductions of 19% (expresses as % GEI) in growing feedlot cattle. Further, these same authors demonstrated that restricted feeding proportionately decreased the amount of CH_4 emitted each day, but results in higher CH_4 emissions as a % GEI (Beauchemin and McGinn, 2006). Enteric CH_4 emissions from dairy and beef heifers were reported at 6.9% GEI when fed ad libitum forage, versus 7.3% GEI when restricted-fed forage at 2% of body weight (Boadi and Wittenberg, 2002). Therefore, promoting maximum DMI will improve the efficiency of energy utilization by the animal, resulting in higher productivity and reduced CH_4 production during its lifetime (Beauchemin and McGinn, 2006).

Feeding frequency influences CH_4 production through changes in ruminal pH and microbial populations. Infrequent feeding of concentrate-based diets, such as once or twice per day, increases diurnal fluctuations in rumen pH (Sutton et al., 1986), thereby causing pH to frequently drop below the range (pH of 6.0 – 8.0) for methanogen survival (Kumar et al., 2009). It also decreases and increases the formation of acetate and propionate, respectively (Sutton, et al., 1986). With methanogen survival hindered and propionate production increased, it stands to reason that CH_4 production would decline (Sutton et al., 1986; Boadi et al., 2004). Alternatively, more frequent feeding helps stabilize rumen pH since feedstuffs continually enter the rumen and allow continual microbial fermentation; increases acetate formation, decreases propionate formation and results in a larger A:P ratio (Sutton et al., 1986) which leads to higher CH_4 production (Kumar et al., 2009). Casper et al. (1999) demonstrated this with lactating dairy cows fed protein supplements either twice or five times daily, with the cows undergoing more frequent feeding demonstrating higher rumen pH and lower propionate concentration than the cows fed less frequently. An earlier study measured CH_4 emissions from lactating dairy cows supplemented with concentrates twice or six times daily and reported that frequent feeding generated 10% more CH_4 emissions (% GEI) than infrequent feeding (Müller et al., 1980).

2.2.3.3.3. Rate of passage of rumen digesta

Factors such as diets with higher concentrate:forage, higher DMI and cold exposure tend to increase ruminal ROP and reduce CH_4 emissions (Kennedy and Milligan, 1978; Okine et al., 1989; Johnson and Johnson, 1995; Kumar et al., 2009).

Faster ROP increases the rate at which consumed feedstuffs are passed out of the rumen, thereby reducing the extent and rate of ruminal fermentation, and shifting fermentation towards propionate formation (Johnson and Johnson, 1995). In turn, CH_4 production is reduced due to the lower A:P ratio, as well as a reduction in the time that methanogens can access ingested feed and generate CH_4 (Mathison et al., 1998; Kumar et al., 2009). However, increased DMI is believed to have a limited influence on ROP when forage-based diets are fed (Mathison et al., 1998), as the size of fibre particles must be reduced sufficiently prior to being filtered through the rumen mat (Zebeli et al., 2007). By feeding a 50 bromegrass: 50 alfalfa (DM basis) chopped hay mixture (11.5% CP and 57.3% NDF) and placing weights in the rumen of Hereford steers to imitate higher DMI and stimulate faster ruminal passage rates, Okine et al. (1989) identified an inverse relationship between ruminal ROP and CH_4 emissions; 63 and 43% faster particulate and fluid passage rates, respectively, resulted in a 29% reduction of CH_4 production. This same study also concluded that since 28 and 25% of CH_4 variation was due to particulate and fluid ROP, respectively, passage rate is solely capable of reducing emissions; that is, without the additional effects of DMI and diet composition (Okine et al., 1989). Kennedy and Milligan (1978) reported a 30% reduction in CH_4 emissions of closely shorn sheep with constant DMI, and 68 and 54% faster particulate and fluid ROP after cold (2 – 5°C) exposure for 35 days. More recent studies have also reported that shorter mean retention time of digesta in the rumen results in lowered enteric emissions (Christophersen et al., 2008).

2.2.3.3.4. Effect of protein (nitrogen) supplementation

Feeding protein supplements is an effective means to improve the quality of pasture and forage-based diets so as to increase animal productivity and decrease CH_4 production (Leng, 1991; IPCC, 2007a). Protein supplementation improves nitrogen (N) intake, which not only improves fermentation efficiency and reduces CH_4 emissions (Leng, 1991), but also decreases N excretion and manure N_2O emissions (Clark et al., 2005). Conversely, research by DeRamus et al. (2003) has demonstrated that beef cows grazing lower-quality forage (< 7% CP) and receiving two different 14% CP supplement mixtures emitted 41 and 30 g d^{-1} more enteric CH_4 than cows not receiving protein supplements for cottonseed meal-corn (CMC) and urea-corn (UC) supplements, respectively. Further, when these supplement mixtures were each fed at two different levels (1.6 and 2.5 kg), enteric CH_4 emissions were increased by 54 and 64 g CH_4 d^{-1} for CMC and UC supplements, respectively (DeRamus et al., 2003). Increased CH_4 emissions occur in response to protein supplementation of low-quality forage diets because increased protein intake increases diet digestibility and CH_4 production at similar rates (Johnson and Johnson, 1995). However, this response can be misleading unless considered in terms of CH_4 losses per unit of product (i.e. maintenance, growth or lactation), as more efficient animal productivity from improved protein intake would actually result in an overall reduction of CH_4 emissions per unit of product (Johnson and Johnson, 1995). It is estimated that implementation of improved feeding practices, such as protein supplementation, has potential to reduce enteric CH_4 production by North American beef cattle by 11% (IPCC, 2007a; Smith et al., 2008).

2.2.4. Nutrient excretion

As described above, low-quality forages that are fed as overwintering diets to beef cattle are commonly low in protein and do not adequately meet the animals' N requirements. Cattle that are N deficient experience inefficient rumen fermentation and utilization of dietary nutrients (Maeng et al., 1976; Köster et al., 1996). Supplementing low-quality forage-based diets with protein sources may serve to improve fermentation efficiency and the efficiency at which cattle can utilize dietary nutrients (Gilbery et al., 2006). Beef steers fed low-quality grass hay (5.1% CP) and supplemented with 0, 5, 10 or 15% corn condensed distillers solubles (CCDS; 86.7% rumen degradable protein) as a mixed ration demonstrated greater CP intake, total tract CP digestibility, microbial CP synthesis and fibre digestion with increasing CCDS intake, thereby indicating that CCDS supplementation improves nutrient availability and utilization of low-quality forages (Gilbery et al., 2006). However, excessive supplementation or supplementation with feedstuffs that contain relatively unavailable nutrients may serve to increase nutrient excretion. A study by Horn and Beeson (1969) demonstrated this concept by replacing cracked corn with DDGS, which is a source of highly degradable protein (Leupp et al., 2009). Steers fed a basal diet containing 53% digestible energy (DE) and 11% CP (56% of CP supplied by urea) excreted significantly more N than when fed the basal diet with 5% DDGS (57% DE; 11% CP; 46% of CP from urea) in lieu of cracked corn, regardless of these diets being isocaloric and isonitrogenous (Horn and Beeson, 1969). Further, some supplements that are an excellent source of dietary N may provide excessive levels of other nutrients, such as P, in the diet and result in greater excretion of those nutrients even if N retention is improved (Simpson et al., 2008). This is precisely the concern with

DDGS (Spiehs et al., 2002). Dairy rations with less than 20% inclusion of DDGS experience 0.5% higher dietary P concentration than average dairy diets without DDGS, which is 0.14 to 0.17% higher than recommended dietary P levels (National Research Council, 2001). Although DDGS has potential to serve as a beneficial N source for ruminant livestock, its high P concentration effectively increases fecal P excretion and the risk of P loss as runoff to soil and ground water (Sharpley et al., 2005; Simpson et al., 2008). Therefore, in order for protein supplementation to reduce nutrient excretion, care must be taken in selecting the type and quantity of protein source to feed in conjunction with low-quality forages.

In recent years, more stringent legislation regarding nutrient release from livestock operations has focused significant attention on reducing nutrient pollution of soil and ground water. Historically, N was the main focus of such relief efforts, but in more recent years P has become an additional nutrient of concern (Van Horn et al., 1996). Phosphorus loading of soils is an environmental issue as runoff of these nutrients contaminates ground water, and eventually causes algae growth and eutrophication of water bodies (Van Horn et al., 1996; Johnston and Roberts, 2001). Society has held agricultural livestock production as largely responsible for the N and P loading of soil and water bodies (Bunting et al., 2011), therefore the livestock industry must employ environmentally sustainable management practices that will reduce livestock nutrient excretion.

2.3. PROTEIN NUTRITION AND METABOLISM IN CATTLE

2.3.1. Definition of protein deficiency

Protein is an important nutrient in the diet of beef cattle as it is required for growth and maintenance of body tissues, reproduction and milk production, as well as for microbial protein (MP) synthesis. Dietary CP can be supplied as either rumen degradable protein (RDP) or rumen undegradable protein (RUP). Rumen degradable protein, also known as digestible intake protein (DIP), is the protein in the diet that can be degraded in the rumen and available to rumen microbes, whereas RUP is the protein that escapes rumen degradation, is flushed from the rumen unused, and degraded and absorbed in the small intestine or excreted from the hind gut (NRC, 2000b). When feeding cattle to meet protein requirements, it is difficult to identify the optimum amount of CP to use as a minimum guideline to ensure requirements are met because the degradability of CP varies largely depending on the type and quantity of each feedstuff in the diet, and animal requirements vary depending on physiological state and production level (Köster et al., 1996; Bodine et al., 2001). Rather, diets must be formulated to include sufficient RDP, and intake of those diets must be sufficiently high in order to provide enough N to rumen microbes (Maeng et al., 1976; NRC; 1984; Köster et al., 1996; Bodine et al., 2001; Gilbery et al., 2006; Bohnert and DelCurto, 2008).

To satisfy the N demands of rumen microbes, cattle have requirements for not only amino acids and peptides, but also for ammonia (Maeng et al., 1976). Ammonia (NH_3) is needed by most rumen microbes to grow and function, and is critical for assimilation and degradation of dietary CP in the rumen (Bryant and Robinson, 1961; Bryant and Robinson, 1962; Hungate, 1966). In fact, NH_3 is the most vital source of N

for rumen microbes. Research by Bryant and Robinson (1962) found that 82% of rumen microbes grown on relatively non-selective medium could be grown with NH_3 as their sole N source, 25% grew only if NH_3 was present and 56% could be grown using either NH_3 or amino acids. Another study by Al-Rabbat et al. (1971) found that 69% of MP was produced using ammonia nitrogen (NH_3-N), whereas the remaining 31% was generated from amino acid and peptide N. As discussed subsequently, it should be noted that microbes require a balanced supply of CP and energy; therefore dietary energy supply and its relationship with dietary CP influences rumen microbial growth and fermentation and, as such, also plays a role in protein deficiency in cattle (Del Curto et al., 1990; Bohnert and DelCurto, 2008).

Supplying rumen microbes with adequate N is critical to ensure maximized MP synthesis since MP, which is absorbed in the animal's small intestine, is actually a main source of N for grazing cattle (Bohnert and DelCurto, 2008). According to the NRC (1984), MP makes up approximately 50% of the protein required by ruminant animals, whereas Sniffen and Robinson (1987) stated that proportion ranges from 40 to 80% of daily protein requirements. Consequently, if rumen NH_3 concentration is too low to satisfy the N requirements of rumen microbes, microbial growth and yield will be restricted, resulting in reduced MP synthesis, as well as limited fibre fermentation, digesta outflow from the rumen and DMI when the animal is fed with low-quality forage-based diets (Maeng et al., 1976; NRC, 1984; Gilbery et al., 2006). If this restriction is not corrected, the animal will develop a protein deficiency characterized by factors such as reduced DMI, weight loss, retarded growth, compromised fertility and reduced milk yield (Merck and Company Inc., 2011). As a result, it is evident that sufficient rumen

NH_3-N concentration is imperative to ensure acceptable protein (N) status in cattle, and as such, is the major determinant in deciding whether or not a protein deficiency exists. Therefore, protein deficiency in cattle can be defined as an inadequate supply or intake of rumen degradable dietary CP that results in rumen NH_3-N concentrations less than the lowest acceptable concentration of rumen NH_3-N that can be present in ruminant livestock without impairing microbial growth and function.

2.3.2. Rumen ammonia nitrogen as an indicator of nitrogen status

Rumen microbes use rumen NH_3-N as a N source to grow and synthesize MP. In ruminant livestock, acceptable protein status is determined through measurements of rumen NH_3-N concentrations. Research has determined that the acceptable concentration range required to permit microbial growth is from 2.0 to 5.0 mg NH_3-N/ 100 ml of rumen fluid. Concentrations less than 2.0 mg NH_3-N/ 100 ml of rumen fluid result in restricted microbial growth and the animal is considered to be protein deficient (Satter and Slyter, 1974; Slyter et al., 1979). However, rumen NH_3-N concentrations can impose additional limitations even in protein sufficient animals. Optimum voluntary DMI of low-quality forages does not occur until rumen NH_3-N concentrations approach 20.0 mg 100 ml^{-1} rumen fluid (Krebs and Leng, 1984; Boniface et al., 1986; Perdok et al., 1988). Further, if rumen NH_3-N concentrations are less than 5.0 mg 100 ml^{-1} rumen fluid, microbial efficiency is not optimized and methane production parallels total VFA concentrations (Satter and Slyter, 1974).

There remains some indecisiveness regarding optimal concentrations of NH_3-N in protein sufficient ruminants. The NRC (1985a) has indicated that the precise rumen

NH_3-N concentration required for optimal microbial growth in ruminants is unclear. Hume et al. (1970) identified that rumen NH_3-N concentrations between 8.8 and 13.3 mg 100 ml^{-1} rumen fluid were required to stimulate MP synthesis. Conversely, research conducted by Ørskov et al. (1972) concluded that maximum microbial growth occurs with NH_3-N concentrations between 4.0 and 8.0 mg 100 ml^{-1} abomasal fluid, which is believed to accurately represent rumen NH_3-N concentrations (Satter and Slyter, 1974). Satter and Slyter (1974) have stated that although microbial growth occurs with ruminal NH_3-N concentrations between 2.0 and 5.0 mg/ 100 ml of rumen fluid, it is not maximized until concentrations meet or exceed 5.0 mg 100 ml^{-1} rumen fluid.

2.3.3. Serum urea nitrogen as an indicator of nitrogen status

For over 45 years, serum urea nitrogen (SUN) has been recognized as a reliable, accurate indicator of protein status of ruminants because of its relationship to the urea cycle (Preston, 1965; Huntington and Archibeque, 1999; Huntington et al., 2009). Serum urea nitrogen is effective at measuring protein status of cattle consuming different types of diets and exposed to different environmental conditions (Ndlovu et al., 2007). Nitrogen, in the form of amino acids and NH_3, is primarily absorbed from the rumen and small intestine (Preston et al., 1965; NRC, 1985b), and at least 50 to 100% of ruminal N enters the NH_3 pool of ruminant animals (Huntington and Archibeque, 1999). To prevent NH_3 toxicity, NH_3 absorbed from the rumen and gastrointestinal tract (GIT) into the bloodstream is transported by the hepatic portal vein into the liver where the urea cycle takes place to detoxify the NH_3 and transport it through the body in the form of urea (Huntington and Archibeque, 1999; Reynolds and Kristensen, 2008). As a result, SUN is

highly correlated to ruminal NH_3 concentration (Hammond, 1997). The liver releases urea into the bloodstream (thereby facilitating its measurement as SUN) where it is either recycled to the rumen or absorbed by the kidneys for excretion in urine (Hammond, 1997; Hristov and Pfeffer, 2005). In general, SUN concentrations between 2.1 and 7.9 mmol L^{-1} are classified as "within range" and suggest acceptable nitrogen status, whereas concentrations $\leq$ 2.0 mmol L^{-1} are classified as "low" and suggest nitrogen deficiency (Ortho Clinical Diagnostics, 1993). Typically, low SUN concentrations occur when cattle consume diets with low CP content, which causes urea in the bloodstream to be cycled back to the rumen (Säkkinen, 2005). According to Ndlovu et al. (2007), SUN concentrations $\geq$2.1 and $\leq$ 3.6 mmol L^{-1} are ideal as they indicate that dietary RDP is adequate, whereas concentrations > 3.6 mmol L^{-1} suggest high CP intake or excessive muscle mobilization (Chimonyo et al., 2002).

2.3.3.1. Relationship between serum urea nitrogen and urine and fecal nitrogen excretion

Serum urea nitrogen is capable of being absorbed back into the rumen, particularly in ruminants fed low-quality forage, as this process aids in increasing rumen NH_3-N concentrations to acceptable levels for microbial growth (Godwin and Williams, 1984; Säkkinen, 2005). It is critical for SUN to be maintained at acceptable concentrations because if it gets too high and causes rumen NH_3-N concentrations to increase in excess of 80 mg/ 100 ml of rumen fluid (Bondi, 1981), the liver will be unable to keep up with converting the NH_3-N to urea (Symonds et al., 1981), and NH_3 toxicity may occur (Bondi, 1981; Godwin and Williams, 1984). To aid in maintaining acceptable SUN

concentrations, the body excretes urea that is not being recycled to the rumen via the urine and feces (Huntington and Archibeque, 1999).

Urine excretion has an important relationship with SUN as SUN concentrations are maintained through increased urine volume thereby increasing urea or N excretion (McIntyre, 1970; Godwin and Williams, 1984). Greater N intake is associated with greater water intake and urine excretion, which was demonstrated by Devendra (1976) by adding urea to the diet of sheep. The reabsorption of urea is higher in ruminants fed low CP diets than when fed high CP diets (Schmidt-Nielsen, 1958; Godwin and Williams, 1984), but the reason for this is uncertain. It was originally hypothesized that urea is actively reabsorbed, but it may be more likely caused by reabsorption in the renal pelvis when urine outflow rates are low in response to low N intake (Pfeiffer, 1968; Godwin and Williams, 1984). In the medulla, or the innermost part of the kidney, blood is only separated from the pelvic urine by endothelium and simple epithelium lining the renal fornix (Pfeiffer, 1968). According to Cirio and Boivin (1990), rhythmic contractions of the renal pelvis may stimulate a backflow of urine into renal fornices entering the outer medulla, and the close proximity of blood to urine in the renal pelvis and medulla facilitates urea reabsorption into the bloodstream. When adequate (6.8%; AP) and low (4.1%; LP) CP diets were fed to sheep, the amount of urea excreted decreased from 161 ± 39 to 16 ± 9 μmol min^{-1} in AP and LP sheep, respectively, and renal pelvis reabsorption of urea accounted for 4% of total renal reabsorption when AP was fed, but was increased to 10.9% in sheep fed LP diets (Cirio and Boivin, 1990). Further, these authors reported that LP sheep had 31% less urea flow from the renal pelvis versus AP sheep, which was associated with urinary urea concentrations that were eight times lower in LP than AP

sheep (27 ± 8 versus 226 ± 48 mmol/L; Cirio and Boivin, 1990). However, enhanced retention of urea in ruminants fed low CP diets cannot be solely attributed to increased renal pelvic reabsorption, but also to significantly reduced glomerular filtration of urea from blood to urine (Cirio and Boivin, 1990). As demonstrated by Cirio and Boivin (1990), glomerular filtered urea decreased from 435 ± 193 μmol min^{-1} in AP sheep to 74 ± 35 μmol min^{-1} in LP sheep, which was speculated to occur in response to low CP intake causing lower plasma urea nitrogen (PUN) concentrations, as well as slower renal plasma flow and glomerular filtration rate.

Electrolyte intake is important in influencing urea retention through urine output (Godwin and Williams, 1984). Diets lower in CP normally also have lower Na^+, Cl^- or K^+ concentrations, therefore lower N intakes are typically associated with lower electrolyte intakes (Godwin and Williams, 1984). Electrolytes increase urine outflow, which increases urine N excretion, and reduces SUN and PUN concentrations (since SUN and PUN are approximately equivalent; Godwin and Williams, 1984). In a study by Godwin and Williams (1984), sheep fed a lucerne chaff-based ration (N intake of 10.8 g d^{-1}) containing a standard vitamin-mineral premix (intake of 1.1 g d^{-1}) and intraruminally infused with urea to create and additional "N intake" of 20.6 g d^{-1} developed PUN concentration of 68.0 ± 14.8 mg/100 ml, but addition of 500 mmol of NaCl and KCl to the urea infusion caused increased urine outflow rates and urine N excretion which substantially decreased PUN to 35.2 ± 3.1 and 37.3 ± 3.3 mg/100 ml, respectively. Decreased electrolyte intake is known to reduce urine flow and excretion, and increase urea reabsorption in the bloodstream of ruminants consuming low CP diets, but this same response has not been identified in ruminants with high CP intake (Ergene and Pickering,

1978; Godwin and Williams, 1984). Cirio and Boivin (1990) reported that sheep with low CP intake (41.1 g d^{-1}) had 28% slower urine flow compared to sheep with adequate CP intake (67.5 g d^{-1}).

Unlike urine N excretion, information regarding the relationship between SUN and fecal N excretion is much more tenuous. Urine N excretion includes the urea in the bloodstream that was excreted rather than recycled to the rumen, whereas fecal N excretion includes urea N that was recycled to the rumen from the bloodstream but not utilized for MP synthesis nor returned to the urea cycle (Reynolds and Kristensen, 2008). This less direct relationship between SUN and fecal N excretion makes fecal N excretion an unreliable indicator of not only SUN concentrations, but also N intakes and animal performance (Bodine and Purvis, 2003).

2.3.4. Effect of protein deficiency on microbial fermentation

Research has suggested that RDP is the critical dietary component that promotes increased microbial fermentation, DMI and nutrient flow to the small intestine due to its "first-limiting" role in ruminant protein metabolism (Köster et al., 1996). Diets deficient in RDP cause limited microbial fermentation since rumen microbes degrade RDP into its constituent amino acids and subsequently produce VFAs for energy, as well as NH_3 for a N source. Then using this NH_3, microbes synthesize additional amino acids and when they die, the microbes become MP (NRC, 2000b; Cheeke, 2005). Therefore, if RDP is deficient in the diet, then rumen N concentrations will be low and consequently, so will rumen NH_3-N concentrations. Deficient rumen NH_3-N limits MP synthesis and bacterial growth, which limits capacity for microbial fermentation of feedstuffs (Maeng et al.,

1976; NRC, 1985a; Köster et al., 1996; Bodine et al., 2001; Gilbery et al., 2006). Limited microbial fermentation slows the rate of passage of digesta from the rumen, thereby increasing the amount of digested energy that is used for microbial maintenance (Russell et al., 1992), reducing the efficiency of MP synthesis (NRC, 1996) and extending retention time of rumen digesta (Gilbery et al., 2006). Köster et al. (1996) fed Angus x Hereford cows low-quality forage (1.9% CP, 77% NDF) twice daily and supplemented with 0, 180, 360, 540 and 720 g RDP d^{-1} (sodium caseinate; 90% CP) via intraruminal infusion to find that MP flow and efficiency, rumen fluid dilution rate and total rumen VFA and NH_3 concentrations were significantly lower in cows not receiving RDP supplementation as compared to when the cows received RDP supplementation to alleviate protein deficiency. Gilbery et al. (2006) fed cannulated beef steers a mixed ration of low-quality forage (3.3% CP, 42.5% ADF) with 0, 5, 10 or 15% (DM basis) corn condensed distillers solubles (CCDS; 21.6% CP, 86.7% RDP) and no observed change in microbial efficiency, but decreasing RDP supplementation caused microbial CP synthesis (as measured by using rumen purines as microbial markers) to significantly drop from 0.16 kg d^{-1} with 10 and 15% CCDS inclusion to 0.14 and 0.12 kg d^{-1} with 5 and 0% CCDS inclusion, respectively.

2.3.4.1. Volatile fatty acids as energetic indicators of microbial protein synthesis

As indicated above, volatile fatty acids (mainly acetate, propionate and butyrate) are major end products of microbial fermentation, providing 50 to 75% of available energy to the animal, and therefore acting as the main energy source for ruminant animals (Faverdin, 1999). Rumen microbes obtain energy for growth from VFA, which is

necessary to ensure replenished microbial populations and continual MP synthesis as MP is passed from the rumen to the small intestine (Walker, 1968). The stimulation of microbial growth by VFA increases DMI, which results in greater MP synthesis, faster ROP of ingesta from the rumen and increased MP flow to the small intestine (Sniffen and Robinson, 1987). Hemsley and Moir (1963) reported that the increase in microbial growth from branched-chain VFA supplementation of low-quality forage (4.4% CP) fed to sheep resulted in significantly improved DMI due to improved fibre digestion, greater rumen VFA concentrations and faster ingesta ROP from the rumen. As VFA provide energy for greater microbial growth and increasing passage rates of MP from the rumen, VFA may serve as an energetic indicator of MP synthesis (Walker and Nader, 1975). By incorporating radioactive sulphate into microbial sulphur amino acids, Walker and Nader (1975) reported MP synthesis in mature sheep fed fresh or dried forages as 20.4 ± 2.3 g and 16.1 ± 3.4 g per mole of VFA produced in the rumen, respectively.

2.3.4.2. Purine derivatives as indicators of microbial protein synthesis

In cattle, essentially all nucleic acids in the hind gut are synthesized by rumen microbes and the purines of these nucleic acids are absorbed, degraded and excreted in the urine as purine derivatives (PD), including allantoin and uric acid (Chen and Gomes, 1992). Of these two PD, allantoin contributes 90% of total PD excretion in urine (Giesecke et al., 1994). Since excretion of PD and purine absorption are directly related, urine excretion of these PD provides an estimation of purine absorption within the body and subsequently, microbial N absorption can be determined (Chen and Gomes, 1992). Although endogenous PD contributes to urine PD excretion, urine PD excretion data may

be corrected to account for endogenous PD and therefore, only reflect PD originating from MP synthesis (Chen and Gomes, 1992). Since MP flow to the small intestine and urine PD excretion have a strong, positive correlation (Moorby et al., 2006; Tas and Susenbeth, 2007), total PD (allantoin and uric acid) excretion successfully indicates the yield and efficiency of rumen MP synthesis (Moorby et al., 2006; Pina et al., 2009), where greater, or more efficient, MP synthesis results in higher urine total PD excretion and vice versa (Valadares et al., 1999; Pina et al., 2009).

Either dietary CP or energy can be the limiting nutrient for MP synthesis; therefore a balanced CP to energy ratio (CP:E) is critical to ensure that neither CP nor energy are limiting on microbial growth and function, and to optimize nutrient utilization and MP synthesis (Oldham, 1984; Gabler and Heinrichs, 2003a). In general, a higher CP:E results in improved feed efficiency, nutrient utilization and MP synthesis (Gabler and Heinrichs, 2003b; Gabler and Heinrichs, 2003c). In research conducted by Moorby et al. (2006), mature, lactating Holstein-Friesen dairy cows fed ad libitum ryegrass silage (12.2% CP, DM basis) supplemented with a standard dairy concentrate (22.8% CP, DM basis) at forage: concentrate ratios of 80:20, 65:35, 50:50 and 35:65, demonstrated significant linear increases in DMI of 12.6, 15.6, 17.7, 19.8 kg d^{-1}, N intake of 303, 392, 505 and 603 g d^{-1}, and metabolizable energy (ME) intake of 31.1, 37.1, 44.4 and 50.8 Mcal d^{-1} across treatments. These diets represented corresponding CP:E of 60.9, 66.0, 71.1 and 74.2 g CP $Mcal^{-1}$ ME with increasing concentrate inclusion (Moorby et al., 2006). In response, significant linear increases in total PD excretion (197, 199, 334 and 370 mmol L^{-1}) and MP flow (0.151, 0.192, 0.254 and 0.249 kg d^{-1}) to the duodenum occurred with increasing CP:E (Moorby et al., 2006). Isocaloric (2.6 Mcal ME kg^{-1} DM)

corn silage-based TMR with CP content of 12.0, 15.2, 17.4 or 19.7%, and corresponding CP:E of 48.3, 59.1, 67.5 or 76.5 g CP $Mcal^{-1}$ ME, were fed to Holstein heifers and resulted in improvements in feed efficiency (expressed as the ratio of kg of feed to kg of gain) of 4.76, 4.42, 4.35 and 4.33, respectively, with increasing CP:E (Gabler and Heinrichs, 2003b). In a separate study, Gabler and Heinrichs (2003c) fed Holstein heifers corn silage-based total mixed ration (TMR) containing CP:E of 45.0, 63.3, 69.4 or 77.3 g CP $Mcal^{-1}$ ME, which was achieved by maintaining the dietary ME inclusion at 2.6 Mcal kg^{-1} DM and adding CP at 11.9, 16.7, 18.1 or 20.1% of the diet. Increasing MP synthesis of 150.6, 161.3, 189.4 and 238.1 g d^{-1} were reported with increasing CP:E, and the authors attributed these increases to greater dietary RDP concentrations with the higher CP:E diets (Gabler and Heinrichs, 2003c). These results are consistent with the understanding that dietary CP supply affects MP synthesis and total PD excretion (Clark et al., 1992; Moorby et al., 2006), but other research has also identified similar effects in response to dietary energy supply. Pina et al. (2009) fed Nellore heifers a corn silage-based TMR with concentrate supplemented at 20 (12.5% CP and 2.4% ether extract, DM basis) or 40% DM (12.5% CP and 2.8% ether extract, DM basis) and high or low (5.0 versus 2.8% DM) levels of RUP, and concluded that dietary concentrate supplemented at 40% of DM generated MP yield and urine allantoin excretion that were, on average, significantly higher by 11.0 g d^{-1} and 12.8 mmol d^{-1}, respectively, regardless of RUP level, but did not affect uric acid excretion. Since DMI and CP intake did not differ between treatments, the authors explained that this affect on MP synthesis and urine PD excretion was a result of increasing dietary energy with 40% concentrate inclusion, as

total digestible nutrients (TDN) intake increased from an average of 2.33 to 2.74 kg d^{-1} (P = 0.01) for the 20 and 40% treatments, respectively (Pina et al., 2009).

2.3.5. Effect of protein supplementation on dry matter intake

Low-quality forages tend to contain dietary CP levels that are inadequate to meet cattle protein (N) requirements and in turn, cattle consuming diets low in dietary CP have lower DMI than if they were consuming diets with adequate protein content (Köster et al., 1996; Bodine et al., 2001; Gilbery et al., 2006). This is explained by the fact that microbial fermentation in the rumen is suppressed when protein is fed below requirement (refer to section 2.3.4 above), causing rumen fill to last for longer periods and preventing greater DMI (Gilbery et al., 2006). Since RDP is considered the critical dietary component that promotes increased DMI (Köster et al., 1996), especially with low-quality forages (Bohnert et al., 2002), it makes sense that protein supplementation alleviates DMI restrictions by ensuring adequate rumen NH_3-N concentrations to meet microbial requirements, therefore enhancing DMI and digestion of low-quality forage-based diets (Del Curto et al., 1990). In fact, protein supplementation is said to increase DMI of low-quality forages by 25% or more while leaving digestibility unchanged or improved by $\leq$ 6% (Bohnert and DelCurto, 2008). In the previously discussed study by Köster et al. (1996), significant increases in forage organic matter (OM) intake occurred in response to increasing RDP supplementation, with intakes of 29.3, 48.1, 57.3, 64.7 and 61.6 g kg $BW^{0.75}$ observed when supplemented with 0, 180, 360, 540 and 720 g RDP d^{-1}, respectively. Likewise, in an experiment conducted by Bodine et al. (2001), cannulated steers fed low-quality hay (4% RDP, 73% NDF, 40% ADF) ad libitum and individually

supplemented with monensin-containing protein supplements with 19 (control), 335, 340 or 360 g RDP steer^{-1} day^{-1} demonstrated significantly greater total OM intake with supplemented treatments (22.6, 23.3 and 24.2 g kg BW^{-1}, respectively) versus control (14.1 g kg BW^{-1}).

Although protein supplementation is proven successful at increasing DMI of low-quality forages, improvements in DMI eventually reach a maximum with greater RDP supplementation (Köster et al., 1996; Gilbery et al., 2006). As such, it is important to identify the optimal dietary RDP inclusion required to maximize digestibility of forages in cattle of different physiological states as RDP supplementation in excess of this amount will result in unnecessary financial expense (Köster et al., 1996). Bohnert and DelCurto (2008) suggest that dietary RDP inclusion is ideal at 10 – 12% of TDN intake for dry beef cows; whereas the study by Köster et al. (1996) concluded that mature, non-pregnant and non-lactating beef cows in that study required dietary inclusion of 11% RDP, or 4 g kg BW$^{0.75}$, to maximize DMI of low-quality forage diets.

2.3.6. Effect of protein supplementation on nutrient digestibility

Protein supplementation of low-quality forages serves to increase rumen NH_3-N concentrations (Maeng et al., 1976), improve microbial growth and fermentation, and increase diet DMI and digestibility (Maeng et al., 1976; Köster et al., 1996). With digestibility improved, dietary nutrients such as N and P can be more efficiently utilized by the animal, which has been demonstrated by several researchers. In a study by Gilbery et al. (2006), beef steers fed low-quality forage (3.3% CP, 42.5% ADF) mixed with 0, 5, 10 or 15% (DM basis) CCDS (21.6% CP, 86.7% RDP) improved nutrient

availability and use of low-quality forages since significant increases were observed in rumen OM and fibre digestion following CCDS supplementation. Demonstrating an improvement in nutrient digestibility in response to CP supplementation, these authors also identified significant linear increases in total CP intake and total tract CP digestibility, with intakes and digestibilities reported as 98, 152, 227 and 228 g d^{-1} and 27.5, 42.8, 44.9 and 57.8% of intake across treatments, respectively (Gilbery et al., 2006). Similarly, the previously discussed study by Köster et al. (1996) reported significant increases in total N intake of 13.4, 48.6, 80.5, 110.9 and 137.8 g d^{-1} across treatments, respectively. They also observed significant increases in total GIT - N digestibility in response to increasing RDP supplementation of -39.8, 39.0, 51.1, 60.5 and 70.4%, with the greatest increase occurring at the 180 g RDP d^{-1} supplementation level, with smaller incremental increases occurring thereafter (Köster et al., 1996). Finally, significant increases, but at a decreasing rate, were observed across treatments in fecal N excretion (18.0, 29.7, 39.5, 47.1 and 45.2 g d^{-1}, respectively; Köster et al., 1996).

Research examining the influence of CP supplementation on P digestibility in cattle specifically is much less common than that conducted to explore N digestibility. However, as overall diet digestibility, as well as digestibility of other dietary nutrients, has been reported to increase in response to CP supplementation (Guthrie and Wagner, 1988; Del Curto et al., 1990) it can be expected that P digestibility would also improve. The study by Gabler and Heinrichs (2003c) supports this claim, as Holstein heifers fed isocaloric corn silage-based TMR with 2.6 Mcal ME kg^{-1} DM and 11.9, 16.7, 18.1 or 20.1% CP demonstrated N intakes of 62.1, 88.0, 96.0 and 105.7 g d^{-1}, respectively, P intakes of 12.7, 13.1, 12.9 and 12.9 g d^{-1}, respectively, and P digestibility of 51.4, 57.3,

60.7 and 54.9%, respectively, across treatments. Typical N and P digestibility for beef cows consuming forage diets was demonstrated in a study by Estermann et al. (2002). Lactating Simmental and Angus beef cows fed an 11% CP forage mixture (ratio of 1 meadow grass silage: 0.7 meadow hay: 0.3 barley straw, DM basis) and 1:1 mixture of salt and mineral premix ad libitum had N intakes of 309 and 297 g d^{-1}, respectively, apparent N digestibilities of 53.4 and 57.9%, respectively, apparent P intakes of 54.7 and 53.3 g d^{-1}, respectively, and P digestibilities of 24.1 and 32.6%, respectively (Estermann et al., 2002).

2.4. CATTLE RESPONSE TO COLD ACCLIMATIZATION

2.4.1. Effect of cold on thyroid hormone concentrations

Cold ambient temperatures increase the activity of the thyroid hormones (TH) triiodothyronine (T_3) and thyroxine (T_4) (Westra and Christopherson, 1976; Kennedy et al., 1977; Christopherson and Kennedy, 1983; Young, 1989; Dauncey, 1990; Delfino and Mathison, 1991; Ekpe and Christopherson, 1999). Immediately after the onset of cold exposure, thyroid stimulating hormone secretions from the pituitary gland increase, and thyroid releasing hormones respond by increasing T_3 and T_4 secretions from the thyroid gland (Reichlin et al., 1972; Evans and Ingram, 1974). However, the increase in TH concentrations may not be the only factor influencing the physiological response of ruminant animals to cold ambient temperatures (Dauncey, 1990). Upon initial cold exposure, behavioural and physiological changes occur, such as huddling and shivering, which may cause body temperatures to actually increase rather than decrease (Dauncey, 1990). In the long term, thickening of hair coats to increase insulation, and in newborn

calves, non-shivering thermogenesis to generate body heat, are normal responses (Dauncey, 1990). Nevertheless, it has been reported that the response of TH occurs more rapidly with exposure to cold versus hot environments (Yousef et al., 1967; Macari et al., 1983). Yousef et al. (1967) monitored shaved, non-lactating dairy cows that were housed in a thermoneutral (18°C) environment and had acclimation factors other than heat production and thyroid function controlled during sudden exposure to cold (1°C) for 1 wk. These cows demonstrated an 86% increase in thyroid activity between 12 and 36 hr after cold exposure, but then thyroid activity gradually returned to thermal-neutral values thereafter, and an unexplained lack of increase in heat production was reported (Yousef et al., 1967). In a more long-term study by Westra and Hudson (1981), numerical decreases in T_3 (123.2, 101.6, 79.1, 69.3 and 57.1 ng %) and T_4 (6.24, 6.11, 5.69, 4.88 and 4.15 mg %) were identified in calves from October through June, with the highest and lowest concentrations for both T_3 and T_4 occurring in October and June, respectively. With T_3, concentrations decreased significantly from October through February, whereas the T_4 concentration in October was not significantly different from the concentrations in December and February, but was significantly higher than the concentrations reported for April and June (Westra and Hudson, 1981).

The effect of cold on TH concentrations may be limited by DMI (Westra and Christopherson, 1976; Kennedy et al., 1977; Young, 1981; Ekpe and Christopherson, 1999). Cold ambient temperatures cannot solely maintain the elevated concentrations of T_3 and T_4 that have been frequently reported in the literature (Dauncey, 1990). Rather, energy intake and digestibility play a major role in determining energy availability to the animal and the number of T_3 receptors in skeletal muscle (Dauncey and Morovat, 1989;

Dauncey, 1990). Without an accompanying increase in energy intake, cold ambient temperatures eventually decrease muscle responsiveness to T_3 (Dauncey and Morovat, 1989). This requirement for greater energy intake in the cold is expected, as ruminants acclimatizing to the cold require extra energy to support thermogenesis (Dauncey, 1990). As such, feed restriction commonly reduces thyroid activity in ruminant animals, with T_3 concentrations normally decreasing and T_4 concentrations being unaffected (Kennedy et al., 1977; Ekpe and Christopherson, 1999). This has been demonstrated in a study by Ekpe and Christopherson (1999), where lambs housed in warm (23 ± 2°C) or cold (0 ± 2°C) environments and fed ad libitum or restricted (1.35 x maintenance) diets for 3 – 5 wk periods had 29 and 13% higher T_3 and T_4 concentrations, respectively, in response to cold exposure, but 48% lower plasma T_3 concentrations and unchanged T_4 concentrations in response to feed restriction.

2.4.2. Effect of cold on dry matter intake

The NRC (1981) indicates that DMI becomes more variable and less predictable as ambient temperatures stray from 20°C. In general, normal DMI occurs when cattle are acclimated to temperatures between 15 and 25°C. Intakes typically increase as temperatures decrease below 15°C with an 8 to 25% increase in DMI occurring in environments colder than -15°C, but exposure to blizzards, storms or temperatures less than -25°C result in a temporary depression of intake (NRC, 1981). With cold stress, the digestive tract response, including increased rumen motility and digesta passage, must take place before greater feed intake can occur (Kennedy et al., 1986). Young (1986) has indicated that this general response to temperature can vary depending on diet, sensitivity

of the animal to thermal fluctuations and state of acclimatization. Therefore, it is important to recognize the differences that may exist between the responses of acute cold-exposed versus cold-acclimatized animals. Cold-exposed animals are housed in cold environments for short periods of time (hours to days; Yousef et al., 1967; Kennedy and Milligan, 1978) and demonstrate rapid, short-term responses to temperature change, whereas cold-acclimatized animals are housed in cold environments for extended periods (several weeks to months; Westra and Hudson, 1981; Kennedy, 1985) and have completed gradual, long-term responses to temperature change (Encyclopaedia Britannica, 2011). Beef cows winter grazing native rangeland (5.5% CP; 50.3% ADF) from November 28 to December 30, 1983 that were exposed to minimum daily temperatures between -7.2 and -38.9°C (average -19.5°C) demonstrated a linear decrease in grazing time by almost 50% and a decrease in forage intake by 42% with progressively colder temperatures over the course of the study (Adams, 1986). Conversely, for thermally acclimatized grazing cows exposed to average daily ambient temperatures that were different (warmer or colder) than average temperatures from one to three days previous, Beverlin et al. (1989) reported only minor increases in forage intake of < 0.0005% BW per day per °C deviation when ambient temperature fluctuated from 8° to -16° C.

2.4.3. Effect of cold on microbial fermentation

Shorter retention times of rumen digesta are associated with reduced microbial fermentation in the rumen (Christophersen et al., 2008). Since cold acclimatization increases rumen motility and decreases rumen retention (Westra and Christopherson,

1976; Kennedy et al., 1977), it also reduces rumen fermentation (Young, 1981). Demonstrating this relationship between rumen retention time and fermentation, Kennedy et al. (1982) fed closely shorn sheep three different diets (barley-canola meal, lucerne hay or bromegrass hay) in either warm (22-24°C) or cold (1-5°C) environments and concluded that, for all three diets, OM fermentation was closely related to rumen retention time, which tended to be reduced by 17, 33 and 2% across diets, respectively, with cold exposure. Kennedy and Milligan (1978) have indicated that cold exposure (2 – 5°C for 35 d) of closely shorn sheep with constant DMI resulted in an approximate 33% reduction in rumen fermentation but unchanged post-rumen fermentation. It is believed that rumen fermentation is reduced during cold exposure because ingested feed is accessible to fermentative microbes for shorter duration as a result of faster rumen ROP (Young, 1981). According to Mertens (1977), slowly fermented dietary components, such as fibre, are most influenced by reduced rumen fermentation, whereas more rapidly fermented components are less affected.

2.4.4. Effect of cold on the rate of passage of rumen digesta

Exposure to cold temperatures increases the duration (Okine et al., 1989) and frequency of biphasic contractions in the reticulorumen (Westra and Christopherson, 1976; Kennedy, 1985). Fluid passage rates often become faster, but an increase in particulate passage is commonly prevented by the filtration effects of the ruminal mat (Zebeli et al., 2007). Large (> 1.18 mm), undigested fibre particles are filtered and trapped within the rumen mat to allow greater time for ruminal digestion (Zebeli et al., 2007). Particles digested to approximately1.18 mm or smaller escape from the ruminal

mat, mix with the fluid fraction and are flushed from the rumen by subsequent contractions (Nisa et al., 1999). In fact, greater reticulorumen motility with cold exposure serves to increase the mixing and propulsion of small particles in the fluid fraction above that which is found during exposure to thermal-neutral temperatures (Gonyou et al., 1979). Therefore, the rumen mat regulates the retention time of the rumen particulate fraction but permits the fluid fraction and small particles (≤ 1.18 mm) to flow through unimpeded (Zebeli et al., 2007). In closely shorn sheep exposed to thermal-neutral (22 – 25°C) or cold (1 – 4°C) ambient temperatures for 4 – 45 d periods, reticulorumen contractions significantly increased from an average of 61.3 to 109.6 contractions h^{-1} with cold exposure, representing a 79% increase in contraction rate (Kennedy, 1985). According to Westra and Christopherson (1976), greater concentrations of TH, T_3 and T_4, are believed to cause the increase in rumen passage rates and decrease in mean retention time of digesta. To investigate this claim, Kennedy et al. (1977) injected 0, 0.125 or 0.250 mg T_3 d^{-1} into sheep with normally functioning thyroid glands in a thermal-neutral environment (22 – 25°C) or exposed these sheep to cold ambient temperatures (2 – 5°C), and concluded that rumen and total tract mean retention time of digesta significantly decreased by 13 and 18%, respectively, with injection of 0.250 mg T_3 d^{-1}; and by 34 and 20%, respectively, with cold exposure.

2.4.5. Effect of cold on nutrient utilization

The efficiency of supplementation of overwintering diets is additionally impacted by cold temperatures during the winter months, and the resultant cold acclimatization that cattle undergo when exposed to these temperatures for extended periods. Some

researchers have concluded that cold-acclimatized cattle demonstrate reduced nutrient excretion, while others report that nutrient excretion actually increases. Nisa et al. (1999) theorized that cold-acclimatized cattle may have improved hepatic portal blood circulation and therefore better nutrient utilization. However, Kennedy et al. (1976) suggested that cold-acclimatized cattle experience a reduction in microbial degradation and an increase in the amount of dietary nutrients that escape rumen fermentation, therefore suggesting an increase in nutrient excretion. Likewise, Ames (1976) demonstrated that protein content of ruminant diets should be decreased during the winter months to reduce nutrient excretion, while Adams (1987) reported that low forage digestibilities in cold-acclimatized ruminants dramatically reduces nutrient utilization.

2.4.5.1. Effect of cold on nutrient digestibility

Limitations in diet and nutrient digestibility are reported to occur in cold-acclimatized ruminants as a result of greater reticulorumen motility causing shorter rumen retention time of digesta and therefore, less opportunity for microbial degradation of feedstuffs (Kennedy et al., 1976; Kennedy and Milligan, 1978; Young, 1981). Lower rumen digestibility results in greater proportions of dietary nutrients escaping the rumen unutilized by microbes, which has been demonstrated by several studies. In the previously discussed study by Kennedy et al. (1982), cold-exposed sheep fed the lucerne and bromegrass hay diets experienced 20 and 23% increases, respectively, in the escape of unutilized dietary N to the hind gut which accommodated corresponding reductions of 5 and 7%, respectively, in N digestibility. Similarly, Kennedy and Milligan (1978) concluded that N digestibility significantly reduced from 62 to 59 – 60%, and dietary N

escape from the rumen significantly increased from 0.28 to 0.46 – 0.50 g N g N intake^{-1} in warm (22 –25 °C) and cold (2 – 5°C) exposed sheep, respectively, when the sheep were closely shorn and fed bromegrass pellets at low (1410 g DM d^{-1}) or high (2350 g DM d^{-1}) intakes. Further, these authors identified a significant reduction in rumen NH_3-N concentration from 100 mg N L^{-1} in warm exposed sheep to 78 – 82 mg N L^{-1} in cold-exposed sheep, which is believed to have occurred in response to the increase rumen N escape (Kennedy and Milligan, 1978). In 1981, Westra and Hudson exposed Wapiti calves to outside ambient temperatures from October through June in Alberta, Canada, and observed significant reductions in apparent ADF digestibility from December to February, where the lowest ADF digestibility reported (27.3%) in December was less than that in October (43.4%) and June (44.6%). Further, these authors also reported that (unlike the aforementioned sheep studies) apparent digestibilities of DM, GE and N slowly increased from October to June, which they explained as compensatory digestion in response to the low ADF digestibility, since these dietary fractions are more readily digestible than ADF (Westra and Hudson, 1981). Beef cows fed a long hay diet and exposed to -11°C for 4 weeks did not demonstrate changes in apparent DM, gross energy (GE) or N digestibility compared to when exposed to 20°C (Christopherson, 1976). Christopherson (1976) also fed beef calves and steers a 50 chopped hay: 50 grain ration while housed outdoors during winter (average temperature range of -17 to 1°C) or indoors in a heated barn (10 – 19°C) and observed significant reductions in DM digestibility (8%) and N digestibility (4%) in calves housed outdoors; as well as 4 and 10% lower DM and ADF digestibilities, respectively, in outdoor steers as compared to indoor steers. In comparing the responses of the calves and steers to cold acclimatization,

Christopherson (1976) found that DM digestibility decreased by 0.21 and 0.08% per degree decrease in temperature for the beef calves and steers, respectively, suggesting that calves are more sensitive to temperature fluctuations than more mature cattle. However, an earlier study by Kennedy et al. (1976) reported that closely shorn sheep fed bromegrass pellets every hour in warm (18 – 21°C) or cold (-1 – 1°C) environments for 28 d experienced faster ROP with cold exposure, which increased the efficiency of MP synthesis and escape of non - NH_3-N to the hind gut, but apparent N digestibility was not affected by differences in environmental temperature.

It is understood that diet type influences the digestibility response in cold-acclimatized ruminants (Kennedy et al., 1982), as studies which have fed ruminants more readily fermentable diets (such as pure concentrate or barley-alfalfa pellets) as compared to lower-quality forage-based (i.e. bromegrass) diets have concluded that temperature has no effect on diet digestibility (Christopherson and Kennedy, 1983; Delfino and Mathison, 1991). Further, the digestibility of ground and pelleted forage diets is more negatively affected by cold temperatures than chopped forages (Christopherson and Kennedy, 1983) as the former has sufficiently small particle size to respond to faster rumen ROP with the fluid fraction, whereas the latter has larger particle size and is therefore trapped by the rumen mat until degraded to ≤1.18 mm in size (Zebeli et al., 2007). Forage type is also an important factor, as more readily digestible forages experience less reduction in digestibility, and vice versa (Christopherson and Kennedy, 1983).

2.4.5.2. Effect of cold on nutrient excretion and balance

Although nutrient digestibility normally decreases with cold acclimatization and greater proportions of nutrients escape rumen degradation, increased nutrient excretion corresponding with decreases in nutrient balance has not been consistently reported. Kennedy and Milligan (1978) reported that N balance did not change between warm and cold-exposed sheep despite significant reductions in N digestibility, increases in ruminal N escape, and lower rumen NH_3-N concentrations in cold-exposed sheep. However, urinary N excretion increased significantly in cold-exposed sheep with high intake (23.5 g d^{-1}) versus warm or cold-exposed sheep with low intakes (13.9 and 14.1 g d^{-1}, respectively; Kennedy and Milligan, 1978). Likewise, in a study by Kennedy et al. (1976) greater ROP did not change N digestibility and N balance was also unaffected by differences in environmental temperature. This lack of response in nutrient excretion may be explained by an improvement in the efficiency of microbial growth associated with cold acclimatization of ruminants (Kennedy et al., 1976; Kennedy and Milligan, 1978). Since greater microbial growth and faster rumen ROP occur, the flow of MP to the small intestine hastens and increases the supply of MP for intestinal absorption (Sniffen and Robinson, 1987), which increases blood urea recycling (Christopherson and Kennedy, 1983; Reynolds and Kristensen, 2008), and may explain why significantly greater (2.2 g N d^{-1} more) blood urea has been found to recycle to the rumen of cold-acclimatized ruminants (Kennedy and Milligan, 1978). These factors combined allow the increase in ruminal escape of dietary N to be offset by the improvement in recycling and utilization of non-dietary N, which improves or maintains the N balance of the animal (Christopherson and Kennedy, 1983).

2.4.5.3. Effect of cold on blood urea nitrogen concentrations

Research over the years has reported varying responses of blood urea nitrogen (BUN) to cold acclimatization in ruminant animals. Kennedy and Milligan (1978) reported no significant difference in PUN concentrations of warm versus cold-exposed sheep, but cold exposure caused 29 – 32% increases in PUN transfer to the rumen NH_3-N pool. Kennedy et al. (1982) also discovered that PUN concentrations increased, although not significantly, from 3.4 to 5.4, 4.9 to 5.6, and 3.1 to 4.1 mmol L^{-1} between warm and cold-exposed sheep fed barley-canola seed meal, lucerne hay or bromegrass hay, respectively. Furthermore, urea transfer between the blood and the rumen were not affected by cold exposure (Kennedy et al., 1982). Studies on reindeer found that the lowest PUN concentrations occurred during the winter (2.5 mmol L^{-1}) versus summer (17.4 mmol L^{-1}) months, but these results were associated with a reduction in feed quality and low N intake during the winter period (Säkkinen, 2005). Bull et al. (1991) studied the effect of warm or cold (21 or 0°C) rearing temperature on neonatal calves up to 72 hours of age and reported BUN concentrations in warm and cold calves of 12.0 and 14.3 mg dl^{-1}, respectively, representing a significant 19% increase in BUN concentration in calves housed in the cold. Whether BUN concentrations increase or not, it is evident that cold temperatures encourage increased urea N recycling within ruminant animals, which is believed to be closely associated with the decrease in rumen NH_3-N due to increased dietary N escape from the rumen (Kennedy and Milligan, 1978; Westra and Hudson, 1981). However, increased BUN concentrations may occur in response to the restrictions that are imposed on energy digestibility in cold-acclimatized ruminants (Christopherson and Kennedy, 1983; Säkkinen, 2005). When energy availability is limited, the

catabolism of endogenous proteins increases to generate an energy supply, renal absorption decreases and/or haemoconcentration occurs, which causes BUN concentrations to climb (Valtonen, 1979; Warren et al., 1982; Wolkers et al., 1994; Säkkinen, 2005). This also supports the observation made by Kennedy and Milligan (1978) whereby increases of 3.9 – 4.7 g urea d^{-1} occurred in the amount of endogenous urea that entered the rumen of cold-exposed sheep as compared to sheep in warm environments.

2.5. SUMMARY

Western Canadian beef cattle are typically overwintered on low-quality forages while being exposed to subzero temperatures for several consecutive months. The increased physiological demands in cold-acclimatized cattle coupled with inadequate nutrient intake can lead to compromised animal production. Therefore, protein supplementation may improve the nutrient status of beef cows in thermal-neutral or cold environments, while increasing nutrient utilization and animal productivity. Protein supplementation alleviates N restrictions on rumen microbes, thereby permitting more efficient nutrient utilization and microbial fermentation. The close interaction between dietary N, rumen microbes and N recycling and excretion is well understood and permits the use of parameters such as SUN, rumen NH_3-N, VFA and PD as indicators of N status in ruminant animals.

The production of enteric CH_4 emissions responds to numerous factors including intake and nutrient status of the animal. Although cattle responses to improved N intake

are understood, the relationship between protein supplementation, cold acclimatization and enteric CH_4 production is neither well documented nor well understood.

Cold ambient temperatures cause a temporary increase in T_3 and T_4 activity in ruminant animals, which is the main trigger for faster reticulorumen motility and rumen ROP. Shorter rumen retention times have induced variable responses in DMI and BUN concentrations, and reduced microbial fermentation and nutrient digestibility. These responses to cold temperatures have been extensively documented for sheep, but many studies have examined cold-exposed, rather than cold-acclimatized animals. Cattle response to cold acclimatization is not well understood.

Supplementing beef cows during the overwintering period provides an opportunity to reduce enteric CH_4 emissions and improve nutrient utilization of low-quality forages, but also presents a risk of excessive nutrient excretion and nutrient loading of the environment if all dietary nutrients are not appropriately balanced to meet animal requirements. Examining the relationships between CP supplementation and cold acclimatization will facilitate assessment of the improvement in environmental sustainability and nutrient utilization of beef cows in a typical Western Canadian production system.

3. RESEARCH HYPOTHESES AND OBJECTIVES

3.1. Hypotheses

Mature, dry and non-pregnant beef cows in a cold-stressed environment will have higher CP and energy requirements than cows in a thermal-neutral environment. In turn, cold-acclimatized beef cows will increase DMI relative to cows in thermal-neutral conditions, but nutrient utilization (as measured by nutrient intake, SUN, rumen NH_3-N, ROP, as well as apparent N and P digestibility) and rumen fermentation efficiency (as measured by VFA, PD and enteric CH_4 emissions) will be reduced as a consequence of increased metabolic rate and ROP of consumed feedstuffs in cold environments. Cold-acclimatized cows will excrete more N and P, except for enteric methane (CH_4, % GEI), than cows acclimatized to thermal-neutral environments in response to more rapid rumen particulate and fluid ROP. Enteric CH_4 emissions (% GEI) from cows in a cold-stressed environment will be lower than current emission estimates provided by the IPCC for cows housed in cold environments.

Beef cows fed low-quality forage in a thermal-neutral and cold-stressed environment will increase DMI with greater protein (DDGS) supplementation due to improved rumen microbial growth and function. Nutrient utilization and rumen fermentation efficiency will be most improved by supplementation with 20% DDGS as cow CP requirements would be exceeded, whereas supplementation with 10% DDGS will marginally meet CP requirements and therefore will improve the efficiency of nutrient utilization and fermentation above that which occurs without supplementation, but not to the same extent as with 20% DDGS supplementation. Excretion of N and P

will increase with greater protein supplementation, but this increase will be complimented with a decrease in enteric CH_4 emissions (% GEI) as protein supplementation will provide borderline adequate or excess dietary CP to meet rumen microbial N requirements and promote efficient rumen fermentation.

3.2. Objectives

The overall objective of this study was to assess nutrient utilization (including DMI, nutrient intake and N and P digestibility), fermentation efficiency and nutrient excretion in beef cows overwintered on low-quality forage diets supplemented with protein in thermal-neutral and cold-stressed environments. Specific objectives were to: 1) determine if nutrient utilization, rumen fermentation efficiency and nutrient excretion by beef cows fed low-quality forages in a cold-stressed environment were different compared to cows in a thermal-neutral environment; 2) assess the response to CP supplementation by beef cows fed low-quality forage diets in cold-stressed and thermal-neutral environments.

4. MANUSCRIPT

Impact of Cold Acclimatization on Nutrient Utilization and Enteric Methane Emissions of Beef Cows Overwintered on Low-Quality Forage Diets Supplemented with Dried Distillers Grain with Solubles

4.1. INTRODUCTION

Cold-acclimatized beef cows have been reported to increase particulate and fluid passage rates in the GIT, which may potentially decrease the efficiency of nutrient utilization during the overwintering period (Kennedy et al., 1982). While this is undesirable from the perspective of animal performance in cold-stressed environments and environmental sustainability, cold acclimatization has also been suggested to beneficially decrease energy losses in the form of enteric CH_4 emissions (Christopherson, 1976; Kennedy et al., 1977). This is important as CH_4 is produced by inefficient enteric fermentation in ruminant livestock and represents a loss of productive energy from the animal in the range of 2 to 12% GEI (Johnson and Johnson, 1995). However, in estimating enteric CH_4 production by Canadian beef cattle, the IPCC currently only accounts for the impact of cold exposure by suggesting increased CH_4 production due to higher DMI estimates (IPCC, 2006) and does not account for potential reduction in emissions from faster ROP associated with cold-acclimatized cattle. However, IPCC (2007a) does estimate that implementation of improved feeding practices, including protein supplementation with ethanol by-products such as DDGS, may suppress enteric CH_4 production (% GEI) of North American beef cattle by up to 11%.

Beef cows are typically overwintered on low-quality forages which tend to contain dietary CP levels that are inadequate to meet cattle CP requirements (Köster et al., 1996; Gilbery et al., 2006). Deficient dietary CP in beef cow diets can compromise rumen microbial function and/or animal metabolism. The former may affect rumen function and reduce fermentation efficiency, including MP synthesis, whereas the latter

may reduce nutrient recycling and utilization (Clark et al., 1992; Köster et al., 1996; Gilbery et al., 2006). In turn, CP deficiency may lead to increased nutrient excretion. Protein supplementation of low-quality forages serves to alleviate CP deficiency, increase rumen NH_3-N concentrations (Maeng et al., 1976), and improve microbial growth and fermentation, thereby increasing diet DMI and digestibility (Maeng et al., 1976; Del Curto et al., 1990; Köster et al., 1996).

In recent years, society has held agricultural livestock production as largely responsible for the N and P loading of soil and water bodies (Bunting et al., 2011), therefore more stringent legislation regarding nutrient release from livestock operations has focused significant attention on reducing nutrient pollution of soil and ground water (Van Horn et al., 1996). Agriculture is considered the greatest contributor to N and P loading of surface and ground water in Canada, with 1996 estimates of 293,000 tonnes of surplus N and 55,000 tonnes of surplus P remaining in the soil each year (Chambers et al., 2008). Although N was originally the nutrient of concern, P loading has increased dramatically in recent years (Van Horn et al., 1996), and projected increases in livestock and crop production are predicted to continue to increase nutrient losses to soil and water sources in the future (Chambers et al., 2008). Agriculture is also considered the greatest source of CH_4 emissions, generating about 50% of global anthropogenic CH_4 emissions in 2005 (IPCC, 2007d). From the total 2008 Canadian GHG emissions, 13% were attributed to CH_4 (Environment Canada, 2010). Enteric emissions from all ruminant livestock in Canada increased by 29% between 1990 and 2008, with enteric emissions from beef cattle increasing by 36%, or from 14 to 19 Mt CO_2 eq (Environment Canada,

2010). Global livestock CH_4 emissions have been projected to increase by 21% between 2005 and 2020 (IPCC, 2007a).

As of January 2011, Canada's total cattle population was approximately 12.5 million head, with 4.3 million beef cows (Statistics Canada, 2011a). Due to the large, fluctuating population of beef cows in Canada, accurate estimation of nutrient utilization and nutrient excretion, including enteric CH_4 emissions, from these animals is of great value to gauge productive efficiency, as well as understand and mitigate the environmental impact of beef cattle production.

The objectives of this study were to 1) determine if nutrient utilization, rumen fermentation efficiency and nutrient excretion by beef cows fed low-quality forages in a cold-stressed environment are different compared to cows in a thermal-neutral environment; 2) assess the response to CP supplementation by beef cows fed low-quality forage diets in cold-stressed and thermal-neutral environments.

4.2. MATERIALS AND METHODS

4.2.1. Feeding Strategy and Animal Management

Thirty mature, dry and non-pregnant Simmental and Gelbvieh beef cows weighing 675.4 ± 51.75 (SEM) kg were used to assess the effect of protein supplementation of low-quality (low CP and high fibre) forage diets and cold acclimatization on nutrient utilization, fermentation efficiency and nutrient excretion. Prior to arrival at the research site, cows were vaccinated with the Pfizer Gold program (Pfizer Animal Health, Exton, PA) and treated with Ivermectin pour-on solution. Vitamin A and D injections were given half way through the study. After an initial 5-wk adjustment period to the feedlot facilities and 5-d adjustment period to the metabolism unit facilities, cows were assigned, on the basis of BW, to one of three dietary treatments consisting of low-quality forage supplemented with 0% (6.0% CP, 67.7% NDF, 46.4% ADF; control; CON), 10% (8.7% CP, 64.4% NDF, 44% ADF) or 20% (11.6% CP, 60.1% NDF, 40.8% ADF) DDGS (as-fed basis; Table 2). Cows were housed in three feedlot pens, each containing four GrowSafe feeder nodes (GrowSafe Model 4000E feed monitoring system, GrowSafe Systems Ltd., Airdrie, Alberta) and a heated watering bowl. The GrowSafe bunks and watering bowls in each pen were protected from the elements by the lean-to roof structure of the feedlot barn which covered one half of the pen and provided shelter to the cows.

Cows were fed their respective test diets, consisting of low-quality forage (6.3% CP grass hay and 5.2% CP oat straw) and DDGS (37.8% CP) combined as a TMR, using

Table 2: Actual ingredient and nutrient composition (DM basis) of TMR fed to cows in thermal-neutral and cold-acclimatized periods.

	Fall (Thermal Neutral)			Winter (Cold-Acclimatized)		
	CON	10%DDGS	20%DDGS	CON	10%DDGS	20%DDGS
Ingredient, %						
Grass hay, chopped	39.8	35.6	31.3	39.7	35.5	30.8
Oat straw, chopped	46.3	41.1	35.7	46.4	41.3	35.8
50 wheat: 50 corn DDGS[1]	-	9.4	18.7	-	9.4	19.0
Cane molasses	1.5	1.3	1.3	1.5	1.3	1.3
Limestone	-	0.1	0.5	-	0.1	0.5
Dicalcium phosphate	0.1	0.1	0.1	0.1	0.1	0.1
Nutrient Composition						
DM, %	84.8	81.1	84.6	81.3	82.0	82.5
CP, %	5.6	8.7	11.4	6.3	8.6	11.7
ADI-CP, % of CP	14.3	18.1	20.0	14.6	17.6	15.7
ACP, [2] %	5.2	7.6	10.4	5.4	8.1	10.3
NDF, %	67.7	63.4	58.6	67.7	65.3	61.6
ADF, %	45.0	43.1	39.9	47.8	44.9	41.6
TDN, %	55.7	56.6	58.2	54.3	55.7	57.4
Ca, %	0.49	0.47	0.60	0.40	0.38	0.44
P, %	0.13	0.20	0.28	0.13	0.21	0.29
K, %	1.45	1.46	1.38	1.14	1.24	1.20
Mg, %	0.21	0.23	0.26	0.19	0.21	0.24
NE_m, Mcal kg^{-1}	1.16	1.19	1.25	1.11	1.16	1.22
GE, Mcal kg^{-1}	4.01	4.06	4.13	4.05	4.10	4.13

[1] Starch content of the 50 wheat: 50 corn DDGS was 9 g kg^{-1} (0.9%), DM basis.

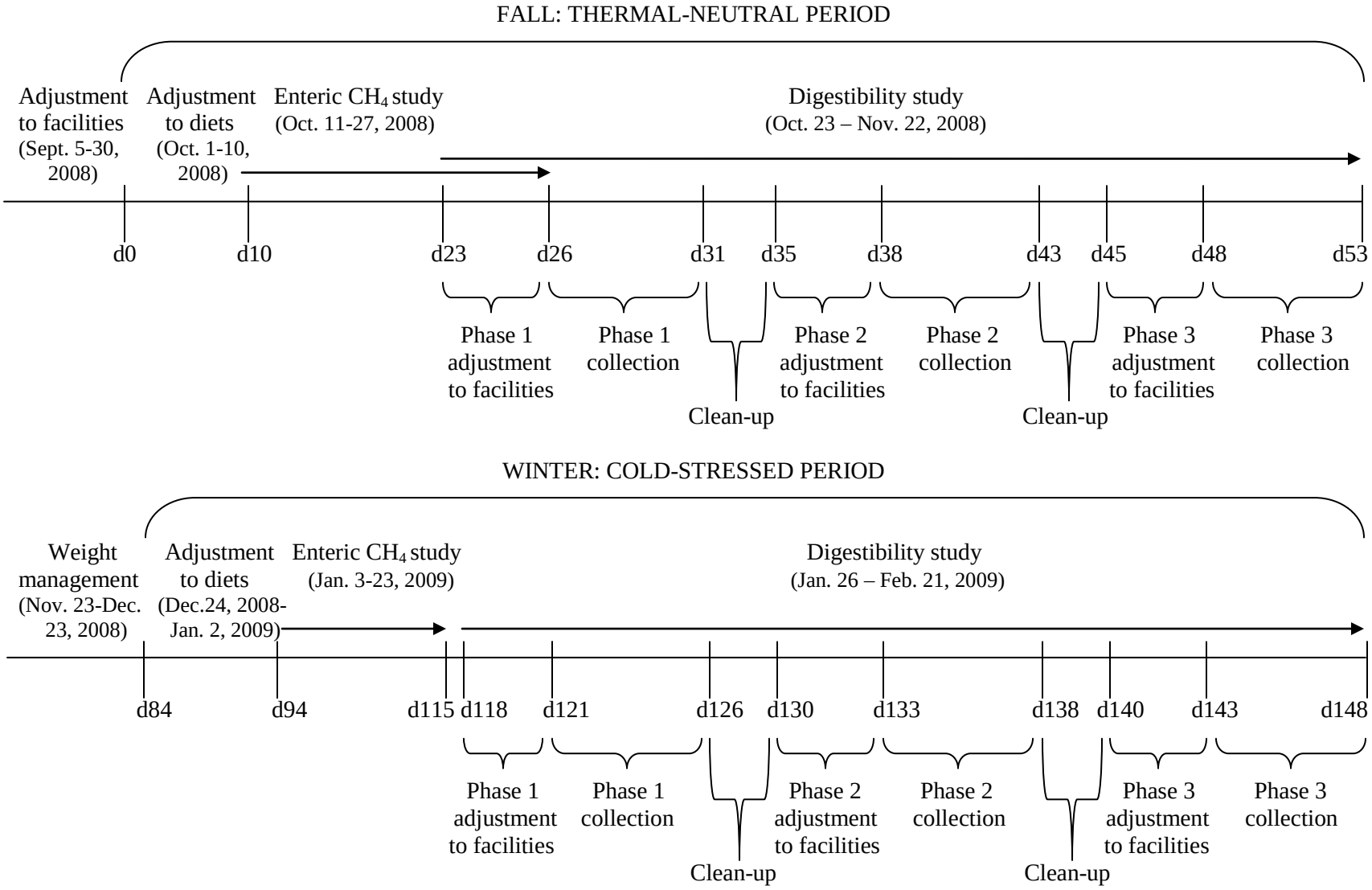
[2] ACP = Available CP, where ACP = [CP (%, DM) * [100 – (ADI-CP (% of CP) – 10%)] / 100].

cane molasses (10.2% CP) as a binder to minimize ingredient separation, during a 10-d dietary adaptation phase at the onset of each of the two feedlot (CH_4 collection) periods: a thermal-neutral period during the fall (October 11 to 27, 2008) and a cold-stressed period during the winter (January 3 to 23, 2009; Figure 1). Feeding occurred as frequently as needed throughout each day to ensure ad libitum intake and 5% orts at all times. Cows had ad libitum access to fresh water and trace mineralized salt blocks (Appendix 1).

Metabolism stalls equipped with feed bunks, watering bowls and padded floor mats were available on site and utilized to conduct ROP and nutrient balance studies. Following the feedlot period, eight cows were selected from each treatment group and randomly assigned to one of three, 8-d digestibility study phases to determine the impact of protein supplementation and cold acclimatization on digesta ROP and nutrient balance (fecal and urinary nutrient excretion). In digestibility study phases one and three, two cows from the CON and 20%DDGS treatments were randomly selected for ROP sample collection, whereas all eight cows in each phase were used to collect rumen fluid and nutrient balance data.

Each phase of the digestibility study consisted of a 3-d adjustment period and 5-d collection period. Ambient temperature in the metabolism unit was maintained between 5 and 18°C in the fall (October 23 to November 22, 2008) and winter (January 26 to February 21, 2009). Diets were fed at 85% of each cow's average ad libitum daily DMI, which was calculated using DMI values for the last 10 days prior to entering the metabolism unit. Cows were fed twice daily at 1100 and 1800 h. Cows had ad libitum access to fresh water. After completion of the fall study, diets to promote weight gain, loss or maintenance were fed so the cows were at their pre-test weights for initiation of

Figure 1: Timeline of study



the winter period. Canadian Council on Animal Care guidelines were followed in the care and management of the cows (CCAC, 1993).

Ambient temperatures in the feedlot and metabolism unit were recorded throughout the duration of the study using HOBO temperature loggers (HOBO U12 Stainless Temperature Data Logger, Onset Computer Corporation, Pocasset, MA). Two HOBOs on opposite ends of each feedlot pen and the metabolism unit logged temperatures on an hourly basis to facilitate determination of daily average temperatures.

4.2.2. Feed Samples and Analysis

During the enteric CH_4 collection and digestibility studies, feed and ort samples were collected daily and frozen at -20°C until analyzed. Frozen samples were thawed and dried in a forced-air oven at 60°C for at least 48 h to determine DM content. After drying, all feed and ort samples were composited using a sample splitter (Model CL-280, Soiltest Inc., Chicago, Illinois). Feed samples from the enteric CH_4 emission study were composited by week for each treatment. Feed samples from the digestibility study were composited by cow and treatment for each of the 5-day collection periods. Once composited, samples were ground through a 1-mm screen (Cyclotec Tecator 1093 Sample Mill, Foss Analytical, Denmark). Thirty grams of each ground composite was submitted to Central Testing Laboratory Ltd. (Winnipeg, Manitoba) for chemical analyses. Composites were analyzed in duplicate with a forage reference standard to validate accurate analytical results.

Feed samples were analyzed for moisture using the Association of Official Analytical Chemists (AOAC, 1990) method 930.15. Crude protein was determined with

a Leco FP-428 (LECO Corporation, St. Joseph, MI) using a modification of Leco Version 2.2 method and acid detergent insoluble-CP (ADI-CP) with the ANKOM 8/98 method (ANKOM Technology, Macedon, NY). Available CP (ACP) was calculated using acid detergent insoluble crude protein (ADI-CP) to adjust for heat damaged protein when ADI-CP was > 10% of CP. The macrominerals Ca, P, Mg and K were determined using modified AOAC (1990) 968.08 and 935.13A methods. Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined with an ANKOM 2000 automated fibre analyzer using the ANKOM 08-16-06 method of analysis (ANKOM Technology, Macedon, NY). Total digestible nutrients and net energy for maintenance (NE_m) were determined using NRC equations (Appendix 3).

4.2.3. Blood Sampling and Analysis

During the enteric CH_4 emission study, blood samples were collected once per week via tail vein puncture into 10-mL serum separator vacutainers (containing gel and clot activator) and plasma vacutainers (containing heparin). Samples were also collected on days 5 and 7 of each phase of the digestibility study. Serum urea nitrogen was analyzed from serum samples using a colorimetric test with a Vitros 250 (Ortho Clinical Diagnostics Inc., Pub. No. MP2-9, Rochester, NY) by Veterinary Diagnostic Services (Manitoba Agriculture, Food and Rural Initiatives, Winnipeg, Manitoba). Plasma samples were centrifuged at 2000 x *g* and 4°C for 20 minutes immediately after collection. Separated plasma was transferred to 2 mL tubes using transfer pipettes and frozen at -20°C until later analysis. Thawed samples were analyzed by radioimmunoassay (RIA) with a Wallac 1470 Wizard automatic gamma counter

(PerkinElmer Canada, Inc., Vaudreuil-Dorion, Quebec) using a Clinical Assays™ Gamma Coat™ M T_3 ^{125}I RIA kit and a Clinical Assays™ Gamma Coat™ M Total T_4 ^{125}I RIA kit (DiaSorin Inc., Stillwater, Minnesota) for determination of the concentrations of thyroid hormones (TH), triiodothyronine (T_3) and thyroxine (T_4), respectively.

4.2.4. Enteric Methane Emissions

Enteric CH_4 emissions were collected using the sulphur hexafluoride (SF_6) tracer gas technique (Boadi et al. 2002). Sulphur hexafluoride was released at a known release rate from a stainless steel permeation tube (12.5 x 40 mm) that was administered orally into the rumen via a speculum 10-d prior to the initial CH_4 gas collection to allow sufficient time for SF_6 release to stabilize. The average tracer gas release rate for permeation tubes was 380.7 ± 17.2 ng min^{-1}. Twenty-four hour gas collection was accomplished using pre-evacuated (40 mmHg) stainless steel canisters (130 mm diameter) connected to 900-mm capillary tubing (128 µm internal diameter) with a 15-µm filter and flexible nose piece mounted onto nylon cattle halters to collect the exhalation from the mouth and nose of each cow. During the collection period, cows were released back into their assigned feedlot pens while wearing the collection system. Two collection systems were also placed on opposite sides of each pen to collect background CH_4 and SF_6 samples. After 24 h, all animal and background canisters were pressure-checked to ensure successful collections and the absence of blocks or leaks in the pressure systems. Canisters were subsequently pressurized with 110 kpa N_2 to prevent contamination of the samples before gas analysis. Collections were repeated to obtain a minimum of three successful samples per cow per period.

4.2.4.1. Methane and SF_6 Analysis

Methane and SF_6 were quantified using flame ionization and electron capture detectors, respectively, in a Varian CP-3800 gas chromatograph (Varian, Mississauga, ON; Boadi et al. 2002). The presence and concentration of gases were determined from the peak area and retention time of the sample following instrument calibration with prepared standards (100.1 ppm CH_4 – Supelco, Mississauga, ON; 20.73 ppt SF_6 – Scott-Marrin Inc., Riverside, CA). Enteric CH_4 emission of each sample was calculated by the following equation:

$$CH_4 \text{ (L min}^{-1}\text{)} = \text{permeation tube } SF_6 \text{ release rate x } [CH_4]/\ [SF_6]$$

where $[CH_4]$ and $[SF_6]$ are concentrations of CH_4 and SF_6 adjusted by the removal of background concentrations of CH_4 and SF_6. Background samples contained low levels of CH_4 and SF_6 that could have been collected from the exhalation of a nearby animal or presence of CH_4 or SF_6 in the atmosphere.

After a 24-h CH_4 collection, canisters with pressures below 228 mmHg and above 700 mmHg were excluded from statistical analysis to ensure inclusion of measurements only from complete collections that were not compromised by blocks or leaks in the collection system. Likewise, measurements that reached atmospheric pressure prematurely due to damage to the collection systems were removed. One CON, one 10%DDGS and two 20%DDGS cows were removed from the enteric CH_4 emissions study due to non-functioning permeation tubes.

4.2.5. Rumen Fluid Sampling and Analyses

Rumen fluid samples were collected at 0900 and 1500 h, corresponding to 2 h pre-feeding and 4 h post-feeding, on days 5 and 7 of each phase of the digestibility study. A Geishauser oral probe (Duffield et al., 2004) was used to aspirate 100 ml of fluid which was discarded to prevent saliva contamination of the sample, and a subsequent 250 ml was collected, immediately measured for pH, subsampled for ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA) analysis, and frozen at -20°C.

Frozen rumen fluid samples were thawed at room temperature and analyzed for NH_3-N concentration according to the method described by Novozamsky et al. (1974). In a test tube containing 50 µl of vortexed rumen fluid, 1.5 ml of reagent I (100 ml alkaline phenolate, 200 ml 0.05% sodium nitroprusside and 10 ml 4% Na_2 EDTA) was added and vortexed. An additional 2.5 ml of reagent II (400 ml phosphate buffer and 100 ml 10% NaOCl) was added and the mixture was vortexed again. Test tubes were covered and incubated in darkness for 30 minutes. Absorbency of each sample was read at 630 nm on a spectrophotometer and NH_3-N concentrations were calculated with the regression equation determined from a standard curve.

Volatile fatty acid concentrations were determined using the method of Erwin et al. (1961). Frozen rumen fluid samples were thawed at room temperature, combined with 25% metaphosphoric acid in a 5:1 ratio of rumen fluid:metaphosphoric acid, mixed and frozen at -20°C overnight. Samples were thawed to room temperature, centrifuged at 3000 x *g* for 20 minutes and approximately 2 ml of supernatant was pipetted into autosampler vials. The capped vials were submitted for determination of VFA concentrations by gas chromatography (PerkinElmer Clarus 500, PerkinElmer Canada,

Inc., Vaudreuil-Dorion, Quebec) with injector, detector, and initial and final column temperatures of 170, 195, 120 and 165°C, respectively.

4.2.6. Particulate and Fluid Rate of Passage

Chromium-mordanted alfalfa (Cr_2O_3) and Co-EDTA were used as the respective markers for measuring outflow rates of the rumen particulate and fluid fractions of the cows, and were prepared following Uden et al. (1980). Feed was withheld for 7.5 h prior to the onset of sample collection on day 4 of each phase of the digestibility study to ensure complete consumption of the markers. Markers, delivered as 90 g Cr-mordanted alfalfa and 25 g Co-EDTA mixed with 250 g corn silage, 800 ml cane molasses and 100 g DDGS, were fed at midnight (0 h) during phases one and three of the digestibility study, and consumed within one hour of administration. Assigned diets were fed thereafter. Pre-weighed stainless steel collection trays were inserted beneath the cleaned manure grates of each stall at 0 h on day 4 and total fecal collection was initiated. Fecal samples were collected at 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 78, 84, 90, 96, 102, 108, 114 and 120 h after feeding the marker. At the end of each collection, feces were weighed, mixed, subsampled and frozen at -20°C until later analysis.

Fecal samples from the ROP collections were dried for 96 h at 60°C in a forced-air oven to determine DM content. Samples were subsequently ground through a 1-mm screen (Cyclotec Tecator 1093 Sample Mill, Foss Analytical, Denmark). Analytical lab DM was determined using AOAC method 934.01 (1990) and samples were analyzed for Cr and Co concentrations using inductively coupled plasma optical emission

spectroscopy (ICP-OES; Vista MPX, Varian, Walnut Creek, CA). Chromium was analyzed using the method described by Williams et al. (1962) and Co with AOAC method 968.08 (1990).

Digesta kinetics for particulate and fluid ROP were evaluated by fitting excretion curves for the Cr and Co markers to a series of two-compartment models using the Proc NLIN procedures of SAS (Iterative Marquardt method, SAS Institute, Inc., 2008) as described by Pond et al. (1988) and Moore et al. (1992). Models evaluated the slow (rumen) and fast (post-rumen) compartments of the ruminant digestive tract. The models included no age dependency (G1G1) or increasing levels of gamma age dependency (GnG1, n = 2 to 4) in the fast compartment, where G1G1 is optimal for fluid passage, G2G1 and G3G1 for rapidly and moderately ascending excretion curves, respectively, and G4G1 for excretion curves that ascend slowly, indicative of lower DMI and slower post-rumen ROP (Moore et al., 1992). Chromium and Co concentrations < 0.1 ppm were removed from each data set as they were below the accurate detection limit of the inductively coupled plasma – optical emission spectrometer (ICP-OES), and outliers identified on the excretion curves were removed. As suggested by Moore et al. (1992), model selection occurred by simultaneously running all four GnG1 models for each cow in each season and selecting the model which demonstrated the majority of the following criteria; (1) best fit of actual vs. predicted data points, (2) lowest mean square error, (3) largest F-value and (4) narrowest range in 95% confidence intervals. Similar to Moore et al. (1992), the G4G1 model was selected for CON and 20%DDGS in both fall and winter, and for both Cr and Co markers.

4.2.7. Fecal and Urine Sampling and Analyses

Total fecal nutrient collection occurred concurrently with fecal ROP collection. Pre-weighed collection trays were inserted beneath the manure grates of non-ROP cows at 0 h on day 4 of each phase of the digestibility study and exchanged for clean trays at 12 h intervals throughout the 5-d collection period. At 1200 h of each day, feces were weighed, mixed and 25% of the fecal weight was subsampled into sealed tubs stored in a cool location to minimize N volatilization as ammonia. At midnight, feces were weighed again, mixed and 25% of the feces were subsampled and added to the 1200 h sample. The combined subsamples were mixed thoroughly and a final subsample representing total 24 h fecal excretion was collected and frozen at -20°C until later analysis. For cows undergoing intensive ROP collection, 25% of every ROP fecal sample was subsampled, placed in a sealed tub and stored as previously described. At the end of each 24 h period, the accumulated feces in each tub were mixed thoroughly, subsampled and frozen at -20°C.

Frozen fecal samples were thawed and dried at 60°C for 96 h in a forced-air oven to determine DM content. Dried samples were ground through a 1-mm screen (Cyclotec Tecator 1093 Sample Mill, Foss Analytical, Denmark) and composited by cow for the 5-d collection period. Analytical lab DM was subsequently determined using AOAC method 934.01 (1990). Composites were prepared for N and P analyses according to AOAC method 968.08 (1990) and AOAC method 965.17 (1990), respectively. Nitrogen concentration was determined using a Leco CNS 2000 (LECO Corporation, St. Joseph, MI) and P concentration analyzed by ICP-OES with the Vista MPX.

On day 1 (first day of the adjustment period) of each phase of the digestibility study, indwelling bladder catheters were placed in the urethra of all cows to facilitate total urine collection. Catheters were attached to the collection tubing on day 3 of the adjustment period and connected to capped carboys containing 200 ml sulphuric acid for prevention of N volatilization at 0 h on day 1 of the collection period. At the end of each 24 h period, carboys containing urine were replaced with clean carboys and acidified urine was measured for volume, mixed, and subsampled for nutrient and purine derivatives (PD) analyses. The 2 ml PD subsamples were diluted with a 1:5 ratio of urine:distilled water and subsequently frozen at -20°C.

Thawed urine samples were composited by cow across the 5-d digestibility study collection period. Urine samples for nutrient determination were submitted for N analysis using the Leco CNS 2000, whereas P concentration was determined by diluting approximately 0.5 ml of filtered urine with 4.5 ml deionized water (modified AOAC 968.08, 1990) prior to analysis by ICP-OES (Vista MPX). To estimate microbial protein flow to the duodenum, concentrations of the purine derivatives allantoin and uric acid were determined from thawed urine PD samples. Allantoin and uric acid were analyzed following the colorimetric and uricase methods, respectively, as described by Chen and Gomes (1992).

4.2.8. Statistical Analysis

All data were analyzed using MIXED model procedures of SAS Institute, Inc. (2008). The model included the fixed effects of diet and season, and the interaction of diet with season. The effects of cow within diet and cow within season were considered

random. Analysis was performed using the following model: $y_{ijk} = \mu + d_i + s_j + ds_{ij} + e_{ijk}$, where d_i = effect of the i^{th} diet, s_j = effect of the j^{th} season, ds_{ij} = interaction effects of the i^{th} diet and j^{th} season, and e_{ijk} = error deviation of the k^{th} cow of the i^{th} diet in the j^{th} season. In the CH_4 study, i = 1 to 3 (CON, 10%DDGS and 20%DDGS0, j = 1 to 2 (fall and winter), and k = 1 to 10. These parameters were the same in the digestibility study except k = 1 to 8, whereas in the ROP study i = 1 to 2 (CON and 20%), j = 1 to 2, and k = 1 to 4. Least square means were separated using the Bonferonni adjustment and significance was set at $P \leq 0.05$. Trends were identified at $0.05 < P \leq 0.10$. Variance of homogeneity of studentized residuals was tested, as well as normality of distribution using Proc Univariate of SAS (SAS Institute, Inc., 2008). Results were reported as least square means ± standard errors of least square means (SEM).

Outliers were identified during statistical analysis using the variance of homogeneity of studentized residuals with an acceptance range set at ± 3.0 rather than ±2.0 in order to avoid removing several data points from data sets with greater variability. Data points just outside of the ±3.0 range (≤ 3.5 and ≥ 3.0) were removed from each data set only if the normality of distribution was not normal and/or if there was a known issue associated with that data point, such as low DMI. If there was no known reason to remove a data point and the data were normally distributed, it remained in the data set. If a data point just outside of the ±3.0 range was removed, it was ensured that the significance outcomes did not change for diet, season or diet x season, and if they did change, removal of the data point was reconsidered.

The GrowSafe failed to record accurate DMI at certain time points throughout the study. To ensure consistent data management, all intake measurements from cows with

ad libitum DMI < 1.0% of BW were removed from the 10%DDGS and 20%DDGS treatments. Since CON cows consumed diets with low ACP content, cows in this treatment with ad libitum DMI < 1.0% BW were removed only if their DMI $BW^{0.75}$ d^{-1} was lower than the graphical values published by the National Research Council (NRC, 1987). Data for one CON and three 20%DDGS cows were removed from the fall CH_4 study for these reasons.

4.3. RESULTS AND DISCUSSION

Beef cows in western Canada are normally fed low quality forage during the overwintering period, which spans from weaning to the third trimester of pregnancy. Of the cattle categories studied, mature, dry cows are considered to be the least susceptible to cold stress (Young, 1981). Mature beef cows with thick, dry winter coats are subject to cold stress when exposed to ambient temperatures lower than -7.8°C (Marston et al., 1998). In western Canada, sub-zero temperatures may persist for four to five consecutive months, usually from November through March. The present study spanned from October through February, therefore beef cows were examined under thermal-neutral and cold-stressed conditions that are typical of western Canada. Daily ambient temperatures during the fall and winter periods of this study averaged 7.3 and -17.7°C, respectively. Average minimum and maximum daily temperatures were 2.7 and 13.8°C in the fall, and -23.5 and -11.0°C in winter. There was a 21.1°C difference between the warmest and coldest daily ambient temperatures (19.0 to -2.1°C) recorded in the fall, and a -38.6°C difference (0.6 to -37.9°C) in the winter. These extreme temperatures demonstrate the importance of identifying the impact of cold acclimatization on cattle overwintered in Prairie Canada.

4.3.1. Nutrient Utilization

4.3.1.1. Influence of Cold Acclimatization on Nutrient Intake

Dry matter intake was measured to determine if cold acclimatization improved nutrient intake of low-quality forage diets in terms of meeting or exceeding animal

nutrient requirements. Rumen NH_3-N was measured to determine if rumen microbial growth and function improved in cold-acclimatized compared to thermal neutral cows. Particulate and fluid passage rates were measured to determine if potential improvements in nutrient intake were attributed to cold acclimatization increasing the ROP of low-quality forages.

Contrary to expectation, DMI did not differ between thermal-neutral and cold-acclimatized cows in this study (Table 3), nor did the proportion of forage and DDGS consumption differ between seasons. Forage and DDGS DMI were 10.6 ± 0.57 and 1.9 ± 0.11 kg hd^{-1} d^{-1}, and 10.5 ± 0.50 and 1.9 ± 0.09 kg hd^{-1} d^{-1} in the fall and winter, respectively. Similar to DMI, ACPI did not differ between seasons (Table 3).

Cows fed CON diets to 85% of ad libitum feedlot intake had restricted-fed available N (Table 4) and P (Table 5) intakes that were less than animal requirements during the digestibility phase (Figures 2 and 3, respectively; NRC, 1996). Cows fed the 10% DDGS diet had restricted-fed N and P intakes close to requirements. Mean restricted-fed N and P intakes of cows fed 20%DDGS at 85% of ad libitum intake appear to be different for animals in the thermal-neutral as compared to the cold-stressed environment. Review of the data shows that data from three cows with low DMI during the digestibility study in the thermal-neutral period was removed. As such, a statistical increase in restricted-fed N and P intake was observed in the thermal neutral period, but not in the cold-stressed period, for cows fed 20%DDGS as compared to 10% DDGS.

In general, beef cows in a cold-stressed environment require 2% higher energy intake than when in a thermal-neutral environment for every 1° C decrease in ambient temperature below their lower-critical temperature (equivalent to -7.8°C; Marston et al.,

Table 3: Dry matter intake, available CP intake, energy intake (DM basis), serum urea nitrogen and enteric methane production values of feedlot cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.

	Diet			Season		P-values		
	CON	10% DDGS	20% DDGS	Fall	Winter	D	S	D x S
Intakes and SUN								
No. Observations (n)	34	36	26	44	52			
Dry matter intake, kg d^{-1}	10.8 ± 0.68	11.7 ± 0.66	12.9 ± 0.80	11.9 ± 0.62	11.7 ± 0.55	0.1622	0.8391	0.7501
Available CP intake, g d^{-1}	576.7 ± 52.52a	918.9 ± 50.96b	1332.5 ± 61.62c	937.1 ± 47.59	948.2 ± 42.46	<0.0001	0.8626	0.5133
NE_m intake, Mcal d^{-1}	12.5 ± 0.81	14.1 ± 0.79	15.5 ± 0.95	14.3 ± 0.74	13.7 ± 0.66	0.0677	0.5773	0.4997
GE intake, Mcal d^{-1}	44.2 ± 2.78	47.9 ± 2.69	53.2 ± 3.26	48.8 ± 2.51	48.1 ± 2.24	0.1169	0.8323	0.6699
No. Observations (n)	34	36	26	47	49			
Dry matter intake, % BW	1.64 ± 0.106	1.74 ± 0.103	1.94 ± 0.124	1.82 ± 0.096	1.73 ± 0.086	0.2000	0.4797	0.7071
No. Observations (n)	34	35	26	43	52			
SUN, mmol L^{-1}	1.6 ± 0.14a	3.1 ± 0.14b	4.3 ± 0.16c	2.5 ± 0.13d	3.4 ± 0.11e	<0.0001	<0.0001	0.0583
Methane								
No. Observations (n)	30	33	24	36	51			
Methane, L d^{-1}	294.2 ± 8.54	311.9 ± 8.29	280.9 ± 10.02	334.7 ± 7.95e	256.7 ± 6.67d	0.0632	<0.0001	0.8356
Methane, L kg BW^{-1} d^{-1}	0.44 ± 0.010ab	0.45 ± 0.009b	0.42 ± 0.011a	0.50 ± 0.009e	0.37 ± 0.007d	0.0508	<0.0001	0.6583
No. Observations (n)	30	31	24	34	51			
Methane, L kg DMI^{-1} d^{-1}	30.6 ± 1.70b	30.6 ± 1.69b	23.9 ± 1.99a	31.7 ± 1.60e	25.0 ± 1.33d	0.0221	0.0025	0.3392
No. Observations (n)	30	32	25	36	51			
Methane, % GEI	6.5 ± 0.33ab	6.8 ± 0.33b	5.3 ± 0.38a	7.1 ± 0.30e	5.2 ± 0.26d	0.0128	<0.0001	0.2468

a, b, c Means within diet followed by a different letter differ ($P < 0.05$).
d, e Means within season followed by a different letter differ ($P < 0.05$).

Table 4: Nitrogen parameters of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20%DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.

	Diet				Season			P-values		
	CON	10%DDGS	20%DDGS	SEM	Fall	Winter	SEM	D	S	D x S
No. Observations (n)	15	15	13		20	23				
N intake,[1] g d^{-1} DM	71.4a	129.3b	173.5c	6.24	124.1	125.4	5.09	<0.0001	0.8526	0.0044
Available N intake, g d^{-1} DM	68.0a	116.9b	145.4c	6.73	113.6	106.6	5.50	<0.0001	0.3767	0.0008
Fecal N, g 100 g^{-1} DM	1.11a	1.37b	1.69c	0.028	1.40	1.38	0.023	<0.0001	0.5915	0.2391
Urine N, g 100 mL^{-1}	0.25a	0.65b	0.83c	0.041	0.48d	0.67e	0.033	<0.0001	0.0002	0.0004
Fecal N excretion, g d^{-1} DM	41.7a	55.8b	62.6b	3.13	49.8	57.0	2.56	0.0001	0.0524	0.9962
Urine N excretion, g d^{-1}	17.9a	53.4b	91.3c	3.33	48.6d	59.8e	2.72	<0.0001	0.0054	0.0050
Fecal N excretion, % of total	70.1a	51.2b	41.2c	2.17	53.8	54.6	1.78	<0.0001	0.7618	0.0154
Urine N excretion, % of total	29.9a	48.8b	58.8c	2.17	46.2	45.4	1.78	<0.0001	0.7618	0.0154
Apparent N digestibility,[2] %	40.6a	57.1b	61.2b	2.45	53.4	52.5	2.00	<0.0001	0.7366	0.0110
N retention,[3] g d^{-1}	11.9	20.1	19.5	5.20	25.7e	8.6d	4.25	0.4530	0.0075	<0.0001

[1] Restricted intake during digestibility study
[2] Apparent N digestibility = ((N intake – fecal N excretion) / N intake) * 100
[3] N retention = N intake – (fecal N excretion + urine N excretion)

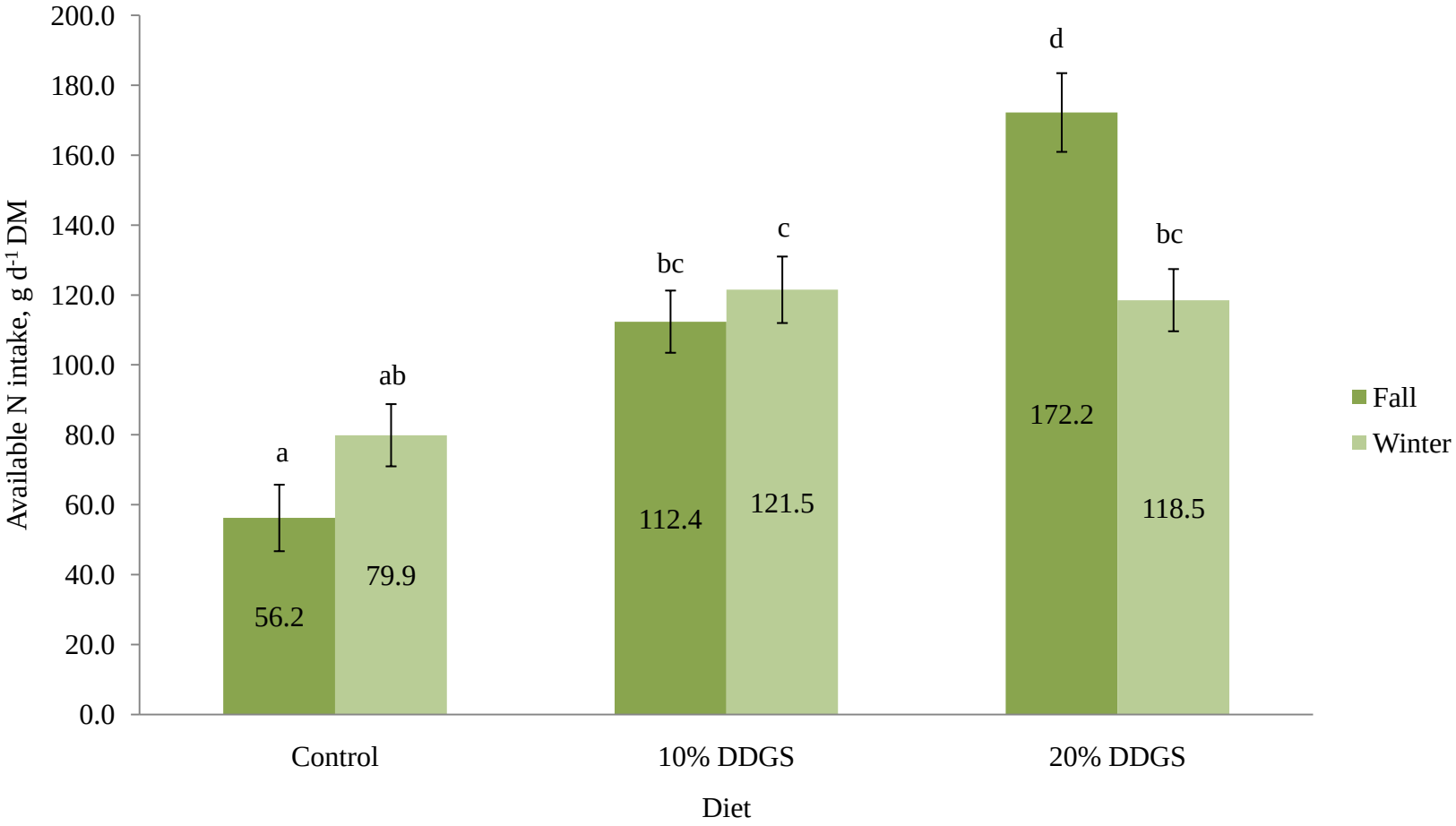
a, b, c Means within diet followed by a different letter differ ($P < 0.05$).
d, e Means within season followed by a different letter differ ($P < 0.05$).

Table 5: Phosphorus parameters and body weight loss of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.

	Diet				Season			P-values		
	CON	10%DDGS	20%DDGS	SEM	Fall	Winter	SEM	D	S	D x S
No. Observations (n)	15	15	13		20	23				
P intake,[1] g d^{-1} DM	9.3a	19.2b	26.8c	0.94	18.4	18.5	0.77	<0.0001	0.8740	0.0025
Fecal P, g kg^{-1} DM	3.0a	4.7b	6.1c	0.24	4.4	4.8	0.20	<0.0001	0.1660	0.7823
Urine P, g L^{-1}	0.04a	0.43a	1.16b	0.128	0.36d	0.74e	0.105	<0.0001	0.0129	0.1067
Fecal P excretion, g d^{-1}	11.5a	19.3b	25.2c	1.52	17.6	19.7	1.24	<0.0001	0.2299	0.1423
Urine P excretion, g d^{-1}	0.3a	3.8a	13.4b	1.56	4.1	7.6	1.28	<0.0001	0.0668	0.2392
Fecal P excretion, % of total	96.6a	84.8b	66.5c	3.69	85.4	79.8	3.01	<0.0001	0.1977	0.4621
Urine P excretion, % of total	5.6a	15.8a	33.5b	4.47	14.6	22.0	3.65	0.0005	0.1592	0.7852
Apparent P digestibility,[2] %	-23.9a	0.2b	5.7b	5.95	-1.4	-10.6	4.86	0.0025	0.1899	0.7407
P retention,[3] g d^{-1}	-2.6a	-3.8a	-11.8b	1.01	-3.4d	-8.8e	0.83	<0.0001	<0.0001	0.0078
Body weight[4]										
No. Observations (n)	9	11	9		21	8				
BW loss, kg d^{-1}	-3.0	-3.4	-3.2	1.13	-3.3	-3.0	0.90	0.9561	0.8332	0.3861

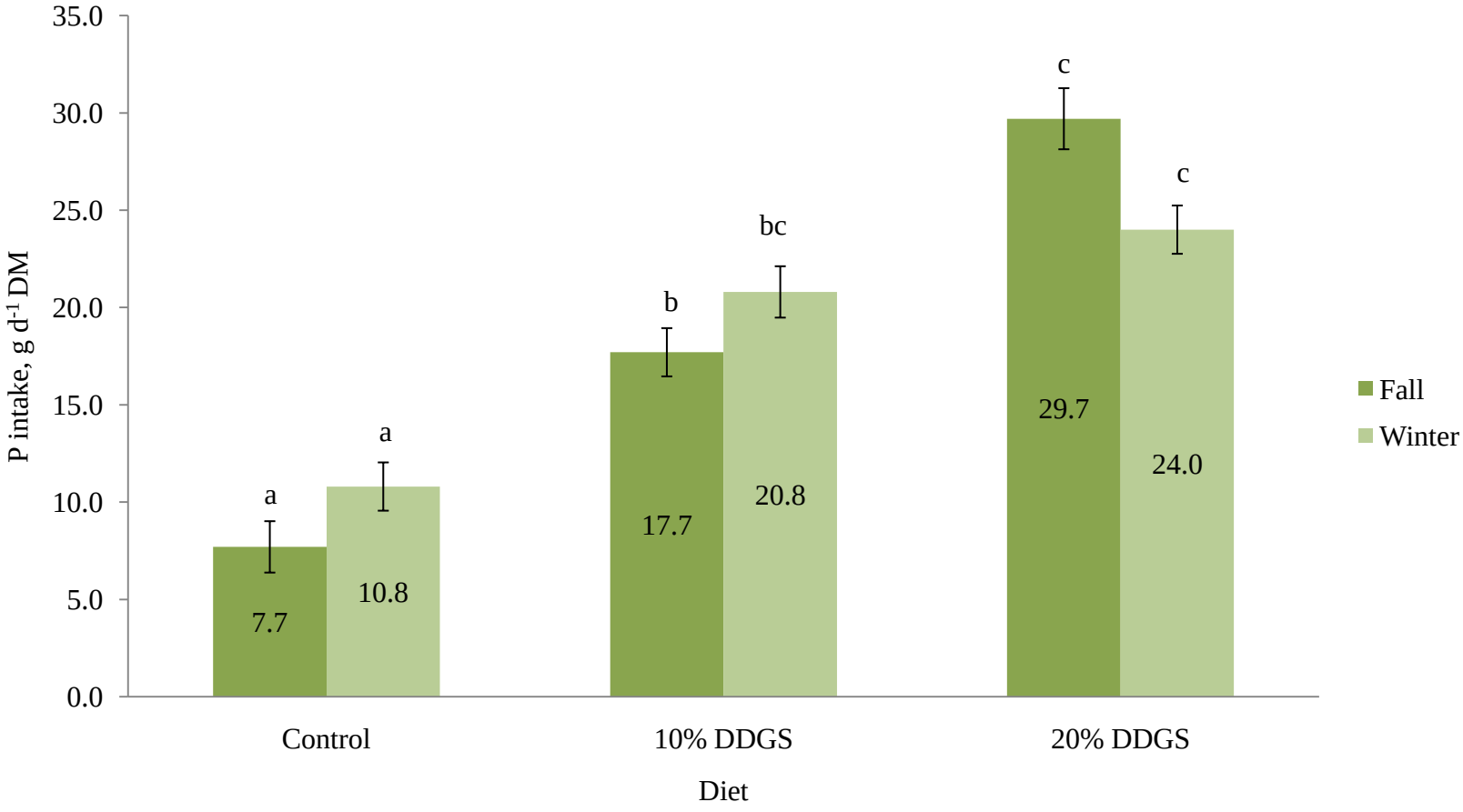
[1] Restricted intake during digestibility study
[2] Apparent P digestibility = ((P intake – fecal P excretion) / P intake) * 100
[3] P retention = P intake – (fecal P excretion + urine P excretion)
[4] BW was measured upon entry and exit from the metabolism unit during the fall and winter periods of the digestibility study. Weigh scale breakdown during phase 1 and 2 of the winter digestibility study permitted acquisition of BW in winter phase 3 only.
a, b, c Means within diet followed by a different letter differ ($P < 0.05$).
d, e Means within season followed by a different letter differ ($P < 0.05$).

Figure 2: Diet by season interaction for restricted-fed available nitrogen intake in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



a, b, c, d Means within diet and season followed by a different letter differ ($P < 0.05$).

Figure 3: Diet by season interaction for restricted-fed phosphorus intake in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



a, b, c Means within diet and season followed by a different letter differ ($P < 0.05$).

1998; Tarr, 2007). Given that energy intake (which was adequate for meeting energy requirements of thermal-neutral cows) did not differ between thermal-neutral and cold-acclimatized cows in the present study (Table 3), this suggests that the energy requirements of cold acclimatized cows were not satisfied by dietary energy intake and therefore energy may have been limiting on rumen microbial function and animal metabolism. Since ACPI did not increase with cold acclimatization, rumen NH_3-N concentrations also remained unchanged, which suggests that microbial growth and function persisted at a similar rate between thermal-neutral and cold-acclimatized cows.

Although it was unexpected that cold-acclimatized cattle in the present study did not increase DMI, and therefore nutrient intake, literature reports indicate considerable variability in DMI between thermal-neutral and cold-acclimatized cattle. The NRC (1981) indicates that DMI becomes more variable and less predictable as ambient temperatures stray from 20°C, and that intake increases as environmental temperature decreases below 15°C. Conversely, Adams et al. (1986) reported that DMI of beef cows grazing native range pasture and exposed to temperatures between 0 and -35°C from mid-November to end of December was reduced by 39% and 44% in 3- and 6-yr old cows, respectively, in accordance with shorter grazing time as minimum daily temperatures decreased.

Cold acclimatized cows lost -111.8 ± 12.82 kg BW over the course of the winter period of the study, as compared to thermal neutral cows which lost -108.1 ± 12.57 kg BW by the end of the fall period ($P = 0.8367$). Further, cold-acclimatized cows lost -0.22 BCS, whereas thermal-neutral cows gained 0.08 BCS ($P < 0.001$), suggesting that mobilization of body reserves was greater during the winter. Graham et al. (1959) and

Thompson and Clough (1972) reported that cold-acclimatized ruminants mobilize and oxidize body stores of adipose tissue to obtain free fatty acids to supply energy for heat production and increased metabolism. This was demonstrated by Young (1975), who fed constant DMI (1.5 kg pelleted alfalfa hay and 3 kg barley grain mixture) for five, 8-wk periods to two non-pregnant, mature beef cows acclimated to warm (20 ± 3°C; periods 1, 3 and 5) or cold (-10 ± 2°C and -25 ± 4°C; periods 2 and 4, respectively) temperatures and reported substantial utilization and mobilization of body reserves in the cold, which resulted in significantly higher plasma free fatty acid concentrations when cows were cold-acclimated (to both -10 and -25°C) than when acclimated to thermal-neutral temperatures. Given that cold-exposed cattle have higher NE_m requirements than thermal-neutral cattle (Ames et al., 1994), the loss of BCS during the winter may have occurred to compensate for the lack of increase in dietary energy intake and to source energy required to meet maintenance requirements.

Cold-acclimatized cows in this study doubled their rate of post-rumen particulate passage compared to thermal-neutral cows (19.8 vs. 9.9 ± 2.04% h^{-1}; P = 0.0081). However, there was no apparent change in rumen particulate ROP and particulate TMRT, or post-rumen fluid ROP and fluid TMRT (Table 6). It is possible that the effect of season on ROP may have been limited by warmer ambient temperatures in the metabolism unit during the digestibility study. However, given that restricted-fed DMI did not decrease between the fall and winter periods of the digestibility study (1.30 vs. 1.32 ± 0.5 0% BW, respectively), and that rumen fluid and post-rumen particulate ROP in cold-acclimatized cows increased rather than decreased with introduction to warmer temperatures in the metabolism unit, temperature change was unlikely to have been an

Table 6: Particulate and fluid rate of passage of cows consuming low-quality forage (CON), and low-quality forage supplemented with 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.

	Diet			Season			P-values		
	CON	20%DDGS	SEM	Fall	Winter	SEM	D	S	D x S
Particulate									
No. Observations (n)	7	6		5	8				
TD,[1] h	0.0	2.2	0.70	1.5	0.7	0.69	0.0569	0.4207	0.4207
K, [2] % h^{-1}	1.3	1.7	0.32	1.7	1.4	0.31	0.3412	0.5544	0.7453
RMRT, [3] h	87.8	79.1	15.93	80.7	86.2	15.84	0.7111	0.8131	0.2859
L, [4] % h^{-1}	13.5	16.1	2.05	9.9d	19.8e	2.04	0.3978	0.0081	0.6380
FMRT, [5] h	32.3	30.6	2.74	41.3e	21.6d	2.73	0.6662	0.0007	0.9603
TMRT, [6] h	120.1	111.9	13.69	123.5	108.5	13.61	0.6820	0.4590	0.2089
Fluid									
No. Observations (n)	7	6		5	8				
TD, h	9.8	10.1	1.16	9.6	10.3	1.16	0.8460	0.7180	0.8575
K, % h^{-1}	8.1	9.2	0.37	7.4d	9.9e	0.37	0.0618	0.0009	0.3295
RMRT, h	12.7	11.4	0.64	13.9e	10.2d	0.63	0.1735	0.0027	0.6908
L, % h^{-1}	75.2	176.6	47.26	136.2	115.6	46.98	0.1641	0.7660	0.6420
FMRT, h	5.8	5.2	1.35	5.6	5.4	1.34	0.7657	0.9069	0.9382
TMRT, h	28.3	26.7	1.40	29.2	25.9	1.40	0.4446	0.1310	0.6891

[1] TD = Time delay to first appearance of marker in feces
[2] K = Rumen passage rate
[3] RMRT = Rumen mean retention time (1/K)
[4] L = Post-rumen passage rate
[5] FMRT = Post-rumen mean retention time (n/L; where age independent model GnG1 = G4G1)
[6] TMRT = Total tract mean retention time (RMRT + FMRT + TD)
a, b, c Means within diet followed by a different letter differ ($P < 0.05$).
d, e Means within season followed by a different letter differ ($P < 0.05$).

issue. Rumen and post-rumen ROP values for fluid and particulate fractions were comparable to those reported by several researchers (Kennedy et al., 1985; Okine et al., 1989; Moore et al., 1992; Villalobos et al., 1997; Zebeli et al., 2003). Ruminal fluid passage rate was 33.8% faster (7.4 vs. 9.9 ± 0.37% h^{-1}; P = 0.0009) in winter than fall.

Exposure to cold temperatures increases the duration (Okine et al., 1989) and frequency of biphasic contractions in the reticulorumen (Westra and Christopherson, 1976; Kennedy, 1985). Given that rumen fluid ROP increased, this suggests that the duration and frequency of reticulorumen contractions did increase in cold-acclimatized cows, even though the filtration effects of the rumen mat prevented an increase in rumen particulate ROP. Although body fluid volume can decrease in cold-acclimatized cattle and sheep (Young, 1975; Degen and Young, 1980), and thereby contribute to an increase in passage rates (Robles, et al., 1981), rumen fluid volume has also been reported to remain unchanged in cold-acclimatized cattle, leaving increased reticulorumen contractions as the cause of increased ROP (Miaron and Christopherson, 1992). As neither reticulorumen contractions nor body fluid volume was measured in the present study, it is uncertain which parameter was more influential on the increased rumen fluid ROP observed in cold-acclimatized cows. However, given that literature reports describe an overwhelming consensus that cold-acclimatized cattle increase ROP due to greater reticulorumen contractions, this suggests that faster rumen fluid ROP in the present study is probably caused, at least to some extent, by increased reticulorumen contractions.

These findings are similar to those observed by Kennedy (1985) where digesta TMRT did not change when shorn sheep were exposed to temperatures of 22 to 25°C (warm) and 1 to 4°C (cold). Further, Barboza et al. (2006) exposed muskoxen to the

climate in central Alaska for one year and did not observe changes in particulate RMRT between seasons. However, unlike the results of this study, Barboza et al. (2006) observed no change in post-rumen particulate ROP. This may be due to the observed slower passage rates in muskoxen compared to cattle (Adamczewski et al., 1994a, 1994b).

Researchers have associated faster or slower passage rates of ruminants in cold environments with increased or decreased DMI, respectively (Kennedy et al., 1977; Kennedy, 1985; Barboza et al., 2006). The response of intake in cold-acclimatized ruminants has been inconsistent, as some researchers have identified increased intakes with faster ROP in sheep and cattle (Kennedy et al., 1977; Kennedy, 1985; Nisa et al., 1999) whereas others have reported lower intakes with slower ROP in cattle (Montgomery et al., 2004) compared to intakes in warm environments. The relationship between particulate ROP and DMI is rumen fill, as consistent particulate ROP maintains rumen fill and prevents increases in DMI (Blaxter et al., 1956). Given that particulate passage from the rumen, RMRT and TMRT remained the same between seasons of the present study, this suggests that consumed feedstuffs passed through the total digestive tract at a similar rate, which permitted rumen and total GIT fill to persist for similar durations in fall and winter, and prevented increased DMI.

Contrary to expectations, cold acclimatization failed to increase nutrient intake, therefore cold-acclimatized cows consumed inadequate energy to meet increased requirements during cold exposure. Given that rumen NH_3-N concentrations were unchanged between seasons, this suggests that cold acclimatization did not affect microbial growth and function, and therefore microbial degradation of the low-quality

forage diets was not improved. In turn, particulate ROP was unaffected by cold acclimatization with consistent DMI between thermal-neutral and cold-acclimatized cows. Given that fluid ROP increased by 33.8% in response to cold acclimatization, this suggests that particulate ROP also could have increased, therefore permitting increased DMI, if microbial dietary degradation had not been limited with inadequate rumen NH_3-N and/or energy. Since cold-acclimatized cows have greater nutrient requirements for maintenance than thermal-neutral cows (Tarr, 2007), these data suggest that increases in DMI in cold-acclimatized cows fed low-quality forage will be obtained only when dietary CP and energy content are high enough to satisfy rumen microbial requirements for increased dietary degradation.

4.3.1.2. Influence of Protein Supplementation on Nutrient Intake

Dry matter intake was measured to determine if CP supplementation improved nutrient intake of low-quality forage diets. Rumen NH_3-N and particulate and fluid passage rates were also monitored to further validate the effect of CP supplementation on DMI.

Protein supplementation did not increase total DMI (Table 3), nor did forage intake change when DDGS was fed. Control cows consumed 10.8 ± 0.68 kg hd^{-1} d^{-1} of forage, 10%DDGS cows consumed 10.6 ± 0.60 kg hd^{-1} d^{-1} of forage and 1.2 ± 0.09 kg hd^{-1} d^{-1} of DDGS, and 20%DDGS cows consumed 10.3 ± 0.73 and 2.6 ± 0.11 kg hd^{-1} d^{-1} of forage and DDGS, respectively. The increase in DDGS DMI was significant across diets ($P < 0.0001$). Although sorting of DDGS from the TMR was possible, actual DDGS intakes were comparable to expected DDGS intakes because frequent feeding constantly

provided appropriately mixed TMR and did not present the opportunity for cows to sort and selectively consume more DDGS that desired. However, DMI did not exceed 2.0% BW for all cows despite CP supplementation. As expected, increases in available CP intake (ACPI) were positive and linear (576.7 ± 52.52, 918.9 ± 50.96 and 1332.5 ± 61.62 g d^{-1} DM for CON, 10%DDGS and 20%DDGS, respectively; $P < 0.0001$; Table 3).

Several factors influence DMI for mature beef cows, including breed, body weight, diet composition, water intake, body composition (fat vs. lean), season, and environmental factors including precipitation, wind chill, humidity and ambient temperature (Bines et al., 1969; Hicks et al., 1990; Mader, 2003). Literature reports of change in DMI in response to protein supplementation to cows and steers have been variable. Protein supplementation has been shown to increase DMI in beef cows consuming 1.9% CP grass forage supplemented with 48.6, 80.5 and 110.9 g N d^{-1}, but no further increases were observed with additional supplementation to 137.8 g N d^{-1} (Köster et al., 1996). Schauer et al. (2005) reported that pregnant beef cows grazing native grass ranging from 5.5 to 9.3% CP (DM) experienced no change in DMI when supplemented with 43% CP cottonseed meal at 0, 4 and 6% (0, 391 and 470 g CP d^{-1}, respectively) of the diet, but DMI tended to be lower for supplemented versus unsupplemented cows. Similar to the current study, Gilbery et al. (2006) fed beef steers 3.3% CP forage mixed with 0, 5, 10 or 15% corn condensed distillers solubles (15.4% CP) and found no difference in forage DMI (% BW) with increasing protein supplementation. These literature reports suggest that DMI eventually reaches a plateau with greater CP supplementation and that factors other than dietary CP content may be limiting on DMI of beef cattle consuming low-quality forages.

Thermal neutral, mature, dry beef cows with a mature BW of 636.4 kg have CP and P requirements ranging from 6.8 to 8.9% CP and 0.13 to 0.17% P (DM basis) between 8 and 12 months post-partum; translating into N and P requirements of 126.1 g N d^{-1} DM and 15.1 g P d^{-1} DM at eight months post-partum (NRC, 1996). The beef cows in the current study were an average of eight months post-partum during the thermal-neutral period (and 10 months post-partum during the cold-stressed period), therefore the diets offered deficient, borderline adequate and sufficient N (71.4, 129.3 and 173.5 ± 6.24 g N d^{-1} DM for CON, 10%DDGS and 20%DDGS, respectively; $P < 0.0001$; Table 4) and P (9.3, 19.2 and 26.8 ± 0.94 g d^{-1} DM for CON, 10%DDGS and 20%DDGS, respectively; $P < 0.0001$; Table 5) for cow requirements when they were restricted-fed, although DMI was fed below requirement. Cows in the present study required a dietary net energy for maintenance (NE_m) content of 0.90 Mcal kg^{-1} DM, or a NE_m intake of 10.5 Mcal d^{-1} DM (NRC, 1996); therefore cow energy requirements were surpassed even with the CP deficient diet. Net energy for maintenance intake tended to differ with greater CP supplementation, with intakes of 12.5 ± 0.81, 14.1 ± 0.79 and 15.5 ± 0.95 Mcal d^{-1} DM ($P = 0.0677$, Table 3) for CON, 10%DDGS and 20%DDGS, respectively, when cows were fed ad libitum in the feedlot. This was attributed to the differences in energy density between the low-quality forage (1.18 Mcal NE_m kg^{-1}) and DDGS (1.60 Mcal NE_m kg^{-1}). Conversely, GEI did not change with CP supplementation.

Supplementation of DDGS increased NH_3-N supply to rumen microbes from 1.5 to 2.1 to 3.1 ± 0.15 mg 100 ml^{-1} rumen fluid ($P < 0.0001$; Table 7) for CON, 10%DDGS and 20%DDGS treatments, respectively. The protein deficient diet successfully generated a rumen NH_3-N deficiency for 93% of CON cows, as a minimum of 2.0 mg

NH_3-N 100 ml^{-1} rumen fluid is required to support optimum growth of rumen microbes (Satter and Slyter, 1974); the remaining 7% demonstrated borderline adequate NH_3-N concentrations for rumen microbial requirements. Supplementation with 10%DDGS provided borderline adequate CP for animal CP requirements and concurrently increased rumen NH_3-N concentration to borderline adequate levels (2.0 ± 1.0 mg 100 ml^{-1}) for microbial growth in only19% of 10%DDGS cows. Thirty-eight percent of the 10%DDGS cows had deficient rumen NH_3-N concentrations (< 1.9 mg 100 ml^{-1}) and 44% had rumen NH_3-N concentrations > 2.1 mg 100 ml^{-1}. Rumen NH_3-N concentrations for the excess protein (20%DDGS) diet was within the acceptable range of 2.0 to 5.0 mg NH_3-N 100 ml^{-1} rumen fluid required to permit optimum microbial growth (Satter and Slyter, 1974; Slyter et al., 1979) for 93% of cows, whereas 7% of the 20%DDGS cows were reported to have deficient (< 2.0 mg 100 ml^{-1}) rumen NH_3-N concentration. Although microbial growth occurs with ruminal NH_3-N concentrations between 2.0 and 5.0 mg 100 ml^{-1} rumen fluid, it is not maximized until concentrations reach the range of 5.0 mg 100 ml^{-1} (Satter and Slyter, 1974) to 13.3 mg 100 ml^{-1} (Hume et al., 1970) of rumen fluid.

Consistent DMI between diets may have been attributed to rumen NH_3-N. According to Song and Kennelly (1989), the NH_3-N concentrations required for maximal microbial growth differ from the concentrations required for maximum dietary degradation. Although studies have indicated that the lowest concentration of rumen NH_3-N to support microbial growth is accepted at 2.0 mg 100 ml^{-1} of rumen fluid (Satter and Slyter, 1974; Slyter et al., 1979), several authors (Krebs and Leng, 1984; Boniface et al., 1986; Perdok et al., 1988) have concluded that the minimum threshold for promoting

Table 7: Rumen pH, ammonia-N and volatile fatty acid concentrations of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.

	Diet				Season			P-values		
	CON	10%DDGS	20%DDGS	SEM	Fall	Winter	SEM	D	S	D x S
No. Observations (n)	15	15	14		24	20				
Rumen pH	6.90	6.84	6.89	0.022	6.88	6.87	0.018	0.1021	0.7636	0.3363
NH_3-N, mg 100 mL^{-1}	1.5a	2.1b	3.1c	0.15	2.2	2.3	0.21	<0.0001	0.6039	0.3889
VFA, mmol L^{-1}										
Acetate	46.5	47.9	45.3	1.19	44.2d	48.9e	0.97	0.3071	0.0014	0.8139
Propionate	11.2	12.3	12.0	0.36	11.1d	12.5e	0.30	0.0923	0.0023	0.9082
Isobutyrate	1.38	1.54	1.45	0.062	1.49	1.42	0.050	0.1757	0.3219	0.9604
Butyrate	5.0	5.5	5.3	0.18	5.0d	5.5e	0.15	0.1586	0.0228	0.6812
Isovalerate	0.55a	0.70b	0.76b	0.028	0.70	0.64	0.022	<0.0001	0.1050	0.7220
Valerate	0.25a	0.36b	0.38b	0.017	0.34	0.33	0.014	<0.0001	0.5495	0.5284
Total VFA	64.9	68.3	65.0	1.72	62.8d	69.3e	1.41	0.2897	0.0024	0.8671
A:P	4.18a	3.96ab	3.82b	0.079	4.04	3.93	0.064	0.0094	0.2313	0.7721

a, b, c Means within diet followed by a different letter differ ($P < 0.05$).
d, e Means within season followed by a different letter differ ($P < 0.05$).

optimum voluntary DMI of low-quality forages is closer to 20.0 mg 100 ml^{-1}. Song and Kennelly (1989) fed a 70 barley silage: 30 concentrate TMR (11.5% CP, DM basis) to non-lactating dairy cows to provide 1.6x energy (31.1 Mcal DE d^{-1}) and 2.4x CP (200 g N d^{-1}) requirements while intraruminally infusing NH_4Cl to increase rumen NH_3-N concentration. Dietary degradation was maximized with a rumen NH_3-N concentration of 16.3 mg 100 ml^{-1} rumen fluid by infusing 122 g NH_4Cl d^{-1}, which represented a dietary N intake of 263 g N d^{-1} (200 g dietary N d^{-1} + 63 g N from NH_4Cl d^{-1}) and generated BUN concentration of 6.5 mmol L^{-1} (Song and Kennelly, 1989). In comparison, the highest ad libitum CP intake and corresponding SUN concentration in the present study were only 238.1 ± 11.99 g N d^{-1} and 4.3 ± 0.16 mmol L^{-1} when CP was fed in excess of requirement. Further, the level of NH_3-N needed by microbes for dietary degradation is related to rumen pH, and therefore, forage quality (Leng, 1990). Low-quality forages, by definition, lack soluble sugars and starch and result in high (6.5 – 7.0) rumen pH, which creates a demand for higher rumen NH_3-N, as compared to concentrate-based diets which generate lower rumen pH, in order to increase microbial forage degradation and optimize DMI (Leng, 1990). Since the wheat-based DDGS fed in this study only contained 9 g kg^{-1} (0.9%) starch, the low-quality forage diets generated high rumen pH (6.90, 6.84 and 6.89 for CON, 10%DDGS and 20%DDGS, respectively; P = 0.1021; Table 7). Thus, the nutrient profile of DDGS, which included low starch and high CP content, may have been responsible for increasing rumen microbial CP and energy requirements for optimal feedstuff degradation. Accordingly, increased DMI did not occur with DDGS supplementation since microbes may have required NH_3-N concentrations closer to 20.0

mg 100 ml^{-1} rumen fluid rather than the modest 3.1 mg 100 ml^{-1} rumen fluid that was provided when CP was fed in excess of requirements.

Comparable rumen and post-rumen ROP between diets resulted in similar particulate rumen, post-rumen and total tract mean retention times (RMRT, FMRT and TMRT, respectively) between protein deficient and sufficient diets (Table 6). It is possible that the effect of DDGS inclusion on ROP may have been limited by restricted feeding during the digestibility study. With DMI restricted to 85% of ad libitum intakes, the rate at which consumed feedstuffs passed through the digestive tract may have been reduced. However, given that the restriction on DMI was proportionate between CON and 20%DDGS diets, it can be reasoned that a decline in ROP also should have been proportionate across diets. As such, if DDGS inclusion did have an effect on ROP, it should have been apparent even if the changes in ROP were less than that which would have occurred when cows were ad libitum fed.

Feedstuff particle size may be a more likely explanation for the lack of increase in particulate ROP between diets. Given that the low-quality forage diets in this study were chopped and presumably contained an abundance of large particulate matter, the rumen mat probably trapped these large particles until microbial degradation decreased particle size sufficiently ($\leq$ 1.18 mm) to permit small particulate matter to filter through the rumen mat and escape the rumen with the fluid fraction (Zebeli et al., 2007). Given that rumen particulate ROP remained constant despite CP supplementation, this suggests that microbial degradation did not increase, and that rumen fill was maintained, which prevented increased DMI (Blaxter et al., 1956; Mertens, 1977). Therefore, the constant particulate passage rates and their corresponding mean retention times indicate that the

rate at which the low-quality forage diets passed through the digestive system and the duration of GIT fill were constant despite CP supplementation (Montgomery et al., 2004). However, CP supplementation should have increased rumen NH_3-N concentrations enough to improve the rate of microbial degradation of the low-quality forage and therefore hasten particulate passage from the rumen, especially when N was fed in excess of requirements. The inability of CP supplementation to increase rumen particulate ROP, and therefore DMI, suggests that rumen microbial requirements were not adequately met. While it is possible that inadequate CP was supplied to meet rumen microbial requirements, perhaps energy was also limiting on rumen microbial requirements and additional provision of energy may have helped improve microbial degradation.

Fluid ROP from the rumen tended to be 13.6% faster in cows fed sufficient versus deficient CP (9.2% vs. 8.1 ± 0.37% h^{-1} for 20%DDGS and CON, respectively; P = 0.0618), whereas post-rumen fluid ROP, RMRT, FMRT and TMRT were unchanged (Table 6). This observed tendency for increased fluid ROP with CP supplementation is peculiar as DMI did not increase across diets, yet according to literature reports, faster rumen fluid ROP is attributed to greater DMI that occurs from increased CP intake (McCollum and Galyean, 1985; Krysl et al., 1987). Perhaps the properties of DDGS, including its highly digestible fibre content, may have been a contributing factor. In a study where beef steers were fed moderate-quality forage (10.6% CP, 65.1% NDF, 37.6% ADF, DM basis) and supplemented with corn DDGS at 0, 0.3, 0.6, 0.9 or 1.2% BW (DM basis), rumen fluid ROP also tended to increase with greater DDGS inclusion

(Lardy et al., 2009). These authors suggested that this response in fluid ROP may have been due to greater digestion of the fibre in DDGS (Lardy et al., 2009).

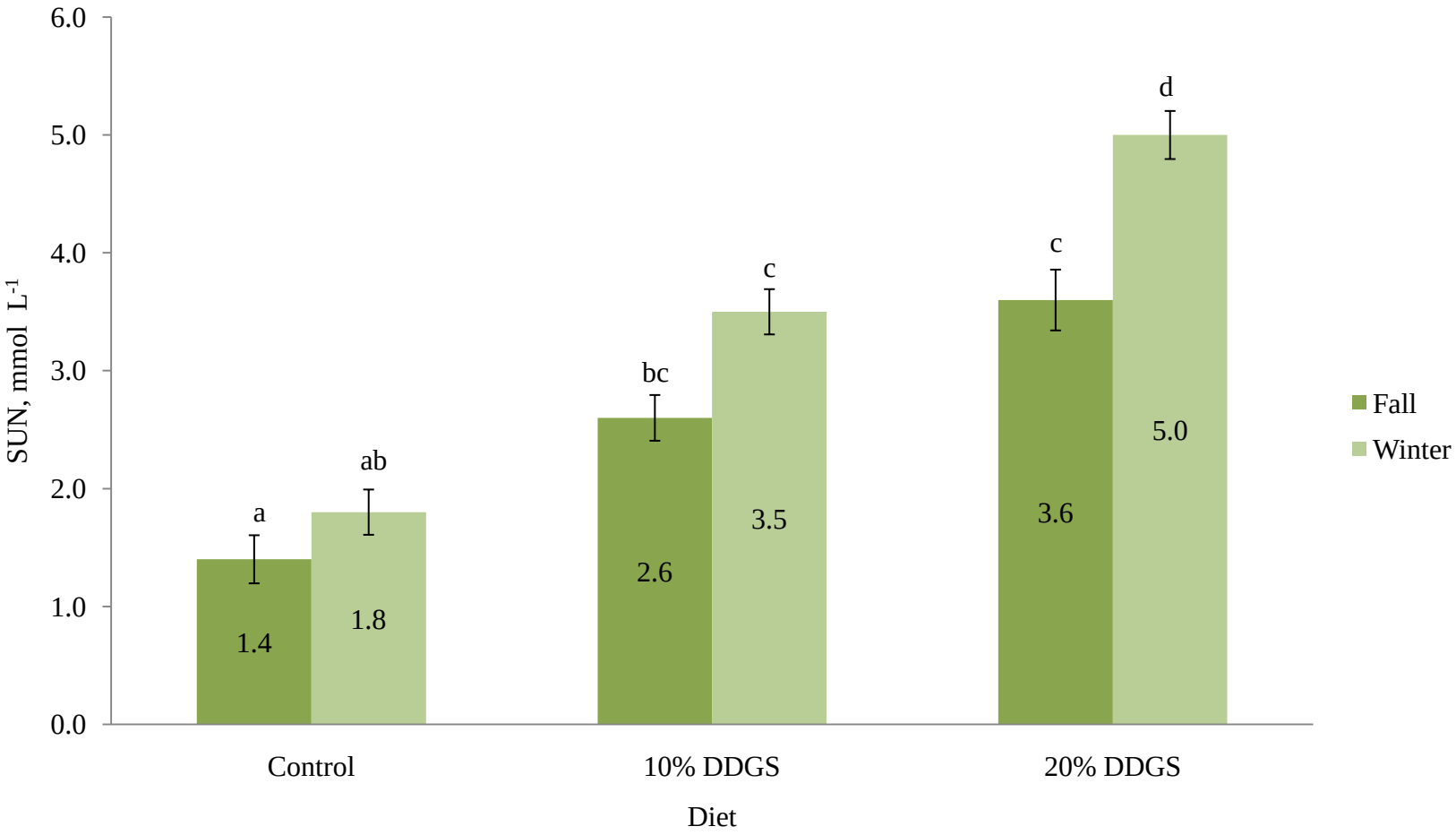
Based on animal nutrient intakes, CP supplementation with 10% and 20% DDGS was successful in providing borderline adequate and sufficient dietary N and P for cow nutrient requirements. Further, all three diets contained adequate NE_m to meet animal energy requirements with adequate NE_m intake. Protein supplementation increased ACPI, however, DMI, and therefore energy intake, failed to increase in cows when fed low-quality forage, whether supplemented with protein or not. Similar rates of rumen particulate passage prevented DMI from increasing as rumen fill persisted for similar duration between cows fed deficient and excess CP. It is known that either CP or energy can restrict microbial growth and function (Satter and Slyter, 1974), and similar rumen particulate ROP suggests that microbes were unable to increase their rate of dietary degradation. Given that DMI did not exceed 2.0% BW even when CP was fed in excess of requirements, this suggests that CP may not have been the only limiting factor affecting DMI. Rather, energy may have also been a limiting factor since the low starch content of the low-quality forage diets maintained high rumen pH and microbial NH_3-N requirements. Concurrently, CP would have been limiting by providing insufficient NH_3-N to meet this high microbial NH_3-N requirement, which failed to promote increased microbial degradation, and therefore optimal DMI, of the low-quality forage diets fed in this study. If both CP and energy were limiting for rumen microbes, then this study suggests that rumen microbial CP and energy requirements may be greater than published animal CP and energy requirements when low-quality forages are fed.

4.3.1.3. Influence of Cold Acclimatization on N and P Recycling and Digestibility

Serum urea nitrogen, rumen NH_3-N, and the digestibility of N and P were measured to determine if nutrient utilization is improved in cold-acclimatized cows consuming low-quality forage. The thyroid hormones, T_3 and T_4, were measured to validate that digestibility parameters obtained during the digestibility study were representative of normal metabolic and physiological function that occurs in cows while housed outdoors. Thyroid hormones were also measured to determine if cold acclimatization potentially increased metabolic rate.

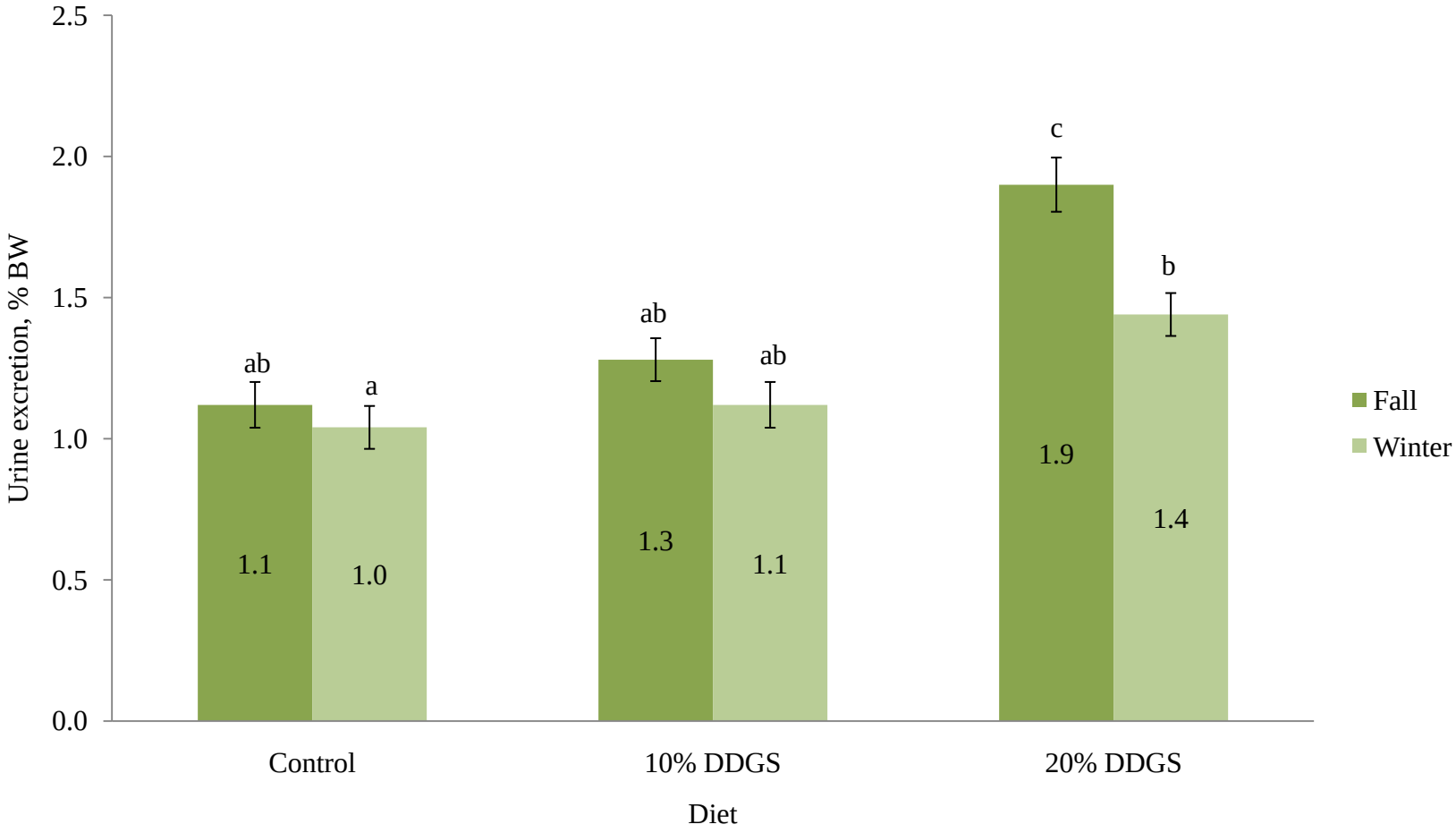
Concentrations of SUN were higher for cold-acclimatized (3.4 ± 0.11 mmol L^{-1}) versus thermal-neutral (2.5 ± 0.13 mmol L^{-1}; $P < 0.0001$; Table 3) cows even though DMI, ACPI and rumen NH_3-N did not increase. However, SUN concentrations tended to demonstrate a diet by season interaction ($P = 0.0583$; Figure 4). By study design, SUN concentrations increased linearly with greater CP supplementation, and cold-acclimatized cows had greater SUN concentrations than thermal-neutral cows, although the differences between thermal-neutral and cold-acclimatized cows was significant only with the 20%DDGS diet. The spread between thermal-neutral and cold-acclimatized SUN concentrations increased with greater CP supplementation, and this was in keeping with a similar trend observed in urine excretion (% BW), which also tended to demonstrate a diet by season interaction ($P = 0.0625$; Figure 5). Urine excretion (% BW), which reflects the changes in SUN concentrations, demonstrated no significant difference between CON and 10%DDGS diets in thermal-neutral and cold-stressed environments. However, when cows were fed the highest level of DDGS, urine excretion was higher relative to CON, with the increase being greatest in the thermal-neutral environment.

Figure 4: Diet by season interaction for serum urea nitrogen in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



a, b, c, d Means within diet and season followed by a different letter differ ($P < 0.05$).

Figure 5: Diet by season interaction for urine excretion (% BW) in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



a, b, c Means within diet and season followed by a different letter differ ($P < 0.05$).

Together, these diet by season interactions for SUN and urine excretion (% BW) suggest that the observed increase in SUN concentration in cold-acclimatized cows fed 20%DDGS occurred in response to a reduction in urine production when cows were in the cold-stressed environment.

In support of the present study, Kohn et al. (2005) evaluated 41 studies using different livestock species and rats, and concluded that BUN concentrations demonstrated a positive linear relationship to urine N excretion in all species. Greater N intake is associated with greater water intake and urine production (Devendra, 1976). In this study, N intake was similar between seasons and as such, it can be reasoned that the impact of N intake on water intake and urine production should have also remained unchanged. Rather, it is likely that reduced volume of body fluid which occurs in cold-exposed animals due to reduced water intake and circulatory volume, as well as cold-induced vasoconstriction, may be responsible for the observed reduction in urine production (Young, 1975; Degen and Young, 1980).

Urea reabsorption increases in the renal pelvis when urine production is low (Pfeiffer, 1968; Godwin and Williams, 1984), the observed increase in SUN concentration may be related to an improved efficiency of N recovery by the kidneys and concentration on N in the body of cold-acclimatized cows. Degen and Young (1980) ad libitum and restricted-fed (1x maintenance) shorn sheep a pelleted, concentrate ration during four - 10-d periods of warm (21°C, periods 1 and 4) or cold (0°C, periods 2 and 3)exposure and reported that ad libitum fed, cold-exposed sheep maintained their body weight and fluid content, while restricted-fed, cold-exposed sheep lost 2.53 kg BW, 1.68 L (representing 66% of total BW loss) of total body water, 1.32 L of reticulorumen fluid,

0.39 L of interstitial fluid and 0.13 L of plasma during the first eight days of cold exposure. The inability for ad libitum fed sheep to gain BW and the ability of restricted-fed sheep to lose body fluid was attributed to dramatic reductions in water intake during cold exposure (Degen and Young, 1980). Although changes in water intake and body fluid volume were not monitored during the present study, these literature reports considered with the fact that urine production was significantly reduced during the winter suggests that cold-acclimatized cows in the present study may have experienced a reduction in body fluid volume.

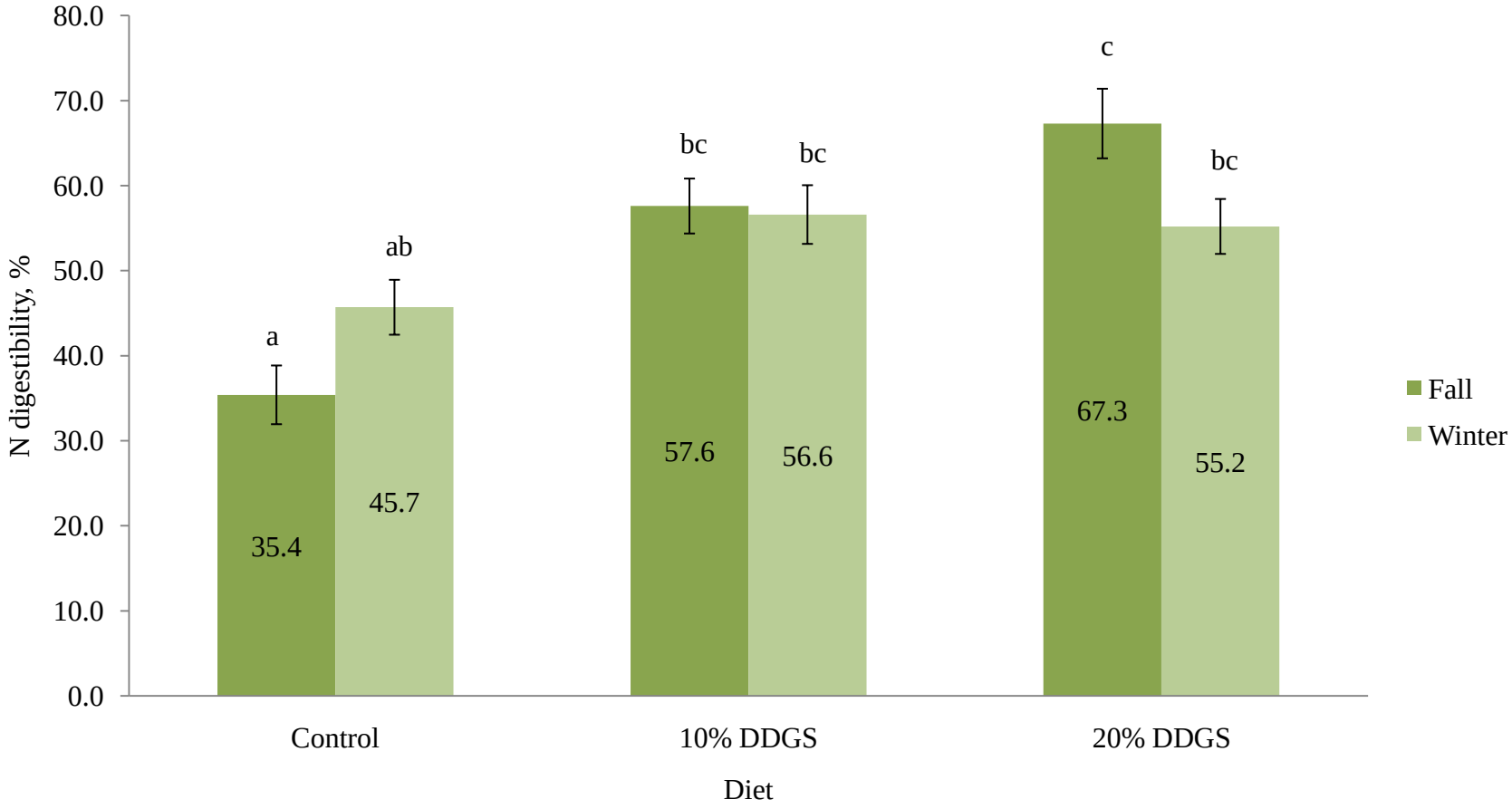
Several literature reports agree with the observed increase in SUN and decrease in urine production in cold-acclimatized, 20%DDGS cows during the present study. Godwin and Williams (1984) fed sheep 23.8 or 29.7 g N d^{-1} and observed that within each intake level, animals that excreted less urine had correspondingly higher plasma urea nitrogen concentrations. In a two year study, Manninen et al. (2007) fed grass silage-based diets (775 and 897 g CP d^{-1} in yr 1 and 2, respectively) to beef heifers housed in cold outdoor temperatures of 0 to -37°C, cold indoor temperatures that were 5 to 7°C warmer than outdoor temperatures, or warm indoor temperatures of 10 to 15°C, and reported significantly higher SUN concentrations in both groups of cold-acclimatized heifers. Similarly, shorn sheep exposed to temperatures from 21.1 to -1.1°C had lower water intake and correspondingly lower urine production in the cold (Pontius et al., 1932). Studies with muskrats have found that urine production decreases in cold environments due to lower water intake (Campbell and MacArthur, 1997), and as observed in other mammalian species, higher concentrations of blood urea can be

attributed to greater urea resorption by the urinary system in response to lower urine volume (Nelson et al., 1975; Harlow 1987).

Apparent N digestibility demonstrated a diet by season interaction (P = 0.0110; Figure 6). Cows fed 10%DDGS demonstrated equal N digestibilities between seasons, and N digestibility was also similar between 10% and 20%DDGS cows in both seasons, demonstrating that improvements in N digestibility plateaued once CP was supplemented to borderline adequate levels for CP requirements. However, thermal-neutral CON cows had significantly lower N digestibility than thermal-neutral and cold-acclimatized cows fed 20%DDGS, which is presumably due to the inadequate CP intake by CON cows and the inclusion of DDGS, which contains highly digestible CP, in the 20%DDGS diet. While apparent N digestibility increased with increased dietary DDGS, and thereby increased CP content, the rate of increase in CON cows was greater when animals were in thermal-neutral conditions. It may be that endogenous N losses were greater for cold-acclimatized cows due to their need to use body energy reserves to cope with cold conditions. Another factor may have been related to a change in rumen fluid and post-rumen particulate ROP, providing less opportunity for degradation and absorption of dietary N by cold stressed animals. Given that apparent P digestibility was consistent between thermal-neutral and cold-acclimatized cows, it is likely that the former explanation was responsible for higher apparent N digestibility in thermal-neutral than cold-acclimatized cows.

Apparent P digestibility was similar between thermal-neutral and cold-acclimatized cows of the current study (Table 5). These results are similar to those reported in the study by Sano et al. (1995), in which shorn sheep fed 15.8% CP

Figure 6: Diet by season interaction for apparent nitrogen digestibility in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



a, b, c Means within diet and season followed by a different letter differ ($P < 0.05$).

orchardgrass hay and housed in thermal-neutral (20°C) or cold (0°C) environments for 18-d each demonstrated similar N digestibilities (70.7 ± 1.9% and 72.8 ± 1.2%, respectively). There is wide consensus that cold-acclimatized ruminants experience reductions in feedstuff digestibility in response to faster digesta ROP (Christopherson, 1976; Westra and Christopherson, 1976; Kennedy et al., 1977; Gonyou et al., 1979). Further, the digestibility of low-quality forage diets is reported to respond readily to changes in ROP induced by exposure to varying temperatures (Von Keyserlingk and Mathison, 1993). Kennedy and Milligan (1978), Young (1981) and Okine et al. (1989) have associated faster digesta ROP with lower ruminal digestibility, whereas Okine et al. (1989) reported that increased passage rates had no impact on apparent digestibility in beef steers regardless of increased frequency of ruminal contractions.

In the present study, cold exposure doubled post-rumen particulate ROP (9.9 versus 19.8 ± 2.04% h^{-1} in fall and winter, respectively; P = 0.0081; Table 6) and resulted in 47.7% shorter particulate FMRT (41.3 versus 21.6 ± 2.73 h in fall and winter, respectively; P = 0.0007), but failed to increase rumen particulate ROP. Given that thermal-neutral and cold-acclimatized cows in the current study demonstrated similar rumen NH_3-N concentrations, and therefore similar particulate rumen ROP, this suggests that microbial digestion in the rumen may have occurred at a similar rate and duration as in thermal-neutral cows. The particulate fraction of digesta is that which is digested throughout the ruminant digestive tract and since its ruminal ROP was unaffected by exposure to the cold-stressed environment, neither was its digestibility. This was confirmed with the similar N and P digestibilities observed between thermal-neutral and cold-acclimatized cows in this study (Table 4 and 5, respectively).

Changes in thyroid hormone concentrations have been proven to affect the metabolic and physiological function of all tissues in humans and animals alike (Yen, 2001). In the current study, TH concentrations were compared between housing types to determine whether metabolic and physiological function of the cows remained constant when removed from the feedlot (outdoors) and placed in the metabolism unit (indoors) for the 8-d digestibility study. Thyroid hormone concentrations are known to increase or decrease when ambient temperatures stray from the thermal-neutral range and induce cold or heat stress, respectively (Yousef et al., 1967; Dauncey, 1990). Therefore, T_3 and T_4 were measured to validate that digestibility parameters measured during the digestibility study were representative of normal metabolic and physiological function that occurs in cows while housed outdoors.

Triiodothyronine concentrations in cows housed outdoors in the feedlot or indoors in the metabolism unit were 1.35 versus 0.97 ± 0.044 nmol L^{-1} ($P < 0.0001$; Table 8) in the fall, and 1.37 versus 0.82 ± 0.035 nmol L^{-1} ($P < 0.0001$) in winter, respectively. For both seasons, the concentration of T_3 was significantly lower in cows housed in the metabolism unit than in the feedlot. This reduction in T_3 concentration was presumably in response to caloric restriction (Palmblad et al., 1977; Beer et al., 1989; Silvestri et al., 2005), which was imposed upon the cows as they were restricted-fed to 85% of their ad libitum feedlot intakes while housed in the metabolism unit. Similarly, Macari et al. (1983) reported that 10-wk old pigs housed at 10°C for six-wks and consuming high (H) or low (L) levels of energy intake (where H = 2L) demonstrated reductions in plasma T_3

Table 8: Comparison of thyroid hormone concentrations between outdoor (feedlot) and indoor (metabolism unit) housing of cows in thermal-neutral (fall) and cold-stressed (winter) environments.

	Housing			
	Feedlot	Metabolism Unit	SEM	P-values
Fall				
No. Observations (n)	20	21		
T_3, nmol L^{-1}	1.35b	0.97a	0.044	<0.0001
T_4, nmol L^{-1}	39.5	41.9	2.07	0.4210
T_3:T_4	0.035b	0.024a	0.0012	<0.0001
Winter				
No. Observations (n)	24	23		
T_3, nmol L^{-1}	1.37b	0.82a	0.035	<0.0001
T_4, nmol L^{-1}	40.9	40.8	2.06	0.9715
T_3:T_4	0.034b	0.021a	0.0012	<0.0001

a, b Means within housing followed by a different letter differ ($P < 0.05$).

from 1.3 nmol L^{-1} with high energy intake to 0.86 nmol L^{-1} ($P < 0.02$) with low energy intake.

Thyroxine showed no change between housing types in either season of the current study (Table 8). Unlike T_3, T_4 is known to be unresponsive to restrictions in feed intake (Kennedy et al., 1977; Ekpe and Christopherson, 1999; Silvestri et al., 2005), but remains responsive to cold temperatures (Yousef, 1967; Hurley et al., 1980; Dauncey, 1990). The impact of caloric restriction on T_3 and T_4 was demonstrated by Ekpe and Christopherson (1999) by housing lambs in warm (23 ± 2°C) or cold (0 ± 2°C) environments and offering diets either ad libitum or on a restricted (1.35 x maintenance) basis for three, five-week periods to find 48% lower plasma T_3 concentrations and unchanged T_4 concentrations in response to feed restriction.

Although there was no difference between outdoor and indoor ambient temperatures in the fall period of the current study, there was a 30.3°C difference between the average outdoor and indoor temperatures (-17.7 versus 12.6°C) during the winter. Therefore, upon entry into the metabolism unit, cows were suddenly exposed to temperatures that were much warmer than the temperatures they were acclimatized to in the feedlot. If the cows had responded to the increase in ambient temperature with a decrease in T_4 concentration, then metabolic and physiological function would have been altered in cows housed in the metabolism unit. However, the lack of response in T_4 concentrations occurred despite the dramatic change in ambient temperature between the feedlot and metabolism unit during the winter, which demonstrates that cows housed in the metabolism unit maintained similar metabolic and physiological function as when housed outdoors in the feedlot.

It is unknown whether T_4 concentrations decreased upon initial entry into the metabolism unit and returned to pre-entry concentrations thereafter, or if there was no change in concentrations when moved between the feedlot and metabolism unit. It is possible that T_4 may have increased during the three day adjustment period upon entry into the metabolism unit, but returned to previous levels for the duration of the collection period. Past research has reported that TH can revert to normal concentrations within a few days after exposure to different temperatures (Yousef, 1967; Dauncey, 1990). Yousef et al. (1967) monitored shaved, non-lactating dairy cows that were housed in a thermal-neutral (18°C) environment and had acclimation factors other than heat production and thyroid function controlled during sudden exposure to cold (1°C) for 1 wk, and reported an 86% increase in T_4 activity between 12 and 36 hr after cold exposure, but a subsequent, gradual return to thermal-neutral rates within four days of initial cold exposure. Further, T_4 activity of the dairy cows remained constant between 36 hr of cold exposure through to 60 hr after returning to the thermal-neutral environment, and by 108 hr after re-entry to the thermal-neutral environment, T_4 activity was restored to the original rate observed prior to cold exposure (Yousef et al., 1967).

There were no observed differences between seasons for T_3, T_4, or the T_3:T_4 in cows housed outdoors in the feedlot (Table 8). This lack of response in TH activity between the thermal-neutral and cold-stressed environments is in direct opposition to the widely accepted consensus that cattle exposed to cold environments experience elevations in TH concentrations (Westra and Christopherson, 1976; Kennedy et al., 1977; Christopherson and Kennedy, 1983; Young, 1989; Dauncey, 1990; Delfino and Mathison, 1991; Ekpe and Christopherson, 1999). However, these researchers mainly

studied the effect of short-term cold exposure on TH activity, whereas the current study investigated the effect of long-term exposure to extremely cold ambient temperatures.

The previously discussed study by Yousef et al. (1967), which reported that increased TH activity in dairy cows housed in cold environments for one week returned to thermal-neutral rates within four days after initial cold exposure, provides support for the apparent lack of response in TH activity between seasons of the current study. Further, elevated concentrations of T_3 and T_4 that occur in response to cold ambient temperatures can only be maintained by an accompanying increase in energy intake and diet digestibility (Dauncey and Morovat, 1989; Dauncey, 1990), or greater DMI (Evans and Ingram, 1977), which is critical to maintain muscle responsiveness to T_3. Therefore, it is likely that T_3 and T_4 concentrations actually increased when the cows in the current study were initially acclimatizing to the progressively colder winter temperatures, but after a prolonged period of exposure to sub-zero temperatures the cows effectively became cold-acclimatized and without an accompanying increase in DMI, ACPI or energy intake (Table 3) to maintain these elevated levels, their T_3 and T_4 concentrations probably returned to similar concentrations that were present during the thermal-neutral period in the fall.

In the metabolism unit, however, T_3 concentrations underwent a significant reduction from 0.97 ± 0.030 nmol L^{-1} in fall to 0.82 ± 0.030 nmol L^{-1} in winter ($P = 0.0013$; Table 8). This reduction in T_3 caused a significant reduction in the T_3:T_4 ratio between fall and winter (0.024 versus 0.021 ± 0.0010; $P = 0.0444$) even though T_4 concentrations remained unchanged between seasons. Restricted-fed intakes were similar between seasons, as was the dietary content of CP and energy; therefore the observed

decrease in T_3 could not be attributed to a reduction in DMI, ACPI or caloric restriction. Rather, differences in ambient temperature in the feedlot and metabolism unit are presumably responsible for the observed reduction in T_3 cows housed in the metabolism unit during the fall and winter. During the fall, ambient temperatures were similar between the feedlot and metabolism unit, and remained within the cows' thermal-neutral range, therefore T_3 levels may have been unaffected by temperature when cows were moved from the feedlot to the metabolism unit. However, during the winter cows in the feedlot were exposed to a cold-stressed environment (average temperature of -17.7°C) and upon entry to the metabolism unit they were suddenly exposed to a thermal-neutral environment (average temperature of 14.8°C). Sudden exposure to the thermal neutral environment presumably lowered T_3 levels in cows housed in the metabolism unit because they no longer required the high levels produced while housed in the cold-stressed environment. Thus, differences in outdoor and indoor ambient temperatures caused T_3 levels to be lower in cows housed in the metabolism unit during the winter than in the fall, despite no changes in indoor ambient temperature between seasons.

As compared to thermal-neutral cows, apparent N digestibility appeared to be unaffected in cows exposed to the cold-stressed environment because the interactions that existed between CON and 20%DDGS were probably caused by unintentional sorting of DDGS from the 20%DDGS diet during the winter digestibility study rather than by an actual season effect. Likewise, apparent P digestibility did not differ between thermal-neutral and cold-acclimatized cows because consistent rumen NH_3-N concentrations probably maintained the rate of microbial digestion in the rumen. While there was no interaction between diet and season for ACPI or rumen NH_3-N, there was an interaction

for SUN. This response in SUN concentrations may be related to a decline in body fluid volume, causing a reduction in urine production, more efficient recovery of N by the kidneys, and concentration of N in the body of cold-acclimatized cows. Similar T_4 concentrations between cows housed in the feedlot and metabolism unit suggest that metabolic rate and physiological function was unaffected during the digestibility study. Given that TH concentrations for cows in the feedlot were the same between seasons, this suggests that cows became effectively cold-acclimatized, and metabolic rates were comparable to thermal-neutral levels, after prolonged cold exposure.

4.3.1.4. Influence of Protein Supplementation on N and P Recycling and Digestibility

Serum urea nitrogen, rumen NH_3-N, and the digestibility of N and P were measured to determine if CP supplementation successfully established acceptable N status and increased the quantity of N and P that were utilized by cows consuming low-quality forage. The thyroid hormones, T_3 and T_4, were measured to determine if CP supplementation potentially induced changes in metabolic rate.

As previously discussed, a diet by season interaction was identified for SUN concentrations ($P = 0.0583$; Figure 4) in response to greater CP intake across diets and lower urine production in cold-acclimatized compared to thermal-neutral cows. In concert with the increasing ACPI, SUN concentrations increased from 1.6 ± 0.14 to 3.1 ± 0.14 to 4.3 ± 0.16 mmol L^{-1} ($P < 0.0001$) for CON, 10%DDGS and 20%DDGS treatments, respectively (Table 3). Serum urea nitrogen concentrations < 2.1 mmol L^{-1} are considered low and reflect deficient N status due to low dietary CP intake, whereas concentrations between 2.1 and 7.9 mmol L^{-1} represent acceptable N status (Ortho

Clinical Diagnostics, 1993). Ndlovu et al. (2007) defined the description for SUN levels, suggesting concentrations between 2.1 and 3.6 mmol L^{-1} represent optimal CP intake, whereas concentrations > 3.6 mmol L^{-1} result from protein intake in excess of requirements. As anticipated, 94% of protein deficient cows demonstrated deficient N status, while the remaining 6% reported an average SUN concentration of 2.1 mmol L^{-1}. Ninety-seven percent of cows receiving CP supplementation exhibited acceptable N status, where the remaining 3% represented one 10%DDGS cow in the fall which demonstrated N deficiency with an average SUN concentration of 1.9 mmol L^{-1}. Serum urea nitrogen concentration for cows fed 20%DDGS was > 3.6 mmol L^{-1} which confirms this diet was successful at supplementing N in excess of requirement, whereas, according to Ndlovu et al. (2007), the 10%DDGS diet generated the most optimal protein status.

Similar to the results of this study, an experiment by Vasconcelos et al. (2006) observed higher SUN concentrations in finishing steers consuming a 13.0% CP ration than steers fed rations with 11.5 and 10.0% CP. Another study supplemented three different levels of protein (0.50, 0.52, and 1.0 kg d^{-1}) using soybean hulls or soybean meal to spring calving beef cows after parturition and found increasing SUN concentrations (3.2 vs. 6.1 vs. 9.1 mmol L^{-1}, respectively) with higher CP supplementation (Marston et al., 1995). In a two-year study by Poore et al. (2006), beef heifers grazed 16.8 and 12.6% CP stockpiled forage in years one and two, respectively, and received 0.24 and 0.25 kg hd^{-1} d^{-1} supplemental CP from a whole cottonseed and cracked corn mixture. Serum urea nitrogen concentrations of these heifers increased from 3.4 to 3.7 mmol L^{-1} in year one and from 2.8 to 3.5 mmol L^{-1} in year two, in response to higher CP intake from supplementation (Poore et al., 2006).

Apparent N digestibility was 40.6 ± 2.45% in protein deficient cows, but increased to 57.1 and 61.2 ± 2.45% ($P < 0.0001$) when CP was fed to marginally meet and exceed requirements, respectively. These values are similar to those reported by Gilbery et al. (2006), when beef steers fed low-quality forage (3.3% CP, 42.5% ADF) mixed with 0, 5, 10 or 15% (DM basis) CCDS (21.6% CP, 86.7% RDP) demonstrated total tract CP digestibilities of 27.5, 42.8, 44.9 and 57.8%, respectively. Similarly, Köster et al. (1996) fed Angus x Hereford cows low-quality forage (1.9% CP, 77% NDF) twice daily and supplemented with 0, 180, 360, 540 and 720 g RDP d^{-1} (sodium caseinate; 90% CP) via intraruminal infusion and reported significant increases in total N intake (13.4, 48.6, 80.5, 110.9 and 137.8 g d^{-1}, respectively) which coincided with total GIT - N digestibilities of -39.8, 39.0, 51.1, 60.5 and 70.4% in response to increasing RDP supplementation.

The improvement in N digestibility reported for the 10%DDGS and 20%DDGS diets may be attributed to dietary CP becoming more digestible with greater DDGS inclusion, rather than microbial N requirements for digestion being satisfied, as similar particulate ROP between diets suggests that rumen microbial digestion may have remained unchanged despite increasing CP supplementation. This opposes the claim that protein supplementation of low-quality forage improves dietary digestion (Del Curto et al., 1990; Matejovsky and Sanson, 1995; Van Nolte et al., 2003) by removing the first-limiting effect of CP on microbial function (Freeman et al., 1992; Mawuenyegah et al., 1997). In the present study, it is possible that CP may not have been the only first-limiting nutrient on rumen microbial growth and function, but rather, energy may have also been limiting. Thus, without provision of additional dietary energy, rumen microbial

function may have been limited despite CP supplementation. However, the results of the present study are in keeping with those reported by Boniface et al. (1986), suggesting that nutrient digestibility appears to be more closely related to the digestibility of the dietary N content than rumen NH_3-N concentration.

Apparent P digestibility increased from -23.9 ± 5.95% in protein deficient cows to 0.2 and 5.7 ± 5.95% (P = 0.0025; Table 5) in cows consuming borderline adequate and excess CP, respectively. Estermann et al. (2002) fed lactating Simmental and Angus beef cows an 11% CP forage mixture (ratio of 1 meadow grass silage: 0.7 meadow hay: 0.3 barley straw, DM basis) and 1:1 mixture of salt and mineral premix ad libitum to generate P intakes of 54.7 and 53.3 g d^{-1}, and P digestibilities of 24.1 and 32.6% for Simmental and Angus cows, respectively. Chantiratikul et al. (2009) fed Brahman x steers rice straw-based diets containing 11.7% CP and 0.19, 0.24 or 0.31% P and reported P digestibilities of 81.8, 70.6 and 62.5%, which are substantially higher than the P digestibilities reported in the present study. The relatively low P digestibilities in cows of the present study are attributed to high fecal P excretion, which suggests that P in the low-quality forage diets was relatively unavailable to rumen microbial digestion. However, DDGS supplementation increased P digestibility, probably in response to the P content in wheat-based DDGS being more available than in the low-quality forage. The observed impact of diet on apparent P digestibility is similar to that observed with diet and N digestibility. That is, in both instances, digestibility of either nutrient was improved once N and P deficiencies were alleviated by CP supplementation. It has been reported that P deficiency does not cause a reduction in overall diet digestibility, but rather decreases utilization of nutrients after they are digested (Eckles et al., 1926; Eckles

and Gullickson, 1927). The negative P digestibility observed in CP deficient cows in this study suggests that P was unavailable, or tied up, in the low-quality forage. Since DDGS was fed as the CP supplement, the observed improvements in P digestibility with 10% and 20% DDGS supplementation are likely due to DDGS increasing the overall quantity of digestible P in the low-quality forage diet. Therefore, this study suggests that CP supplementation with wheat-based DDGS generates improvements in dietary N digestibility, as well as similar improvements in apparent P digestibility, in response to DDGS inclusion introducing more digestible CP and P fractions to low-quality forage diets.

Triiodothyronine concentrations were increased in beef cows receiving CP supplementation as compared to cows consuming the CP deficient forage diet, and this occurred regardless of being housed outdoors in the feedlot (1.23, 1.41 and 1.44 ± 0.054 nmol L^{-1} ($P = 0.0165$) for CON, 10%DDGS and 20%DDGS, respectively; Table 9) or indoors in the metabolism unit (0.80, 0.95 and 0.94 ± 0.036 nmol L^{-1} ($P = 0.0108$) for CON, 10%DDGS and 20%DDGS, respectively). The consistent response of T_3 to diet is a result of dietary protein content, as T_3 has been shown to decrease when dietary protein is fed below requirement (Hastings and Zeman, 1979). Hastings and Zeman (1979) fed pregnant rats diets ad libitum with adequate or deficient CP content (27 versus 4% casein) and reported that CP deficient rats had 24% lower plasma T_3 (0.61 versus 0.79 nmol L^{-1}; $P < 0.05$) concentrations than rats with sufficient CP intake. Further, these rats were also fed the CP sufficient diet either ad libitum or restricted to 75% of ad libitum intake. Feed restricted rats demonstrated lower plasma T_3 concentrations than CP deficient rats (0.45 versus 0.61 nmol L^{-1}; $P < 0.05$; Hastings and Zeman, 1979). These

Table 9: Comparison of thyroid hormone concentrations between seasons in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in outdoor (feedlot) or indoor (metabolism unit) housing.

	Diet				Season			P-values		
	CON	10% DDGS	20% DDGS	SEM	Fall	Winter	SEM	D	S	D x S
Feedlot										
No. Observations (n)	18	18	16		26	26				
T_3, nmol L^{-1}	1.23a	1.41b	1.44b	0.054	1.35	1.37	0.044	0.0165	0.7574	0.1915
T_4, nmol L^{-1}	36.4	40.9	43.7	2.38	39.6	41.1	1.95	0.1122	0.5970	0.6744
T_3:T_4	0.035	0.036	0.034	0.0016	0.035	0.034	0.0013	0.6944	0.5714	0.0877
Metabolism Unit										
No. Observations (n)	15	15	14		21	23				
T_3, nmol L^{-1}	0.80a	0.95b	0.94b	0.036	0.97e	0.82d	0.030	0.0108	0.0013	0.3150
T_4, nmol L^{-1}	39.7	41.6	42.7	2.50	41.9	40.8	2.04	0.6907	0.7188	0.1906
T_3:T_4	0.021	0.023	0.022	0.0012	0.024e	0.021d	0.0010	0.5483	0.0444	0.2843

a, b, c Means within diet followed by a different letter differ ($P < 0.05$).
d, e Means within season followed by a different letter differ ($P < 0.05$).

findings support the results of the current study as cows that were restricted-fed in the metabolism unit consistently demonstrated lower T_3 concentrations than the CON cows when fed ad libitum in the feedlot. Given that reduced TH concentrations result in reduced metabolic rate (Dauncey, 1990), this suggests that protein deficient cows experienced lower metabolic rates than cows receiving CP supplementation.

Thyroxine concentrations and the ratio of T_3:T_4 were unchanged between diets while cows were housed in the feedlot and metabolism unit. Unlike T_3, T_4 does not respond to protein deficiency or caloric restriction (Hastings and Zeman, 1979; Silvestri et al., 2005) and therefore is not a very accurate indicator of metabolic changes in response to CP supplementation.

The relationship between ACPI, SUN and rumen NH_3-N were positive and linear, which demonstrates that CP supplementation successfully alleviated protein deficiency and established acceptable N status in cows consuming low-quality forage. Given that CP deficiency slowed metabolic rate and decreased N and P digestibility, nutrient utilization was reduced in protein deficient cows. Further, these data suggest that consumption of low- quality forages appears to result in negative P digestibility if supplemental CP is not provided. Nitrogen and P digestibility, and therefore nutrient utilization, was improved from CP supplementation despite limitations on DMI, as the readily digestible CP fraction in wheat-based DDGS served to improve overall diet digestibility with greater inclusion up to 20% (as fed basis). These results demonstrate that beef cows consuming low-quality forage experience improvements in nutrient utilization when CP is supplemented to marginally meet N requirements, and further

supplementation to provide dietary CP content greater than 7.9% ACP (8.7% CP; DM basis) does not generate additional improvements in terms of nutrient utilization.

4.3.2. Rumen Fermentation Efficiency

4.3.2.1. Influence of Cold Acclimatization on Rumen Fermentation Efficiency

Volatile fatty acids and urine PD were measured to determine if cold acclimatization improved the efficiency of rumen fermentation of low-quality forage diets.

Maximum fermentation efficiency occurs when rumen microbial growth, and therefore MP synthesis, is maximized by dietary provision of rumen available protein and energy in the correct proportions to ensure rumen microbe requirements for growth and function are met (IAEA, 2000). Efficient rumen fermentation ensures that the animal receives the maximum amount of digestible nutrients from their diet and involves optimal microbial digestion of feedstuffs into end-products (i.e. propionate, amino acids) that can be used by the animal for maintenance, growth or lactation rather than wasteful end-products (i.e. methane) that are excreted (IAEA, 2000). High MP synthesis represents a high efficiency of microbial growth from digestion (i.e. g MP/ g substrate digested; Eun, 2002). Therefore, VFA, enteric CH_4 emissions and urine PD are indicators of efficient rumen fermentation. High VFA concentrations indicate low microbial biomass, and therefore low fermentation efficiency in the rumen (Leng, 1991; Eun, 2002). Likewise, a VFA profile which results in a high A:P ratio, and therefore high enteric CH_4 emissions, indicates inefficient fermentation (Leng, 1991). Urine PD excretion is an indicator of the

level of MP synthesis, therefore higher total PD concentrations indicate greater microbial biomass and efficient rumen fermentation (Pina et al., 2009).

Total VFA concentrations increased between thermal-neutral and cold-acclimatized cows (62.8 versus 69.3 ± 1.41 mmol L^{-1}, respectively; P = 0.0024; Table 7). Acetate:propionate ratios have been speculated to decrease in cold-acclimatized ruminants, suggesting a shift towards more efficient fermentation and suppression of CH_4 emissions (Okine et al., 1989; Von Keyserlingk and Mathison, 1993). However, no change was observed in A:P ratio between seasons of the current study (Table 7), which suggests no change in fermentation pattern in response to cold acclimatization. Given the lack of differences in DMI, dietary nutrient intake and rumen NH_3-N between seasons, this suggests that rumen fermentation should have remained constant between thermal-neutral and cold-acclimatized cows. Total PD concentrations were also similar between thermal-neutral and cold-acclimatized cows, therefore, the reported increase in total VFA concentrations is not likely explained by a decrease in microbial fermentation efficiency, but rather by the reduction in total body fluids and/or blood flow to the reticulorumen that occurs in cold-acclimatized ruminants (Young, 1975; Schaefer and Young; 1980). However, if reduced rumen volume caused the observed increase in VFA concentration, then it stands to reason that rumen NH_3-N concentration also should have increased. Since both rumen parameters failed to increase in cold-acclimatized cows, perhaps changes in blood flow resulted in the higher total VFA concentrations.

Animals in cold environments maintain thermal balance by shifting blood flow away from the skin of the extremities and digestive tract, and towards skeletal muscle and adipose tissue to support shivering and fat mobilization for thermogenesis (Thompson,

1977; Schaefer and Young, 1980). Schaefer and Young (1980) exposed groups of four shorn sheep to thermal-neutral (18°C for 10 to 12 wk), acute cold (3°C for 12 h) or chronic cold (3°C for 10 to 12 wk) environments and reported that compared to thermal-neutral sheep, acute and chronic cold sheep demonstrated significant reductions of 39 and 33%, respectively, in blood flow to the reticulorumen. Absorption of VFA is affected by blood flow to the rumen epithelium, therefore, low blood flow becomes the main limitation for VFA absorption and reduces the amount of VFA that are absorbed from the rumen into the bloodstream (Storm et al., 2011). As such, the observed increase in total VFA concentrations in cold-acclimatized cows of the current study may be indicative of a reduction in VFA absorption, and therefore an accumulation VFA in the rumen, rather than an increase in rumen fermentation. If this is true, the reason that rumen NH3-N concentrations did not concurrently increase with VFA concentrations may be explained by the ability of NH_3-N to be absorbed and recycled from parts of the digestive tract other than the reticulorumen (Reynolds, 1992). That is, contrary to VFAs, up to 51% of NH_3-N originating from the rumen can be absorbed in the small intestine (Parker et al., 1995), which may permit continued NH_3-N recycling in the animal and consistent rumen NH_3-N concentrations if reductions in blood flow did not also occur in the small intestine.

Allantoin, uric acid and total PD excretion did not differ between seasons (Table 10). Given that the A:P ratio also did not change between thermal-neutral and cold-acclimatized cows, this suggests that cold acclimatization had no affect on fermentation efficiency. Several factors related to MP synthesis may explain these observed similarities, such as ACPI, energy intake, ruminal NH_3-N concentration and particulate

Table 10: Urine excretion and purine derivatives of cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.

	Diet				Season			P-values		
	CON	10%DDGS	20%DDGS	SEM	Fall	Winter	SEM	D	S	D x S
No. Observations (n)	15	15	13		20	23				
Urine excretion, L d^{-1}	7.2a	8.4a	11.7b	0.42	9.7e	8.4d	0.34	<0.0001	0.0102	0.1654
Urine excretion, % BW	1.08a	1.20a	1.67b	0.058	1.43e	1.20d	0.047	<0.0001	0.0013	0.0625
Allantoin excretion, mmol d^{-1}	95.0a	116.6b	142.3c	4.50	117.9	118.0	3.67	<0.0001	0.9808	0.2685
Uric acid excretion, mmol d^{-1}	10.2a	16.2b	16.3b	0.92	13.8	14.6	0.75	<0.0001	0.4842	0.7259
Total PD excretion, mmol d^{-1}	105.2a	132.9b	158.5c	4.97	131.8	132.6	4.06	<0.0001	0.8796	0.2720
Allantoin / Total PD, %	90.2b	87.5a	89.4ab	0.61	89.4	88.7	0.50	0.0074	0.3050	0.9711

a, b, c Means within diet followed by a different letter differ ($P < 0.05$).
d, e Means within season followed by a different letter differ ($P < 0.05$).

ROP. However, literature describing the impact of cold acclimatization on MP synthesis, particularly in cattle, is limited. Reed et al. (2006) fed nursing beef calves either a soybean meal-based supplement or a corn distillers dried grains with solubles-based supplement with comparable CP (30.0 vs. 29.6%) and starch (8.0 vs. 8.8%) concentrations from June through August, and reported no difference in MP synthesis between diets. As previously discussed, when ruminal NH_3-N concentrations are limiting, microbial growth, and the subsequent production of MP synthesis, is slowed (Satter and Slyter, 1974; Maeng et al., 1976; Gilbery et al., 2006). Sniffen and Robinson (1987) have indicated that ruminal particulate ROP is an important regulator of MP synthesis, as the larger, more active bacterial populations are associated with this fraction of rumen digesta. Kennedy et al. (1976) exposed closely shorn sheep to warm (18 to 21 °C) and cold (-1 to 1°C) environments for 28 d and observed that faster particulate ROP in the cold environment increased the amount of MP synthesis. Therefore, similar MP synthesis between seasons likely occurred since ACPI, energy intake, rumen NH_3-N concentrations, and particulate ROP were also unchanged between thermal-neutral and cold-acclimatized cows in the current study.

Fermentation efficiency in cows fed low-quality forage diets was unaffected by cold acclimatization. Fermentation efficiency, including MP synthesis, may have improved if nutrient intake and rumen NH_3-N concentrations had increased and provided rumen microbes with additional nutrients for improved growth and function. Given that total VFA concentrations increased despite no change in rumen fermentation, this suggests that cold-acclimatized cows in the present study shifted blood flow away from the reticulorumen to help maintain thermal balance. Although rumen fluid volume may

have also declined, the lack of increase in NH_3-N concentration suggests that rumen volume may have been unaffected by cold acclimatization.

4.3.2.2. Influence of Protein Supplementation on Rumen Fermentation Efficiency

Volatile fatty acids, urine PD and enteric CH_4 emissions were measured to determine if CP supplementation successfully improved the efficiency of rumen fermentation of low-quality forages.

Total VFA concentrations did not differ between CP deficient and supplemented cows (Table 7), which reflects the non-significant increases observed in DMI and energy intake. Since VFA are major end products of microbial fermentation, this suggests that a dietary CP deficiency did not decrease fermentation. The A:P ratio decreased from 4.18 and 3.96 with CON and 10%DDGS, respectively, to 3.82 ± 0.079 with 20%DDGS ($P = 0.0094$). This suggests that fermentation efficiency was reduced when CP was deficient. Conversely, fermentation efficiency improved when CP was fed in excess of requirements, as lower A:P ratio is indicative of a favourable shift in the pattern of microbial fermentation towards reduced acetate and greater propionate production (Harrison et al., 1976).

Allantoin excretion increased linearly across the CON, 10%DDGS and 20%DDGS diets (95.0, 116.6 and 142.3 ± 4.50 mmol d^{-1}, respectively; $P < 0.0001$; Table 10). Protein supplementation increased uric acid excretion from 10.2 ± 0.92 mmol d^{-1} in protein deficient cows to 16.2 and 16.3 ± 0.92 mmol d^{-1} ($P < 0.0001$) in cows receiving borderline adequate and excess CP, respectively. Total PD excretion increased linearly from 105.2 to 132.9 to 158.5 ± 4.97 mmol d^{-1} ($P < 0.0001$) across CON, 10%DDGS and

20%DDGS, respectively. The relationship between ACPI, SUN, rumen NH_3-N and MP synthesis was positive and linear in response to greater CP supplementation.

Urinary PD excretion is an indicator of the efficiency of microbial CP synthesis (Pina et al., 2009) as strong, positive correlations exist between MP flow to the small intestine and urinary PD excretion (Moorby et al., 2006; Tas and Susenbeth, 2007). Pina et al. (2009) increased concentrate supplementation from 20 to 40% in a corn silage-based ration so as to maintain dietary CP concentration at 12.5% DM while increasing energy (non-fibre carbohydrate) content from 34.0 to 44.4% DM, and found that MP yield increased from 40.9 to 51.9 g d^{-1}, which caused urinary allantoin excretion to increase from 55.1 to 67.9 mmol d^{-1}, but generated no change in uric acid excretion. Satter and Slyter (1974) infused urea into continuous-culture fermentors to imitate increasing rumen NH_3-N concentrations while maintaining consistent ruminal energy concentration, and observed that when rumen NH_3-N concentrations were lower than 2.0 mg 100 ml^{-1}, MP synthesis increased linearly with increasing rumen NH_3-N concentration up to 5.0 mg NH_3-N 100 ml^{-1} rumen fluid. These literature reports, as well as reports from other researchers (Satter and Slyter, 1974; Hoover and Stokes, 1991), demonstrate that MP synthesis is mainly driven by rumen available energy, but also by rumen NH_3-N, that is available to rumen microbes. Maeng et al. (1976) and Gilbery et al. (2006) have suggested that when microbial N requirements are not met, microbial growth slows and reduces fermentation efficiency. More optimal rumen fermentation increases microbial N flow to the small intestine (Clark, et al., 1992; Valadares et al., 1999), which accounts for the observed increase in urinary PD excretion in response to CP supplementation.

The results of the present study demonstrate that feeding low-quality forage diets without DDGS inclusion, and thereby providing deficient CP, resulted in rumen NH_3-N concentrations being limiting on microbial growth and function as MP synthesis was lowest in cows fed the CON diet. Given that NE_m and GE intake did not differ between diets of the current study, the observed increases in microbial CP synthesis did not result from greater energy consumption. Rather, the improvement in MP synthesis is attributed to DDGS supplementation increasing the supply of rumen NH_3-N to meet microbial CP requirements for optimal microbial growth and function. This was reflected in allantoin and total PD excretion increasing in the same pattern as NH_3-N concentration, and in uric acid excretion remaining unchanged, once microbial N requirements were met with 10%DDGS and 20%DDGS supplementation. However, DDGS supplementation increased dietary P in the rumen, and may have also increased rumen available energy, which may have also contributed to the observed improvement in MP synthesis.

Cows consuming CP in excess of N requirements produced 18.5% (5.3 ± 0.38 versus 6.5 ± 0.33% GEI; Table 3) less enteric CH_4 as compared to cows consuming deficient CP. This reduction suggests an improvement in fermentation efficiency, and coincides with the decrease in A:P ratio that occurred when CP was supplemented in excess of requirements. Conversely, CP deficient cows produced 22.6% more enteric CH_4 than cows consuming excess CP. Low-quality forage diets have been reported to generate higher enteric CH_4 emission losses (% GEI) as they are fermented slower than diets containing greater proportions of concentrates (Boadi et al., 2004). Contrary to expectations, cows fed borderline adequate CP produced 28.3% more enteric CH_4 than cows fed CP in excess of requirement, and 4.6% more enteric CH_4 than protein deficient

cows. The similarity in enteric CH_4 emissions between cows consuming deficient and borderline adequate CP suggests that the 10%DDGS diet did not provide adequate rumen NH_3-N for efficient microbial fermentation in the rumen.

A dietary CP deficiency decreased available N for rumen microbes, but did not affect the rate of rumen fermentation. There was, however, a reduction in fermentation efficiency and enteric CH_4 emissions (% GEI) were 22.6% higher than for the diet which provided N in excess of published animal requirements. Protein supplementation with 10%DDGS and 20%DDGS failed to increase the rate of rumen fermentation in cows fed low-quality forage diets, but supplementing CP to provide N in excess of requirements promoted more efficient fermentation as compared to when CP was deficient. These data suggest that the 10%DDGS diet did not provide adequate dietary CP, and therefore did not provide adequate rumen NH_3-N, for efficient rumen fermentation. Accordingly, recommended dietary CP levels in low-quality forage diets for mature, dry, non-pregnant beef cows should be in excess of 7.9% ACP (8.7% CP; DM basis) to ensure efficient rumen fermentation.

4.3.3. Nutrient Excretion

4.3.3.1. Influence of Cold Acclimatization on Nutrient Excretion

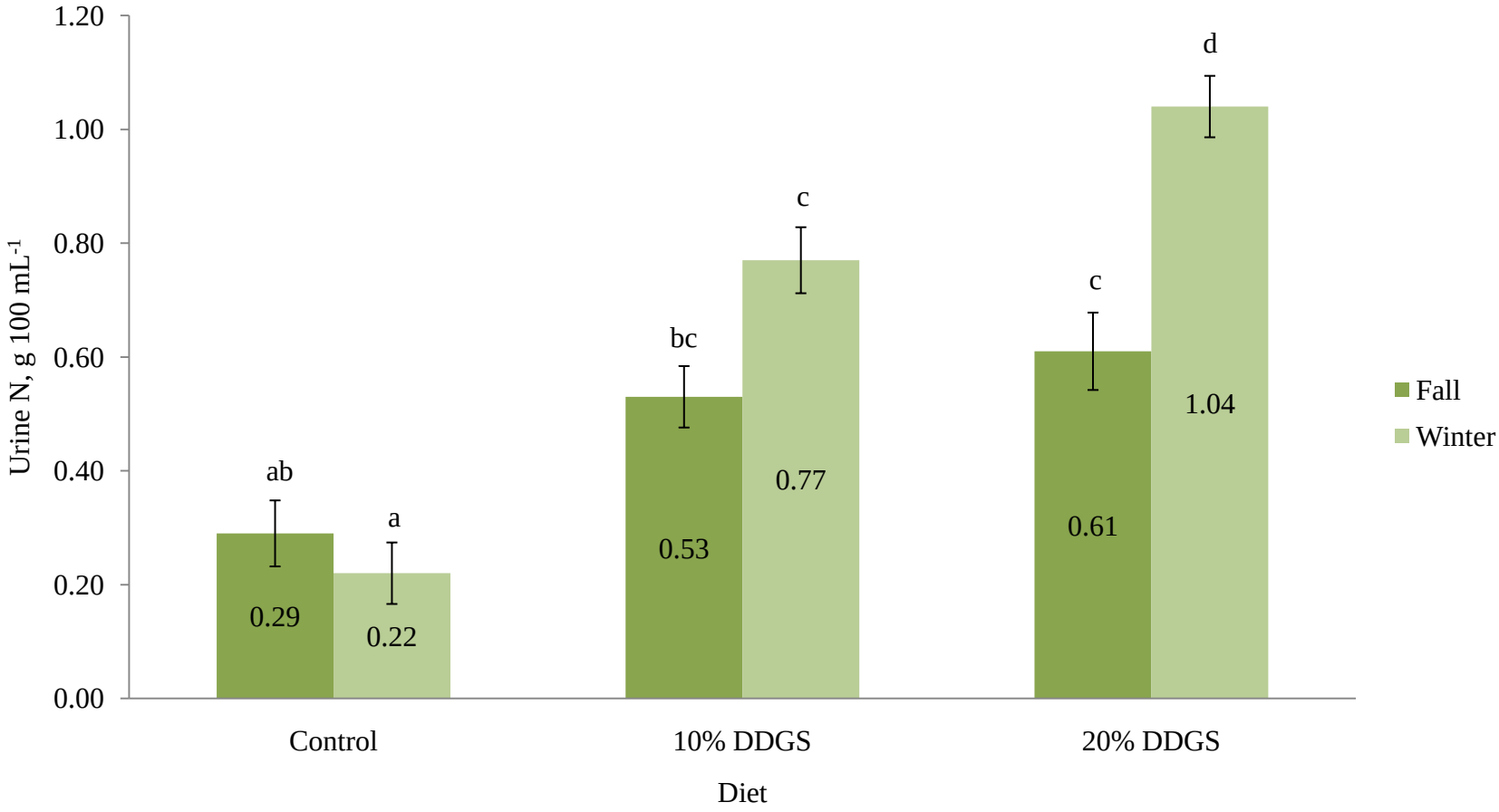
Nitrogen and P excretion were measured to determine if nutrient losses increased in response to cold acclimatization. Enteric CH_4 emissions were monitored to determine if cold acclimatization decreased energy losses from cows fed low-quality forage diets.

Fecal N concentration and excretion were unchanged between seasons, whereas urine N concentration and excretion (Table 4) demonstrated similar diet by season

interactions (P = 0.0004 and P = 0.0050, respectively; Figures 7 and 8). There were numeric, but not significant, increases in urine N concentration and excretion in cold-acclimatized versus thermal-neutral cows fed CON and 10%DDGS, whereas a significant increase occurred when 20%DDGS cows were in the cold-stressed environment. These increases suggest less efficient retention of absorbed N when animas were supplemented with CP. Inefficient retention of absorbed N was most evident when thermal-neutral and cold-acclimatized cows consumed CP in excess of requirement, but 10%DDGS cows in both seasons also demonstrated increased inefficiency in retention of absorbed N compared to CON cows.

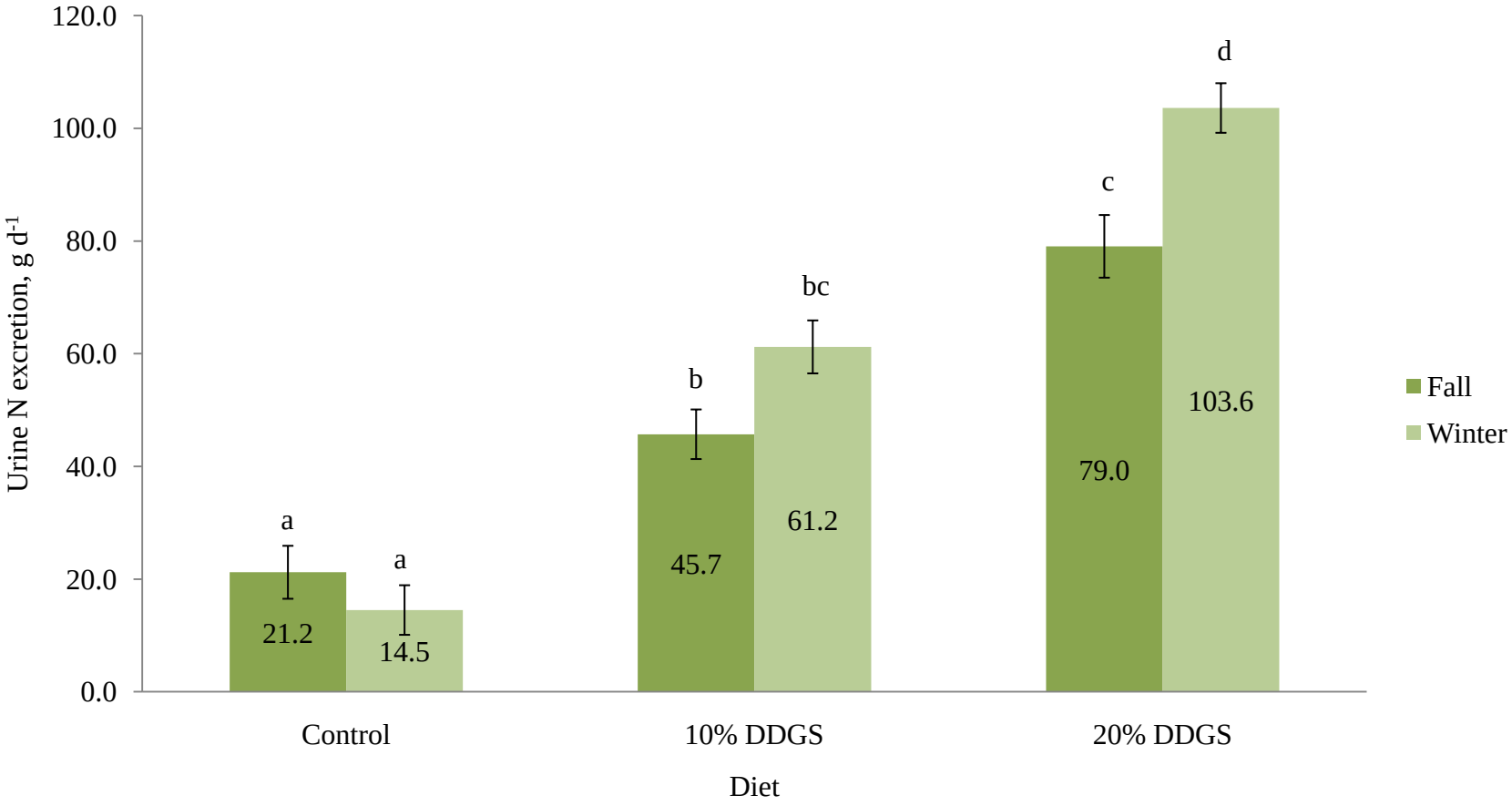
Greater urine N concentration and excretion may have been attributed to increased TH concentrations that occur in cold-stressed ruminants (Christopherson and Kennedy, 1983; Young, 1981; Nisa et al., 1999), and which were numerically, although not significantly, higher in cold-acclimatized versus thermal-neutral cows in the current study (Table 9). That is, increased concentration of TH stimulates the synthesis and degradation of protein, but protein catabolism from skeletal muscle predominates and results in greater urine N excretion (Müller and Seitz, 1984). Further, urine N concentration and excretion were significantly higher in cold-acclimatized than thermal-neutral cows fed 20%DDGS, probably due to increased endogenous N losses from breakdown of soft tissue when the cows lost BW during the digestibility study. Body weight loss (-3.0 to -3.4 kg BW d^{-1}; Table 5) was similar across diets and seasons of the present study, therefore all cows were expected to source similar amounts of endogenous N, but only 20%DDGS cows demonstrated an increase in endogenous N loss via urine N concentration and excretion during the winter. This is presumably due to the level of CP

Figure 7: Diet by season interaction for urine nitrogen concentration in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



a, b, c, d Means within diet and season followed by a different letter differ ($P < 0.05$).

Figure 8: Diet by season interaction for urine nitrogen excretion in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.

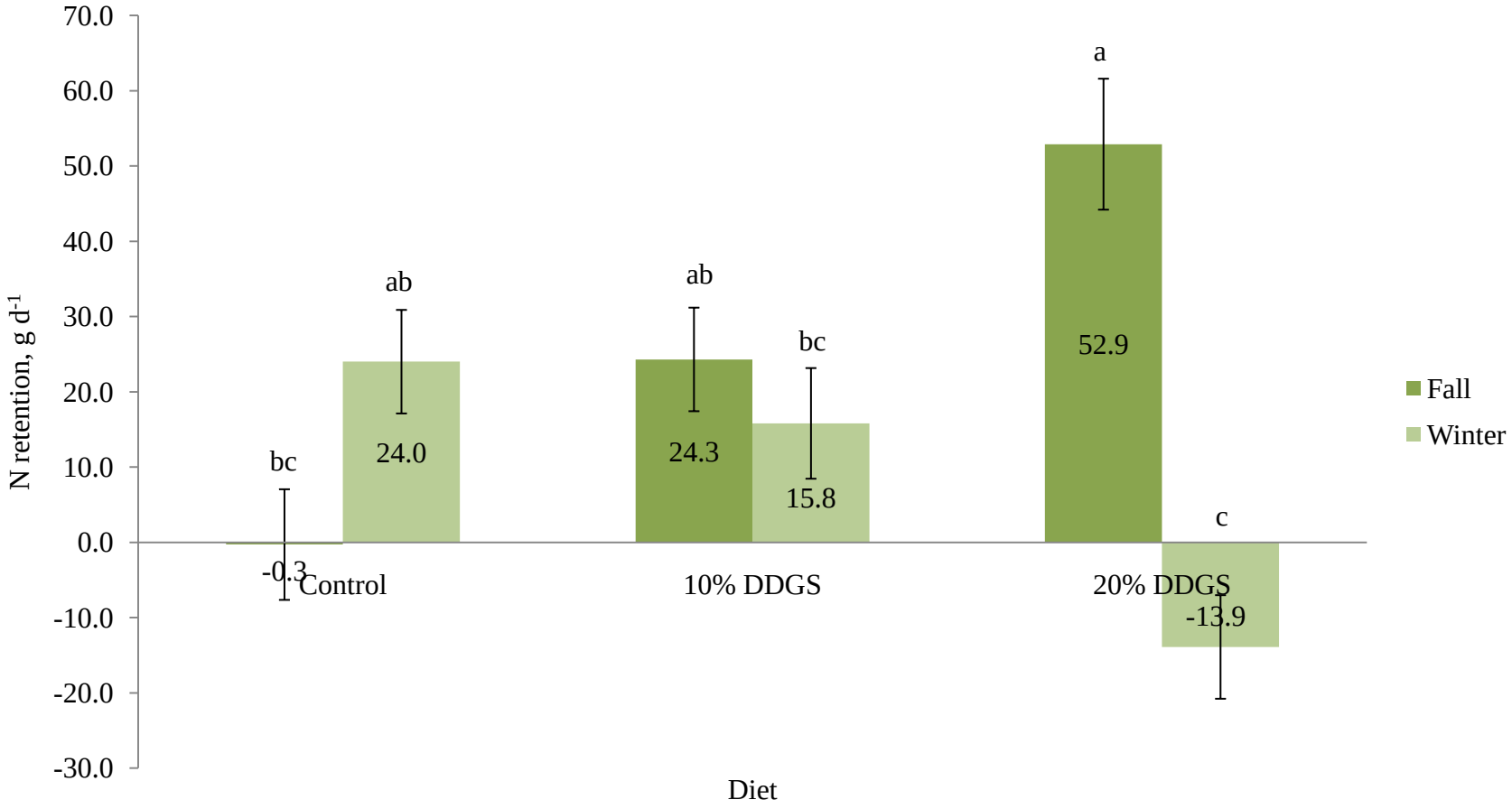


a, b, c, d Means within diet and season followed by a different letter differ ($P < 0.05$).

intake in comparison to CP requirements, as CON and 10%DDGS cows were consuming deficient or marginally adequate CP to meet requirements and therefore, in an effort to meet CP requirements, these cows may have retained absorbed N more efficiently than 20%DDGS cows who consumed excess dietary CP and did not need to retain additional endogenous N. Further, cold-acclimatized cows fed 20%DDGS excreted less urine compared to thermal-neutral cows, thereby contributing to greater urine N concentration

Nitrogen retention was 66.5%, or 1.2x, lower in cold-acclimatized than thermal-neutral cows (25.7 vs. 8.6 ± 4.25 g d^{-1}in fall and winter, respectively; $P = 0.0075$) and demonstrated a diet by season interaction ($P < 0.0001$; Figure 9). A linear improvement in N retention by thermal-neutral cows fed low-quality forage diets and receiving greater levels of CP supplementation was observed, whereas cold-acclimatized cows fed the same diets demonstrated a linear decline in N retention. Greater supplementation with DDGS as the protein source likely contributed to the linear improvement in N retention by thermal-neutral cows as CP intake increased. The CP fraction of DDGS is relatively available to rumen microbes for digestion, and therefore, increasing CP intake did not result in greater N excretion relative to intake. Since both N intake and fecal N excretion were unchanged between seasons, the reduction in N retention observed in cold-acclimatized cows was attributed to the greater urine N excretion (g d^{-1}) observed in cold-acclimatized versus thermal-neutral cows. Increased breakdown of body muscle and proteins may have occurred in response to cold exposure. Therefore, as previously discussed, cold-acclimatized cows probably sourced endogenous N to help meet increased nutrient requirements while exposed to the cold-stressed environment. This, in combination with endogenous losses resulting from BW loss during the digestibility

Figure 9: Diet by season interaction for nitrogen retention in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



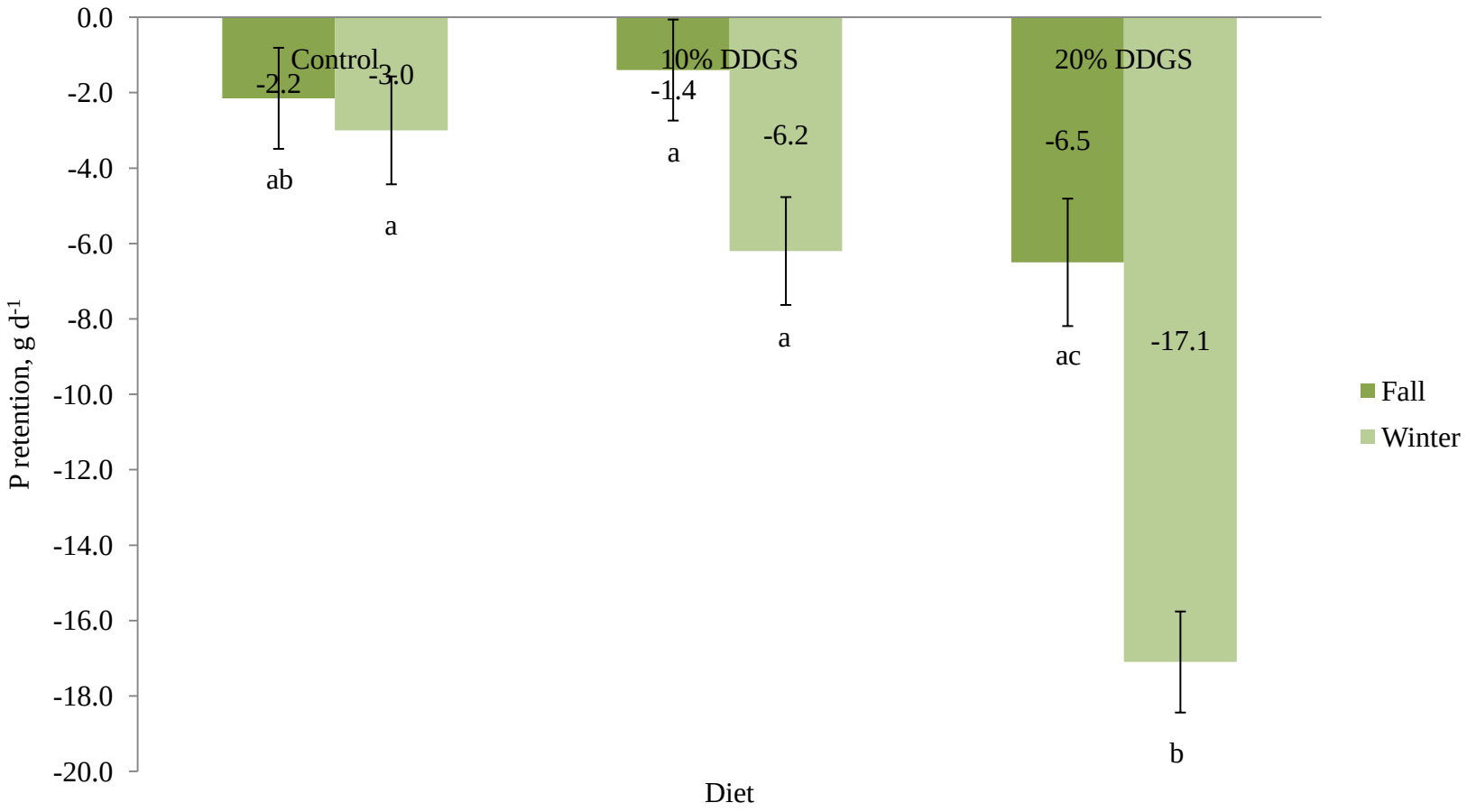
a, b, c Means within diet and season followed by a different letter differ ($P < 0.05$).

study, may have provided cows with endogenous N available to help meet nutrient requirements during cold exposure. Protein deficient cows in the cold-stressed environment likely retained the most endogenous N in an effort to satisfy nutrient requirements, and borderline adequate CP cows would have retained only enough endogenous N to satisfy CP requirements. Conversely, cold-acclimatized 20%DDGS cows presumably excreted all dietary CP consumed in excess of requirements, as well as all unneeded endogenous N, thereby excreting more N than consumed and resulting in the negative N retention observed in this study.

Fecal P concentration and excretion were similar between seasons. However, urine P concentration was significantly greater in cold-acclimatized versus thermal-neutral cows (0.36 versus 0.74 ± 0.105 g L^{-1} in fall and winter, respectively; P = 0.0129; Table 5). Despite lower urine production during the winter, urine P excretion tended to increase from 4.1 to 7.6 ± 1.28 g d^{-1} (P = 0.0668) between fall and winter, respectively. Higher urine P concentration in cold-acclimatized cows is justified with the same explanation as offered for the similar response in urine N concentration. That is, higher urine P concentration occurred because P intake, fecal P excretion and apparent P digestibility remained constant between seasons, which coincided with the reduction in urine production during the winter, meaning that the same quantity of P was consumed and digested in thermal-neutral and cold-acclimatized cows, and although it was not measured, the reduction in urine production in cold-acclimatized cows would have caused a greater concentration of P in the bloodstream followed by a subsequent increase in urine P concentration.

Phosphorus retention demonstrated a diet by season interaction ($P = 0.0078$; Figure 10). Cold-acclimatized cows retained 2.6x less P than thermal-neutral cows (-3.4 versus -8.8 ± 0.83 d g-1, $P < 0.0001$; Table 5), although the difference within diet was only significant between thermal-neutral and cold-acclimatized cows fed excess CP. The increase in urine P concentration during cold-exposure explains the significant reduction in P retention that was observed in cold-acclimatized compared to thermal-neutral cows. Shivering during cold exposure may have contributed to muscle breakdown and mobilization of muscle P, thereby resulting in an increase in endogenous P excretion (Sykes et al., 1969). However, shivering was not measured in the current study and therefore increased endogenous P loss cannot be contributed to shivering with certainty. Further, it is possible that cold-induced mobilization of P from muscles did not contribute significantly to the endogenous P pool, as the cows in the present study may have shivered less with prolonged cold exposure, or once acclimatized to the cold. Sykes and Slee (1968) reported that Scottish Blackface sheep reduced shivering activity once acclimatized to sub-zero temperatures. Further, Sykes et al. (1969) monitored plasma P levels in shorn Southdown and Welsh Mountain female sheep exposed to thermal-neutral (+30°C) or cool (+8°C) temperatures for two weeks prior to and after two acute cold exposures (-20°C, 4 mph wind) that lasted two days each. Sheep in this study demonstrated a significant positive relationship between length of cold exposure and elevation of plasma P concentration during the first cold exposure ($r = 0.58$) but not during the second exposure (Sykes et al., 1969). Therefore, negative P retentions observed in both seasons of the present study may be attributed to increased endogenous P excretion due to mobilization of P from bone and soft tissue, as the cows lost -3.3 and

Figure 10: Diet by season interaction for phosphorus retention in cows consuming low-quality forage (CON), and low-quality forage supplemented with 10% or 20% DDGS in thermal-neutral (fall) and cold-stressed (winter) environments.



a, b, c Means within diet and season followed by a different letter differ ($P < 0.05$).

-3.0 kg BW d^{-1} (P = 0.8332) in thermal-neutral and cold-stressed seasons, respectively. However, it should also be noted that P is pulled from muscle during muscle breakdown sooner than N, thereby explaining why P balance was negative while N balance remained positive in cows of the present study. Further, cows in the cold-stressed environment lost -0.22 BCS, which suggests increased mobilization of body fat and possible contribution of phospholipids from fat breakdown to the endogenous P pool (Bruckmaier et al., 1998).

Cows consuming low-quality forage diets excreted more P than consumed, thereby indicating that endogenous P was being excreted regardless of which level of CP supplementation was received. Cows consuming 20%DDGS retained the least amount of P since they excreted more urine P than CON and 10%DDGS cows. The ability of 20%DDGS cows to excrete more P than CON and 10%DDGS cows is attributed to the fact that they consumed the most dietary P in excess of requirements. This is important to consider as, according to Breves and Schröder (1991), ruminants with P intake below requirement experience reductions in parathyroid hormone secretion, which results in increased dietary P absorption, increased mobilization of P from bones and soft tissue, increased renal P reabsorption and reduced urine P excretion as a P conservation tactic. If this is so, then the similar urine P excretion between CON and 10%DDGS cows in the present study suggests that the 10%DDGS cows did consume borderline adequate dietary P when restricted-fed, and therefore responded as if in a deficient, rather than sufficient, state. As such, both the CON and 10%DDGS cows responded to their respective P intakes with increased dietary P absorption and renal P reabsorption, as well as lower urine P excretion, which was reflected in their P retentions being less negative than the P retention for the 20%DDGS cows. In the case of the 20%DDGS cows, their P intake in

excess of requirements avoided an increase in dietary P absorption and renal P reabsorption as P conservation was not required in order to meet P requirements, and as such, excess P in the bloodstream was excreted via urine and resulted in the most negative P retention in the present study. However, Lomba et al. (1969) cautioned that P retention in both dry and lactating, non-pregnant cows is highly variable regardless of dietary composition because of very inconsistent and unpredictable endogenous P excretion.

As observed with all three diets, P excretion in excess of P intake may be attributed to BW loss experienced in the cows when their DMI was restricted in the digestibility study, since cows fed the CON, 10%DDGS and 20%DDGS diets lost -3.0, -3.4 and -3.2 ± 1.13 kg BW d^{-1} (P = 0.9561; Table 5), respectively. Hogan and Nierman (1927) estimated the P content of the body of 300 kg Hereford steers to be 0.84% P, suggesting a loss of 8.4 g P d^{-1} for each kg BW lost daily. These estimates have also been used with confidence by researchers to approximate the mobile P pool in cows (Kleiber et al., 1950). For example, the mobile P pool for a 400 kg mature, lactating Jersey cow was calculated as 0.54% of P in her body, or 15.6 g P (Kleiber et al., 1950). Although these estimates give an approximation of the amount of P that may have been excreted by the beef cows in the current study, it can be expected that their heavier BW would result in their body P concentration being slightly different than the estimates provided above. Moreover, these predictions do not account for endogenous P losses that may be induced in response to environmental or dietary stressors.

Endogenous P is primarily mobilized from bones and/or soft tissues (Engels, 1981; Breves and Schröder, 1991). In a study by Aziz et al. (1992), wethers fed ad

libitum to stimulate BW gain (+10 kg BW, from 23 to 33 kg) or restricted-fed over 3 – 25 d periods to stimulate BW loss (-10 kg BW, equivalent to -133 g d^{-1}) mobilized more muscle than fat, while maintaining constant bone weight. Further, endogenous P is absorbed into the bloodstream and eventually reflected in urine P concentrations (Tomas, 1974; Williams et al., 1991). Therefore, when BW loss occurred due to intake restriction in the present study, P may have been pulled from soft tissues and bone, which contributed to the endogenous P pool and increased P excretion above the level of P intake to result in negative P retention. Since restricted-fed DMI (8.0, 9.5 and 9.1 ± 0.49 kg d^{-1} for CON, 10%DDGS and 20%DDGS, respectively; P = 0.0859) and BW loss were similar across diets, it may be assumed that the extent of P mobilization from bones and soft tissue also would have been similar, therefore leaving the observed differences in P retention to be explained by differences in urine P excretion. However, Moreira et al. (2009) fed lactating dairy cows forage diets containing Ca and P concentrations that were deficient or in excess of nutrient requirements and reported that P fed in excess of requirements caused prolonged and greater bone mobilization regardless of dietary Ca content. This further confirms the results of the current study in that 20%DDGS cows demonstrated the greatest urine P excretion, and in turn, the most negative P retention.

Results of this study show that mature beef cows under cold-stressed conditions produced 26.8% less enteric CH_4 (7.1 ± 0.30 vs. 5.2 ± 0.26% GEI; P < 0.0001; Table 3) than when exposed to thermal-neutral conditions. Limited literature reports suggest that this significant reduction in CH_4 production may be attributed to increased reticulorumen contractions and corresponding faster digesta passage rate observed in cold-acclimatized ruminants (Christopherson, 1976; Westra and Christopherson, 1976; Kennedy et al.,

1977; Gonyou et al., 1979). However, reductions in rumen fluid volume, which may occur in cattle as a physiological response to cold exposure (Young, 1975; Degen and Young, 1980), may also contribute to an increased fluid passage rate (Robles et al., 1981). However, the low-quality forage diets in this study generated 26.8% less enteric CH_4 in response to cold acclimatization, and this reduction was attributed to rumen fluid ROP and RMRT increasing by similar proportions (33.8% and 26.6%, respectively) in cold-acclimatized compared to thermal-neutral cows. These results are comparable to those of the in vitro experiment by Stanier and Davies (1981), which determined that CH_4 emissions increased by 40% due to a 50% reduction in ruminal fluid passage rate.

Although particulate FMRT was significantly shorter in cold-acclimatized versus thermal-neutral cows, it was unlikely to have contributed considerably to the reduced enteric emissions observed in winter as only 13% of enteric CH_4 production occurs post-ruminally (Murray et al., 1976). Therefore, the reduction in enteric CH_4 emissions from cold-acclimatized cows in this study may be better explained by the change in rumen, rather than post-rumen, passage rates. Even though rumen particulate ROP did not change with cold exposure, the significant reduction in enteric CH_4 emissions may be attributed to the increase in rumen fluid ROP since methanogenic archaea are mainly suspended in the fluid fraction of rumen digesta (Janssen and Kirs, 2008). Thus, cold acclimatization significantly increased rumen fluid ROP and may have reduced the time for methanogens to perform enteric fermentation, thereby decreasing CH_4 emissions.

Cold-acclimatized cows fed low-quality forage diets demonstrated significantly higher N and P excretion than thermal-neutral cows. Cold acclimatization resulted in1.2x lower N retention by cows, and the extent of this decline was dependent on the dietary

CP level. Cold-acclimatized cows also demonstrated dramatically lower (2.5x) P retention, although cold acclimatization cannot be solely held responsible for this reduction as endogenous P losses in response to BW loss and restricted feeding may have also contributed. Compared to thermal-neutral cows, cows in the cold-stressed environment emitted 1.2% less GEI as methane, equivalent to a 26.8% reduction in emissions in response to cold acclimatization. This suggests that the reduction in CH_4 emissions may have provided additional energy to the cow to help meet increased energy requirements during cold exposure. Increased rumen fluid ROP, rather than improved fermentation efficiency, appears to be responsible for the dramatic reduction in enteric CH_4 emissions from cold-acclimatized cows. These data show that current IPCC methodology is significantly over estimating enteric CH_4 emissions for beef cattle housed in cold environments.

4.3.3.2. Influence of Protein Supplementation on Nutrient Excretion

Nitrogen and P excretion from urine and feces, as well as energy excretion in the form of enteric CH_4 emissions (% GEI), were monitored to determine if CP supplementation increased excretion of N and P while decreasing energy excretion.

In concert with increasing ACPI, increases in fecal N (1.11 to 1.37 to 1.69 ± 0.028 g 100 g^{-1} DM for CON, 10%DDGS and 20%DDGS, respectively; $P < 0.0001$; Table 4) and urine N (0.25, 0.65 and 0.83 ± 0.041 g 100 mL^{-1} for CON, 10%DDGS and 20%DDGS, respectively; $P < 0.0001$) concentrations were positive and linear. Protein supplemented cows had greater fecal N excretion (55.8 and 62.6 ± 3.13 g d^{-1} DM for 10%DDGS and 20%DDGS, respectively) than CP deficient cows (41.7 ± 3.13 g d^{-1} DM;

P = 0.0001). Similarly, Köster et al. (1996) reported fecal N excretion of 18.0, 29.7, 39.5, 47.1 and 45.2 g d^{-1}, respectively, when Angus x Hereford cows were fed low-quality forage (1.9% CP, 77% NDF) supplemented with 0, 180, 360, 540 and 720 g RDP d^{-1} (sodium caseinate; 90% CP) via intraruminal infusion. As previously discussed, urine N excretion was positively correlated to urine N concentration (r = 0.89; P < 0.0001) and SUN concentration (r = 0.91; P < 0.0001). This positive, linear association between urine N excretion and SUN concentrations is consistent with reports from Kohn et al. (2005).

Total fecal and urine N excretion was calculated and converted to N_2O emissions using the IPCC (1996) conversion factor of 2% for N originating from the excreta of grazing livestock. Total N_2O emissions estimates were then converted to CO_2 equivalents (CO_2 eq) given that the current GWP of N_2O is 298. Thus, CON, 10%DDGS and 20%DDGS cows demonstrated CO_2 eq for N excretion of 357.6, 655.6 and 923.8 g CO_2 eq d^{-1}(Appendix 4).

In response to the linear increase in P intake, fecal P concentrations (3.0, 4.7 and 6.1 ± 0.24 g kg^{-1} DM for CON, 10%DDGS and 20%DDGS, respectively; P < 0.0001; Table 5) and fecal P excretion (11.5, 19.3 and 25.2 ± 1.52 g d^{-1} for CON, 10%DDGS and 20%DDGS, respectively; P < 0.0001) demonstrated positive, linear increases in accordance with increasing DDGS supplementation. Witt and Owens (1983) fed cottonseed hull-based diets that were low (0.12% P) and adequate (0.23% P) in P to ruminally cannulated steers and reported P intakes of 9.5 and 18.3 g d^{-1}, and corresponding fecal P excretion of 8.5 and 10.0 g d^{-1}, respectively. Similar to the present study, Chantiratikul et al. (2009) fed Brahman x steers rice straw-based diets containing 11.7% CP and 0.19, 0.24 or 0.31% P and reported positive, linear increases in P intakes

(12.8, 19.1 and 25.7 g d^{-1}) and fecal P excretion (2.4, 5.6 and 9.6 g d^{-1}) across diets. Compared to these literature reports, P intakes were similar but fecal P excretion was higher in cows in the present study who received DDGS supplementation, thereby suggesting that the P in wheat-based DDGS may be relatively unavailable. Nevertheless, the close association observed between increased P intake and fecal P excretion may be attributed to absorption in the intestines, but particularly the small intestine, as it is a major site of P absorption and regulation in cattle (Breves and Schröder, 1991; Bravo et al., 2003). When P requirements are not satisfied, P is absorbed and recycled to the body through the intestines, but when P is adequate for requirements, excess P is excreted primarily via feces (Merck Veterinary Manual, 2011).

Cows fed CP in excess of requirement demonstrated higher urine P concentration (0.04, 0.43 and 1.16 ± 0.128 g L^{-1} for CON, 10%DDGS and 20%DDGS, respectively; $P < 0.0001$) and urine P excretion (0.3, 3.8 and 13.4 ± 1.56 g d^{-1} for CON, 10%DDGS and 20%DDGS diets, respectively; $P < 0.0001$) than cows fed deficient or borderline adequate CP. Although urine P excretion is not a major contributor to P regulation in ruminant livestock, as the majority of P regulation occurs via the salivary glands or intestines (Breves and Schröder, 1991), urine P excretion remains important as a means to remove excess P circulating in the bloodstream (Tomas, 1974).The observed increase in urine P concentration and excretion in the present study is attributed to the high P content of wheat-based DDGS, and therefore increasing P intakes with greater DDGS inclusion in the diet.

Enteric CH_4 production by cows consuming deficient, borderline adequate and excess CP was 294.2 ± 8.54, 311.9 ± 8.29 and 280.9 ± 10.02 L d^{-1} ($P = 0.0632$),

representing energy losses of 6.5 ± 0.33, 6.8 ± 0.33 and 5.3 ± 0.38% GEI (P = 0.0128), respectively (Table 3). These emissions as a % GEI are equivalent to calculated CO_2 eq of 5267.5, 5582.5 and 5027.5 g CO_2 eq d^{-1} (Appendix 4). Protein deficient cows were expected to produce higher CH_4 emissions (% GEI) than the supplemented diets as the minimum microbial N requirement, equivalent to 2.0 mg NH_3-N 100 ml^{-1} rumen fluid, was not satisfied from low dietary CP intake. However, contrary to expectation, protein deficient cows produced 4.4% less CH_4 than cows consuming borderline adequate CP. Since N was deficient in the CON diet, fermentation may have been slower due to reduced microbial growth (Maeng et al., 1976; Gilbery et al., 2006), potentially imposing limitations on enteric CH_4 production and in turn, preventing emissions from increasing as much as expected from consumption of the low-quality diet. Furthermore, Satter and Slyter (1974) have indicated that when microbial efficiency is not optimized due to rumen NH_3-N concentrations < 5.0 mg 100 ml^{-1}rumen fluid, methane production parallels total VFA concentrations. This may also explain why enteric CH_4 emissions were not significantly different between the CON and 10%DDGS treatments, as total VFA concentrations were not different across all three diets (Table 7).

Conversely, this theory by Satter and Slyter (1974) does not apply to the reduction in enteric CH_4 emissions from cows consuming excess CP. Rather, it seems that supplementation of low-quality forage diets with CP in excess of animal requirement may have increased NH_3-N concentration sufficiently for near-optimal microbial growth. Satter and Slyter (1974) admitted that although 5.0 mg NH_3-N 100 ml^{-1} rumen fluid is generally accepted as the lower limit required for maximum microbial growth, this concentration is actually an over-estimation and that other researchers have identified

maximum growth at concentrations of 4.0 mg 100 ml^{-1}rumen fluid. National Research Council (NRC, 1985a) indicates the precise NH_3-N concentration required for optimal microbial growth in ruminants is unclear.

Thus, the observed NH_3-N concentrations of 3.0 mg 100 ml^{-1} rumen fluid in 20%DDGS cows of this study may have been nearly sufficient for optimal microbial function, permitting enteric CH_4 production to respond to factors other than total VFA concentrations, such as the concentrations of individual VFA (Table 7). Although acetate concentrations were not significantly different across diets, they followed the same pattern as enteric CH_4 emissions (% GEI), and higher acetate concentrations promote CH_4 production (Johnson and Johnson, 1995). Given that the 20%DDGS diet generated the lowest A:P ratio and highest PD excretion (Table 9), this suggests that supplementing CP in excess of requirements improved fermentation efficiency (Clark, et al., 1992; Valadares et al., 1999), which coincides with the fact that this treatment also produced the lowest CH_4 emissions.

However, the 18.5% (5.3 ± 0.38 versus 6.5 ± 0.33% GEI; Table 3) reduction in enteric CH_4 emissions by cows consuming excess CP compared to cows consuming deficient CP may not only be attributed to improved fermentation efficiency, but also to rumen fluid ROP being 13.6% (9.2 versus 8.1 ± 0.37% h-1; P = 0.0618; Table 6) faster for 20%DDGS than CON. Given that methanogenic archaea responsible for enteric CH_4 production are predominately suspended in the fluid fraction of rumen digesta (Janssen and Kirs, 2008), faster rumen fluid ROP that occurred in cows consuming excess CP increased the rate at which methanogenic archaea were flushed from the rumen, reduced the time for methanogens to ferment fibre into CH_4 and ultimately lowered enteric CH_4

emissions (Kumar et al., 2009). Therefore, 20%DDGS may have had the lowest enteric CH_4 emissions because it had the lowest acetate concentrations, coupled with the lowest A:P ratio, and adequate NH_3-N concentration to allow minimal restrictions on microbial growth and fermentation.

Protein supplementation increased fecal N excretion, and these increases can be accounted for from the relatively unavailable N content of the low-quality forage diets. From an environmental perspective, efficiency of N use was highest when the low-quality forage diets contained CP in excess of animal N requirements, although N losses increased incrementally with greater dietary inclusion of CP. These data suggest that low-quality forage diets appear to result in high endogenous P losses; however, high endogenous losses may have been confounded by BW loss caused by restricted feeding. Excreta N:P ratios for cows fed deficient, borderline adequate and excess CP were 5.05, 4.73 and 3.99, respectively, suggesting that the best nutrient ratios were observed for the excess CP (20%DDGS) diet. Feeding CP in excess of requirements may ensure adequate CP for efficient microbial growth and function and reduce enteric CH_4 emissions by up to18.5% in beef cows consuming low-quality forage diets, but it can also result in 2 and 5 fold increases in fecal and urine N excretion. Net GHG emission estimates (CO_2 eq for fecal and urine N excretion + CO_2 eq for enteric CH_4 emissions) of 5625.1, 6238.1 and 5951.3 g CO2 d^{-1} for CON, 10%DDGS and 20%DDGS cows, respectively, suggest that supplementing CP in excess of requirements reduced CH_4 emissions enough to offset increased N excretion. Thus, this study suggests that feeding CP in excess of animal requirements is the most desirable option for reducing contributions to global warming when low-quality forage diets are fed to beef cows. Further, the nutrient profile of

wheat-based DDGS appears to result in major (2 and 45 fold, respectively) increases in fecal and urine P excretion when fed to provide adequate CP for efficient microbial growth and function.

4.4. CONCLUSIONS

Dry matter intake was prevented from increasing in cold-acclimatized cows as the low-quality forage diets provided inadequate CP and/or energy to promote greater microbial degradation. Since DMI, and therefore energy intake, did not increase, it appeared that cold-acclimatized cows sourced additional energy to cope with increased maintenance requirements by mobilizing body reserves. Protein supplementation with 10% and 20% DDGS satisfied cow nutrient requirements, but failed to meet microbial requirements for optimal degradation of the low-quality forage diets (6.0 to 11.6% CP, DM) and therefore prevented an increase in DMI. Contrary to expectations, nutrient utilization and fermentation efficiency did not differ between thermal-neutral and cold-acclimatized cows fed low-quality forage diets, which may have occurred from a constant rumen NH_3-N concentration preventing increased microbial growth and function during cold exposure. Improvements in N and P digestibility are likely attributed to greater digestible substrate entering the rumen when increasing levels of DDGS were fed, rather than improvements in microbial digestion. Cow CP requirements may be satisfied with a dietary CP content of 7.9% ACP (8.7% CP, DM), but rumen microbes appear to require a dietary CP content greater than 10.4% ACP (11.6% CP, DM) to optimize dietary digestion of low-quality forage diets. This may be caused by the low starch content of wheat-based DDGS (0.9%, DM) increasing rumen microbial NH_3-N requirements despite provision of CP in excess of animal requirements. Fermentation efficiency was not improved when cows consumed low-quality forage diets containing 7.9% ACP, therefore dietary CP levels for mature, dry, non-pregnant beef cows should approach 10.4% ACP

to ensure improved fermentation efficiency. Excretion of N and P was significantly higher (1.2x and 2.5x, respectively) in cold-acclimatized than thermal-neutral cows; however, energy excretion as enteric CH_4 decreased significantly (26.8%) compared to thermal-neutral production values, suggesting that lower CH_4 emissions may have provided cold-acclimatized cows with additional energy to cope with greater maintenance demands. Increasing the inclusion of supplemental CP by feeding 10 or 20% DDGS (as fed basis) incrementally increased excretion of N and P because of the high CP and P content of wheat-based DDGS. Although feeding CP in excess of requirements resulted in the highest N and P excretion, it also resulted in the lowest energy excretion with enteric CH_4 emissions reduced by 18.5% compared to when deficient CP was fed.

5. GENERAL DISCUSSION

5.1. Cold Acclimatization and DMI

Cold acclimatization failed to increase DMI. It was hypothesized that cold acclimatization would increase rumen ROP to allow for greater DMI, but this hypothesis was only partially proven. Cold acclimatization increased the rate of fluid passage from the rumen, but particulate passage was unaffected. Once particulate material was sufficiently small enough to escape the rumen mat, its post-rumen passage was doubled compared to thermal-neutral cows, and this, in conjunction with faster fluid ROP, suggests that cold-acclimatized cows did increase ROP of consumed feedstuffs. However, data from this study suggest that the lack of increase in rumen NH_3-N during cold exposure may have been the limiting factor responsible for preventing greater DMI, and therefore energy intake, in cold-acclimatized cows. To meet greater nutrient demands during exposure to cold-stressed environments, cattle require increased nutrient intake. However, in this study, ACPI and energy intake did not increase between thermal-neutral and cold-acclimatized cows, which caused cows to lose BCS in an effort to source additional energy, and resulted in a lack of increase in rumen NH_3-N which may have limited microbial degradation. If microbial degradation did not increase in cold-acclimatized cows, then it may have prevented faster particulate ROP, which, in turn, prevented cold-acclimatized cows from consuming more feed.

5.2. Protein Supplementation and DMI

Dry matter intake did not increase in response to protein supplementation even though increased dietary CP marginally met or exceeded N requirements. It is possible that the large particle size of the low-quality forage diets may be attributed to this unexpected lack of response. That is, the particulate matter in the diet required a minimum amount of time to be degraded in the rumen, and as such, rumen fill persisted for similar durations between diets regardless of greater CP supplementation. The lack of response in rumen particulate ROP suggests a limiting factor that restricted microbial degradation. Since rumen NH_3-N concentrations in cows consuming excess CP were 6.5 times lower than the proposed concentration (20 mg 100 ml^{-1} rumen fluid) required to promote optimal microbial degradation of low-quality forages, microbial degradation could not increase despite the presence of an abundance of particulate matter in the rumen. Both CP and energy can be limiting to rumen microbes (Satter and Slyter, 1974), and in this study, it is possible that both nutrients restricted microbial degradation. Given that low dietary starch content may have been responsible for the high rumen pH (6.84 – 6.90) observed in CON, 10%DDGS and 20%DDGS cows of this study, this suggests that the low-quality forage diets contained inadequate energy for microbial NH_3-N requirements which may have approached 20 mg 100 ml^{-1} rumen fluid (Leng, 1990). In turn, dietary CP content was also limiting, as the 20%DDGS diet was incapable of generating rumen NH_3-N concentrations near microbial requirements for optimal dietary degradation. This potential for CP and energy to be limiting to microbes was unexpected. It was hypothesized that providing CP in excess of animal requirements would promote increased DMI since restrictive factors that may prevent increased intake should have

been removed. However, this study demonstrates that low-quality forage diets may result in different CP and energy demands between the animal and the rumen microbes. That is, it appears that current published beef cow requirements underestimate CP and energy requirements of mature, dry beef cows when low-quality forages are fed, as rumen microbes may actually require additional nutrients in order to degrade low-quality forage diets at a rate which will permit increases in DMI.

5.3. Cold Acclimatization and N and P Recycling and Digestibility

Contrary to the research hypotheses, cold acclimatization did not increase nutrient utilization in terms of N recycling, or N and P digestibility. However, nutrient utilization also did not decrease, which contradicts the widely accepted hypothesis that cold acclimatization will reduce nutrient utilization due to faster rumen ROP (Kennedy et al., 1976; Kennedy and Milligan, 1978; Young, 1981). Cold-acclimatized cows demonstrated greater SUN concentrations, but this increase in SUN was probably in response to a reduction in body fluid volume which would have caused the significant reduction in urine production that was observed in cold-acclimatized cows in this study. However, body fluid volume was not measured in the present study, so this increase in SUN concentration during exposure to cold can only be speculated as a response to reduced body fluid volume. Similar N and P digestibility between thermal-neutral and cold-acclimatized cows in the present study probably occurred in response to consistent rumen NH_3-N which maintained the rate of microbial digestion, and therefore constant particulate ROP, in the rumen.

Thyroid hormones were measured to validate that cold-acclimatized cows did not start acclimatizing to thermal-neutral temperatures when introduced to the metabolism unit for the digestibility study. Similar T_4 concentrations between the feedlot and metabolism unit suggest that cows maintained a similar metabolic rate despite entering different housing environments. Therefore, these data suggest that the cows were still cold-stressed while in the metabolism unit and that data collected during the digestibility study were representative for cows in the feedlot during the winter. Further, constant TH concentrations between seasons in feedlot cows suggests that metabolic rate was either unchanged in cold-acclimatized compared to thermal-neutral cows, or that it may have returned to baseline rates after cows became effectively cold-acclimatized.

However, TH may not have been the best parameters to measure to obtain useful estimates of cold acclimatization status as T_3 is highly responsive to caloric restriction and protein deficiency, as well as photoperiod changes and stress. As such, it is evident that restricted feeding during the digestibility study, as well as the CP deficient diets fed to CON cows, constant exposure to light, and increased stress levels from being catheterized, restricted-fed and tied in stalls may have confounded T_3 results, making it impossible to use as an accurate measure of cold acclimatization status. However, T_4 is less active than T_3 and does not respond to caloric restriction, but does increase with exposure to cold temperatures. As such, T_4 was relied upon to indicate acclimatization status in the present study, but the strength of study results could have been increased if the more active T_3 could have been referred to in order to validate the responses observed in T_4.

5.4. Protein Supplementation and N and P Recycling and Digestibility

Protein deficiency in ruminant animals is considered restrictive to microbial growth and function, which compromises animal performance by reducing nutrient utilization through lower diet degradation, nutrient digestion, MP synthesis and fermentation efficiency (Bryant and Robinson, 1962; Maeng et al., 1976; Köster et al., 1996). Results of the present study demonstrate that protein deficiency did result in compromised nutrient utilization, including reduced N recycling and apparent N digestibility. Further, it appears that CP deficiency may induce negative apparent P digestibilities in cows fed low-quality forages, further compromising the animal's ability to effectively utilize dietary nutrients.

It was hypothesized that CP supplementation would improve nutrient utilization in beef cows fed low-quality forages, and that cows consuming CP in excess of requirements would demonstrate the most efficient utilization of nutrients. Although the former part of this hypothesis was supported, cows consuming N in excess of requirement did not increase nutrient utilization compared to cows consuming borderline adequate CP. Rather, 10%DDGS and 20%DDGS cows digested dietary nutrients to similar extents, but this was probably due to DDGS inclusion increasing the digestible fraction of the low-quality forage diet. Given that rumen particulate passage did not increase with CP supplementation, this suggests that microbial requirements for improved feedstuff digestion were not satisfied, and therefore, the improvement in nutrient digestibility with CP supplementation is unlikely attributed to improved microbial digestion. As such, this study demonstrates that supplementing low-quality forage with 10%DDGS to provide a dietary ACP content of 7.9% (8.7% CP, DM) and a

rumen NH_3-N concentration of 2.1 mg 100 ml^{-1} is inadequate to meet microbial NH_3-N requirements for optimal growth and digestion, which is in disagreement with predictions provided by Satter and Slyter (1974). Further, the lack of difference in N and P digestibility despite greater inclusion of DDGS up to 20% of the diet (as fed basis) may be attributed to the fact that rumen microbial digestion was not improved. That is, 20%DDGS supplementation would have introduced more fermentable substrate into the rumen as compared to 10%DDGS supplementation, but if microbial digestion was not improved with 20%DDGS supplementation, then improvements in N and P digestibility may have been limited to the capability of rumen microbes to digest the low-quality forage diets, which were presumably similar between 10%DDGS and 20%DDGS diets. Therefore, these data suggest that supplementing DDGS to increase dietary CP content above 10.4% ACP (11.6% CP, DM) is necessary to provide adequate rumen NH_3-N to improve rumen microbial digestion of low-quality forage diets.

5.5. Cold Acclimatization and Fermentation Efficiency

Cold acclimatization did not improve fermentation efficiency as the fermentation profile of VFAs and MP synthesis were both unchanged between thermal-neutral and cold-acclimatized cows. Given that rumen NH_3-N concentrations appeared to have limited optimum rumen microbial growth and function, it stands to reason that fermentation efficiency, including MP synthesis, may have improved if nutrient intake and rumen NH_3-N concentrations had increased and provided rumen microbes with additional nutrients for improved growth and function. This study also suggests that cold-acclimatized cows may have shifted blood flow away from the reticulorumen in an

effort to maintain thermal balance, and/or decreased body fluid volume, as rumen VFA concentrations increased despite no decrease in rumen fermentation efficiency, as indicated by consistent urine PD excretion between seasons.

However, in keeping with the research hypotheses, enteric CH_4 emissions decreased by a significant 26.8% with cold acclimatization, and this reduction was due to faster fluid ROP rather than more efficient rumen microbial fermentation. In fact, it was interesting that fluid ROP increased by 33.8%, which is similar in proportion to the decrease in CH_4 emissions. However, caution should be exercised in comparing the enteric CH_4 emission and ROP data from this study, as these parameters were measured under different experimental conditions and housing types. If enteric CH_4 emissions had been concurrently measured using chambers while cows were housed in the metabolism unit for collection of ROP data, then direct conclusions could be made on how ROP affects enteric CH_4 emissions. Nevertheless, the importance of the association observed between enteric CH_4 emissions and ROP in the present study is that faster rumen fluid ROP decreases CH_4 emissions by increasing the rate of removal of suspended methanogenic microbes from the rumen, thereby reducing the time for microbes to access ingested feed and produce CH_4. Accordingly, it seems that cold acclimatization offers important environmental benefits to western Canadian beef cattle production through the natural ability of cold-acclimatized cattle to adjust physiological function and mitigate CH_4 emissions.

Faster ROP in cold-acclimatized versus thermal-neutral cows of the present study was likely attributed to changes in physiological function in response to prolonged cold-exposure rather than an abrupt introduction to the thermal-neutral environment of the

metabolism unit. Cows during the winter period of the study were removed from the cold-stressed environment and housed in a thermal-neutral metabolism unit for 8-d during the digestibility study, and this may have affected ROP. However, literature reports of ruminant animal response to changes in environmental temperature have consistently reported faster ROP with exposure to cold temperatures, and vice versa (Young, 1981; Kennedy, 1985). Therefore, if changes in ROP between thermal-neutral and cold-acclimatized cows in the present study had occurred in response to the change in thermal temperature between the cold outdoor (feedlot) and warm indoor (metabolism unit) temperatures, it may be expected that ROP should have decreased rather than increased upon entry into the metabolism unit. Given that passage rates became faster between thermal-neutral and cold-acclimatized cows despite sudden exposure to thermal-neutral temperatures for 8-d during the winter period, this suggests that the observed increase in ROP was due to physiological acclimatization by the cows to the cold-stressed environment. This physiological acclimatization to the cold-stressed environment presumably involved increases in the duration and frequency of reticulorumen contractions (Westra and Christopherson, 1976; Kennedy, 1985; Okine et al., 1989) which appear to have persisted for the duration of the 8-d digestibility study.

5.6. Protein Supplementation and Fermentation Efficiency

In keeping with the study hypotheses, fermentation efficiency was improved because of higher MP synthesis with greater CP supplementation and because of a shift towards propionate production when N was fed in excess of requirements. However, reductions in enteric CH_4 emissions behaved unexpectedly, aside from emissions from

20%DDGS which were the lowest of the three diets, as emissions were expected to progressively decrease with increased CP supplementation. Rather, emissions from CP deficient cows were not as high as expected, which may be attributed CP deficiency providing inadequate rumen NH_3-N and therefore limiting microbial function. Protein deficient cows did demonstrate 22.6% higher enteric emissions than cows consuming excess CP. Given that fermentation in CON cows was less efficient with greater production of acetate than propionate, this suggests that microbes functioned inefficiently while performing rumen fermentation.

The 10%DDGS diet resulted in similar emissions compared to the CON diet because borderline-adequate NH_3-N may not have completely removed restrictions on microbes for fermentation, thereby allowing fermentation to persist with similar inefficiency as in CON cows. This was demonstrated by the similar A:P ratios between CON and 10%DDGS cows. Fermentation efficiency, and therefore enteric CH_4 emissions, were most desirable only when N was fed in excess of requirement. In fact, since it is widely accepted that cattle lose 6% GEI as enteric CH_4 (Johnson and Johnson, 1995), the emissions from cows consuming excess N in the present study were improved compared to this standard, with emissions as low as 5.3 ± 0.38% GEI. However, it should be noted that the significant reduction in CH_4 production may have not been solely caused by improved fermentation efficiency, but also by the tendency for fluid ROP to increase and therefore remove methanogenic bacteria from the rumen more quickly, when N requirements were surpassed. Therefore, these data suggest that although dietary CP content of 7.9% ACP with 10%DDGS supplementation was adequate to permit desirable nutrient digestibility, efficient fermentation does not also occur with CP fed at

this level. Rather, to ensure desirable nutrient digestibility and efficient fermentation in mature, dry beef cows consuming low-quality forage diets, dietary CP content should approach 10.4% ACP.

The results of this study demonstrate that the maximum dietary CP inclusion to optimize nutrient utilization and fermentation efficiency in mature, dry, non-pregnant beef cows consuming low-quality forage diets is at least 10.4% ACP (11.6% CP, DM). Given that nutrient digestibility at this level of CP intake was similar to cows consuming 7.9% ACP, but microbial fermentation efficiency did not yet reach a plateau (as indicated by urine PD excretion), this suggests that further CP supplementation may have continued to improve nutrient utilization and/or fermentation efficiency of the low-quality forage diets. Improvements in nutrient utilization, including DMI, and microbial fermentation are expected to plateau once CP intake is sufficiently high, but the CP level at which this would occur cannot be concluded with certainty based on the data from the present study. Similar to the observed responses of cows in the present study, Köster et al. (1996) identified that beef cows fed low-quality forage (1.9% CP, 77% NDF) reached a peak in total tract N digestibility with intakes of 180 g supplemental DIP d^{-1} (11% dietary DIP), but maximum DMI did not occur until 540 g supplemental DIP d^{-1} was consumed, and MP synthesis continued to increase even when supplemental DIP intake reached 720 g d^{-1}. In terms of DDGS inclusion rate, 20% DDGS inclusion (DM basis) is the recommended maximum for optimum nutrient utilization and rumen fermentation in dairy cows as greater inclusion may suppress DMI (Schingoethe, 2006). This study suggests that the recommended inclusion of DDGS in low-quality forage diets for beef cows is greater than 20% (as fed basis) as inclusion up to this rate failed to improve DMI,

and therefore, suppression of DMI is unlikely to be a concern with DDGS inclusion slightly above 20% (i.e. 20 to 30%, as fed basis). In order to determine the optimal dietary inclusion of CP (in the form of wheat-based DDGS) in low-quality forage diets, further research must be conducted examining the changes in nutrient utilization (including DMI) and fermentation efficiency in mature beef cows consuming dietary CP ≥ 10.4% ACP.

5.7. Cold Acclimatization and Nutrient Excretion

Although microbial digestion and fermentation were unchanged by cold acclimatization, urine nutrient excretion was dramatically increased and nutrient retention was decreased. The extent of these increases in N and P excretion were dependent on the level of DDGS supplementation. Urine production decreased in cold-acclimatized cows, and although this reduction may have resulted from reduced body fluid volume in winter, this cannot be concluded with certainty as body fluid volume was not measured in the present study. Further, reduced water intake, which may have been related to lower body fluid volume, cannot be completely ruled out as a potential contributor either as water consumption also was not measured in the present study. This suggests future research experiments investigating the impact of cold acclimatization on ruminant nutrient excretion should consider measuring water intake and body fluid volume to confirm if these may be contributing factors to increased nutrient excretion.

Nevertheless, constant digestibility and fermentation, increased endogenous N and P losses due to cold exposure, and reduced urine production generated higher N and P concentration in the urine, and increased N and P urine excretion in cold-acclimatized

compared to thermal-neutral cows. Further, the significant diet by season interaction for P retention demonstrates that the reduction in P retention across diets was amplified while cows were exposed to the cold-stressed environment. Given that cows lost BW and BCS when exposed to the cold-stressed environment, this suggests that cows may have sourced endogenous P from their body tissues and bones, which may have contributed to an increased P load in the body and, in an effort to maintain blood P balance, cows increased their urinary P excretion. As such, cold acclimatization generated negative environmental impacts by imposing reductions in N and P retention of cold-acclimatized cows, which would also contribute to increased N and P losses to soil and ground water.

Conversely, cold-acclimatized cows appear to offer potential environmental benefits as they decreased enteric CH_4 production by 26.8%. This enteric CH_4 mitigation may also be beneficial in terms of animal performance by providing the animal with additional dietary energy to be used to meet maintenance requirements when in a cold-stressed environment. Whether or not this energetic contribution from reduced CH_4 emissions was enough to satisfy increased energy demands during cold exposure is uncertain. However, the data from this study demonstrate that cold-acclimatized cows emitted 1.2% less GEI as enteric CH_4, suggesting that this amount may have been available for maintenance requirements.

5.8. Protein Supplementation and Nutrient Excretion

Protein supplementation increased excretion of N and P incrementally, but N retention was unchanged between CP deficient cows and cows receiving

supplementation. Losses of fecal N were unchanged between 10%DDGS and 20%DDGS cows, but differences existed in urine N losses, which increased with greater supplementation. Overall, the efficiency of N use was highest when cows consumed CP in excess of requirements, but total N losses from feces and urine were also greatest from these cows. Dried distillers grain with solubles supplementation influenced P excretion differently than N excretion, in that P retention was reduced in cows consuming excess CP. This may be attributed to the high dietary P content of the DDGS, as increased urine P excretion is suggested to occur in response to excessive P intake (Breves and Schröder, 1991).

Energy losses were reduced by 18.5% from CP supplementation, but only when ACPI was in excess of requirements. This demonstrates that feeding CP in excess of requirements results in high, and undesirable, losses of N and P, but much reduced, and more desirable, energy excretion. Nitrogen and P loading of soil and ground water, as well as enteric CH_4 emissions contributing to global warming, are all concerns for the Canadian beef sector, and feeding management is an effective means to help mitigate contributions to these environmental concerns. However, as demonstrated in this study, greater CP supplementation may exacerbate the challenge associated with N and P excretion, while improving excretion of energy. Therefore, it seems there may be an environmental trade-off to make in deciding whether to feed CP at borderline adequate levels, or in excess of animal requirements.

As previously discussed, it appears that providing N in excess of requirements results in the most efficient nutrient utilization, which would generate the most beneficial improvement in animal performance. Therefore, from a production viewpoint, there

appears to be more overall value in feeding CP in excess of, rather than borderline adequate for, animal requirements. Further, conversion of N excretion and enteric CH_4 emissions to CO_2 eq (Appendix 4) allowed comparison between feeding strategies to determine which rate of CP supplementation was least detrimental from the perspective of global warming. Given that net GHG emission estimates were 286.8 g CO_2 eq d^{-1} lower for cows consuming excess compared to borderline adequate CP, this suggests that supplementing low-quality forage diets with excess CP is also the most desirable option in terms of reducing contribution to global warming. However, the overall impact of CP supplementation on environmental sustainability cannot be concluded with certainty as the observed increases in fecal and urine P excretion, and their associated negative impact on P loading of soil and groundwater, are not factored into the net GHG emission estimates and therefore cannot be directly compared to N excretion or CH_4 emissions. In any regard, it may be beneficial to investigate the impact of feeding CP at levels between 7.9 and 10.4% ACP on nutrient excretion, as there may be an optimal dietary CP content within this range that optimizes utilization and excretion of N, P and energy.

While restricted feeding during the digestibility study successfully avoided fluctuations in daily DMI, this feeding strategy was not without fault. Cows lost between 3.0 and 3.4 kg BW hd^{-1} d^{-1} due to restricted DMI, which likely caused mobilization of P from body tissues and bones. As such, P retention in response to CP supplementation was difficult to gauge as endogenous P confounded the concentration of P within the body, particularly in the urine, therefore making it impossible to determine the fractions of P that were of dietary and endogenous origin. It is likely that P balance would not have been negative if cows were not restricted-fed, but without knowing how much

endogenous P was sourced from tissue and bone mobilization, that conclusion cannot be made with certainty. It can only be assumed that since cows in all three treatments had similar restricted-fed DMI and therefore lost similar amounts of BW each day, their endogenous P contribution may have been similar regardless of diet.

5.9. Wheat-based DDGS in Overwintering Beef Cow Nutrition

During Western Canadian winters, beef cows are typically overwintered on low-quality forages that do not meet animal CP requirements. To correct CP deficiencies which cause reductions in DMI, and limit rumen fermentation and nutrient utilization (Maeng et al., 1976; Köster et al., 1996), CP supplementation is necessary and serves to improve animal performance and reduce enteric CH_4 emissions (Johnson and Johnson, 1995; Larson et al., 2009). Diets supplemented with DDGS in the current study were well received by the beef cows, as DDGS is a highly palatable CP supplement. The high concentrations of ACP, energy and readily fermentable fibre allowed the wheat-based DDGS to act as a successful protein supplement capable of generating acceptable N status in cows fed low-quality forage diets when supplemented at both 10 and 20% of the diet (as fed basis). Wheat-based DDGS supplementation improved nutrient utilization and therefore animal performance by increasing apparent N and P digestibility, and increasing fermentation efficiency, including MP synthesis, of low-quality forage-based diets. However, DDGS supplementation did not increase DMI of low-quality forage when included at 10 or 20% of the diet, as the resulting rumen NH_3-N concentrations were not adequate to meet microbial requirements for optimal dietary degradation. Given that wheat-based DDGS is low in starch (0.9%, DM), this may be a potential

disadvantage of its use as a protein supplement with low-quality forages, as low dietary starch has been associated with increasing microbial requirements for optimal feedstuff degradation (Leng, 1990).

In terms of environmental sustainability, wheat-based DDGS inclusion at 20% of the diet increased fermentable fibre content of the diet and tended to increase fluid ROP in cows of the current study, which suggests the potential contribution of DDGS supplementation in lowering enteric CH_4 emissions. Further to this, wheat-based DDGS supplementation increased dietary CP content so as to satisfy rumen microbial N requirements for fermentation, which improved fermentation efficiency by shifting towards propionate (and away from acetate) production and increased MP synthesis, all of which contribute to reductions in CH_4 production. However, the P in DDGS was found to be less digestible than N and although increasing inclusion of DDGS improved P digestibility, it also dramatically increased urine P excretion when fed at 20% of the diet as the high P content of DDGS provided P in excess of requirements.

5.10. Future Research

The substantial reduction in enteric CH_4 emissions observed in cold-acclimatized versus thermal-neutral cows of the present study warrants further exploration in the future. These results are of particular interest to the cattle industry as it demonstrates that the Canadian beef herd may produce less CH_4 than previously predicted as a consequence of the lack of information about fermentation efficiency in cold-acclimatized cattle. Such information is essential for accurate IPCC and nutrient model development, as well as accurate prediction of enteric CH_4 emissions from Canadian beef cattle. Further, wheat-

based DDGS is a relatively new and readily available feedstuff demonstrating potential promise as a suitable protein supplement for Western Canadian beef cattle diets. Therefore future research must be conducted to better understand its impact on nutrient utilization by beef cattle and environmental sustainability.

6. CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that:

- Cold acclimatization did not appear to increase microbial degradation of the low-quality forage diets. In turn, rumen particulate ROP and DMI did not increase when cows were in a cold-stressed compared to a thermal-neutral environment.
- Protein (DDGS) supplementation successfully provided adequate CP, P and energy to marginally meet or exceed cow nutrient requirements, but failed to supply adequate CP and energy to meet microbial requirements for optimal degradation of the low-quality forage diets. This suggests that rumen microbial CP and energy requirements may be greater than published animal CP and energy requirements when low-quality forages are fed.
- Feeding CP at 7.9% ACP (8.7% CP, DM basis) resulted in 38% of cows having rumen NH_3-N concentrations that were deficient for optimal microbial growth and function when low-quality forages were fed. However, 44% of cows consuming low-quality forage supplemented with 10% DDGS (7.9% ACP) had adequate rumen NH_3-N concentrations to support optimal rumen microbial growth and function.
- Restricted microbial degradation of low-quality forage diets prevented faster rumen particulate ROP, maintained constant rumen fill, and prevented increases in DMI despite increasing CP supplementation. When low-quality forages are fed, CP supplementation is unlikely to increase DMI if rumen NH_3-N concentrations do not approach 20 mg 100 ml^{-1} rumen fluid.

- Cold acclimatization had no affect on nutrient utilization of low-quality forage diets supplemented with DDGS because consistent rumen NH_3-N concentrations may have maintained the rate of microbial degradation and digestion in the rumen.
- Cold acclimatized cows demonstrated significantly higher N digestibility than thermal-neutral cows, presumably because endogenous N losses may have been greater in the cold-stressed environment.
- Nutrient utilization was compromised in protein deficient cows, but supplementation of low-quality forage with wheat-based DDGS alleviated CP deficiency and improved nutrient digestibility by increasing the digestible fraction of the TMR.
- Supplementing low-quality forage with 10%DDGS to provide a dietary ACP content of 7.9% (8.7% CP, DM) and a rumen NH_3-N concentration of 2.1 mg 100 ml^{-1} is inadequate to meet microbial NH_3-N requirements for optimal growth and digestion. This may be caused by the low starch content of wheat-based DDGS (0.9%, DM) increasing rumen microbial NH_3-N requirements despite provision of CP in excess of animal requirements.
- Cow CP requirements may be satisfied with a dietary CP content of 7.9% ACP (8.7% CP, DM), but rumen microbes appear to require a dietary CP content greater than 10.4% ACP (11.6% CP, DM) to optimize dietary digestion of low-quality forage diets.
- Cold-acclimatized cows demonstrated no change in fermentation efficiency compared to thermal-neutral cows, which may be attributed to constant microbial growth and function between seasons.

- Protein deficiency reduced fermentation efficiency as the A:P ratio was higher and enteric CH_4 emissions (% GEI) were 22.6% higher than for the diet which provided N in excess of published animal requirements.
- Supplementing low-quality forage diets with wheat-based DDGS improves MP synthesis by increasing rumen NH_3-N concentrations to promote increased rumen microbial growth. Increased rumen P, as well as potentially greater rumen available energy, from greater DDGS supplementation probably also contributed to greater MP synthesis.
- Protein supplementation with 10%DDGS and 20%DDGS improved fermentation efficiency by promoting propionate production and generated 18.5% less enteric CH_4 emissions when CP was fed in excess of requirements.
- Recommended dietary CP levels in low-quality forage diets for mature, dry beef cows should be in excess of 7.9% ACP (8.7% CP; DM basis) to ensure efficient rumen fermentation.
- Higher SUN and VFA concentrations, as well as lower urine production, occurred in cold-acclimatized versus thermal-neutral cows, and may be attributed to reduced body fluid volume and redirection of blood flow away from the reticulorumen.
- As measured by SUN concentrations, feeding supplemental CP to generate dietary CP contents of 7.9 and 10.4% ACP (8.7 and 11.6% CP; DM basis) alleviated CP deficiency in 97 and 100%, respectively, of beef cows fed low-quality forage diets.
- Urine nutrient excretion increased in cold-acclimatized compared to thermal-neutral cows, which suggests potentially negative environmental impacts due to greater N and P losses to soil and ground water during the overwintering period.

- Cold-acclimatized cows fed low-quality forage diets decreased N retention by 1.2x (66.5%), and the extent of this decline was dependent on the dietary CP level. Cold-acclimatized cows also decreased P retention by 2.5x, although endogenous P losses in response to BW loss may have been a contributing factor to increased P excretion.
- Feeding borderline adequate CP decreased total N and P excretion by 29 and 40%, respectively, compared to when CP was fed in excess of requirements, but these improvements were offset with a 28% increase in energy excretion as CH_4.
- From an environmental perspective, feeding CP in excess of animal requirements resulted in the most efficient N use and reduced enteric CH_4 emissions by 18.5%, but it also resulted in major (2 to 45 fold) increases in total N and P excretion by beef cows consuming low-quality forage diets.
- Compared to thermal-neutral cows, cows in the cold-stressed environment emitted 1.2% less GEI as methane, equivalent to a 26.8% reduction in emissions. This reduction in CH_4 emissions may have provided additional energy to the cow to help meet increased energy requirements during cold exposure.
- Increased rumen fluid ROP, rather than improved fermentation efficiency, appears to be responsible for the reduction in enteric CH_4 emissions from cold-acclimatized compared to thermal-neutral cows.
- These data show that current IPCC methodology is significantly over estimating enteric CH_4 emissions for beef cattle housed in cold environments.
- Net GHG emission estimates were 286.8 g CO_2 eq d^{-1} lower for cows consuming excess compared to borderline adequate CP and this suggests that supplementing low-quality forage with excess CP (10.4% ACP) reduces contributions to global

warming as compared to when CP is supplemented at borderline adequate levels (7.9% ACP) for beef cow requirements.

- Wheat-based DDGS is a suitable protein supplement in overwintering beef cow nutrition given its ability to improve overall dietary digestion and fermentation efficiency. The use of DDGS to act as a successful protein supplement with low-quality forages is most effective when included at rates which provide 2.7 to 4.8% CP in excess of requirements.
- Wheat-based DDGS supplementation offers environmental benefits by improving fermentation efficiency and reducing enteric CH_4 emissions in cows fed low-quality forages. However, reductions in P balance may occur when dietary inclusion reaches or exceeds 20% DDGS (as fed basis), which may negatively contribute to environmental sustainability by increasing P loading of soil and ground water.
- The ability of cold acclimatization to substantially mitigate enteric CH_4 emissions must be explored further in the future in order to validate the results of the present study and improve the accuracy of predictions of enteric CH_4 emissions from Canadian beef cattle.
- Future research must be conducted to further characterize wheat-based DDGS as a protein supplement in low-quality forage diets and determine its impact on the environmental sustainability of beef cattle production in western Canada. Given the high CP and P content in wheat-based DDGS, particular attention should be directed towards the impact of DDGS supplementation on N and P loading of soil and ground water.

7. REFERENCES

Adamczewski J. Z., R. K. Chaplin, J. A. Schaefer and P. F. Flood. 1994a. Seasonal variation in intake and digestion of a high roughage diet by muskoxen. Can. J Anim. Sci. **74**: 305-313.

Adamczewski J. Z., W. M. Kerr, E. F. Lammerding and P. F.Flood. 1994b. Digestion of low-protein grass hay by muskoxen and cattle. J. Wildl. Manage. **58**: 679-685.

Adams, D. C. 1987. Influence of winter weather on range livestock. In: Proceedings of the Grazing Livestock Nutrition Conference. pp 23 – 28. Jackson, Wyoming, July 23-24.

Adams, D. C., T. C. Nelsen, W. L. Reynolds and B. W. Knapp. 1986. Winter grazing activity and forage intake of range cows in the northern great plains. J. Anim. Sci. **62**: 1240-1246.

Allard, H., M. Meiner and E. Lindberg. 2009. Methane emissions from Swedish sheep production. Swedish University of Agricultural Sciences, Uppsala, Sweden. [Online] Available: http://stud.epsilon.slu.se/422/1/allard_h_090811.pdf. 27 June 2011.

Allen, M. S. 1996. Physical constraints in voluntary intake of forages by ruminants. J. Anim. Sci. **74:** 3063-3075.

Al-Rabbat, M. F., R. L. Baldwin and W. C. Weir. 1971. In vitro nitrogen-tracer technique for some kinetic measures of ruminal ammonia. J. Dairy. Sci. **54**: 1150-1161.

Alvarez, M. B. and H. D. Johnson. 1970. Urinary excretion of adrenaline and noradrenaline in cattle during heat and cold exposure. J. Dairy. Sci. **53**: 928-930.

Ames, D. R. 1976. Protein adjustments during cold for steers. J. Anim. Sci. **44**: 1342. (Abstr.).

Ames, D. R., D. R. Brink and C. L. Williams. 1994. Adjusting protein in feedlot diets during thermal stress. J. Anim. Sci. **72**: 598-605.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Washington, DC.

Aziz, N. N, D. M. Murray and R. O. Ball. 1992. The effect of live weight gain and live weight loss on body composition of Merino wethers: dissected muscle, fat and bone. J. Anim. Sci. **70**: 1819-1828.

Baile, C. A. and J. M. Forbes. 1974. Control of feed intake and regulation of energy balance in ruminants. Physiol. Rev. **54**: 160-214.

Barboza, P. S., T. C. Peltier and R. J. Forster. 2006. Ruminal fermentation and fill change with season in an arctic grazer: responses to hyperphagia and hypophagia in muskoxen (Ovibos moschatus). Physiol. Biochem. Zoo. **79**: 497-513.

Beauchemin, K. A. and S. M. McGinn. 2006. Enteric methane emissions from growing beef cattle as affected by diet and level of intake. Can. J. Anim. Sci. **86**: 401-408.

Beer, S. F., P. M. M. Bircham, S. R. Bloom, P. M. Clark, C. N. Hales, C. M. Hughes, C. T. Jones, D. R. Marsh, P. R. Raggatt and A. L. R. Findlay. 1989. The effect of a 72-h fast on plasma levels of pituitary, adrenal, thyroid, pancreatic and gastrointestinal hormones in healthy men and women. J. Endocinol. **120**: 337-350.

Benchaar, C., C. Pomar and J. Chiquette. 2001. Evaluation of dietary strategies to reduce methane production in ruminants: A modelling approach. Can. J. Anim. Sci. **81**: 563-574.

Beverlin, S.K., K.M. Haystad, E.L. Ayers and M.K. Petersen. 1989. Forage intake responses to winter cold exposure of free-ranging beef cows. Appl. Anim. Behav. Sci. **23**: 75 – 85.

Bines, J. A., S. Suzuki and C. C. Balch. 1969. The quantitative significance of long-term regulation of food intake in the cow. Br. J. Nutr. **23**: 695-704.

Biswajit, R., B. Brahma, S. Ghosh, P. K. Pankaj and G. Mandal. 2011. Evaluation of milk urea concentration as useful indicator for dairy herd management: A review. Asian J. Anim. Vet. Adv. **6**: 1-19.

Blaxter, K. L., N. Mc. Graham and F. W. Wainman. 1956. Some observations on the digestibility of food by sheep, and on related problems. Brit. J. Nutr. **10**: 69.

Boadi, D. A. and K. M. Wittenberg. 2002. Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF_6) tracer gas technique. Can. J. Anim. Sci. **82**: 201-206.

Boadi, D., C. Benchaar, J. Chiquette and D. Massé. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. Can. J. Anim. Sci. **84**: 319-335.

Boadi, D. A., K. M. Wittenberg and A. D. Kennedy. 2002. Validation of the sulphur hexafluoride (SF_6) tracer gas technique for measurement of methane and carbon dioxide production by cattle. Can. J. Anim. Sci. **82**:125-131.

Boadi, D. A., K. M. Wittenberg, S. L. Scott, D. Burton, K. Buckley, J. A. Small and K. H. Ominski. 2004a. Effect of low and high forage diet on enteric and manure pack greenhouse gas emissions from a feedlot. Can. J. Anim. Sci. **84**: 445-453.

Bodine, T. N. and H. T. Purvis II. 2003. Effects of supplemental energy and/or degradable intake protein on performance, grazing behavior, intake, digestibility, and fecal and blood indices by beef steers grazed on dormant native tallgrass prairie. J. Anim. Sci. **81**: 304-317.

Bodine T. N., H. T. Purvis II and D. L. Lalman. 2001. Effects of supplement type on animal performance, forage intake, digestion and ruminal measurements of growing beef cattle. J. Anim. Sci. **79**: 1041-1051.

Bohnert, D. and T. DelCurto. 2008. Fundamentals of supplementing low-quality forage. 2nd ed. Cattle Producer's Library: Nutrition Section. Western Beef Resource Committee. **317**: 1-4.

Bohnert, D. W., C. S. Schauer and T. DelCurto. 2002. Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: Cow performance and efficiency of nitrogen use in wethers. J. Anim. Sci. **80**: 1629-1637.

Bondi, A. 1981. Metabolism of protein in ruminant animals: A review. Nutr. Rep. Int. **35**: 993-1004.

Boniface, A. M., R. M. Murray and J. P. Hogan. 1986. Optimum level of ammonia in the rumen liquor of cattle fed tropical pasture hay. Proc. Aust. Soc. Anim. Prod. **16**: 151-154.

Bravo, D., D. Sauvant, C. Bogaert and F. Meschy. 2003. II. Quantitative aspects of phosphorus absorption in ruminants. Reprod. Nutr. Dev. **43**: 271-284.

Breves, G. and B. Schröder. 1991. Comparative aspects of gastrointestinal phosphorus metabolism. Nutr. Res. Rev. **4**: 125-140.

Bruckmaier, R. M., L. Gregoretti, F. Jans, D. Faissler and J. W. Blum. 2008. Longissimus dorsi muscle diameter, backfat thickness, body condition scores and skinfold values related to metabolic and endocrine traits in lactating dairy cows fed crystalline fat or free fatty acids. J. Vet. Med. Series A. **45**: 397-410.

Bryant, M. P. and I. M. Robinson. 1961. Studies on the nitrogen requirements of some ruminal cellulolytic bacteria. Appl. Microbiol. **9**: 96-103.

Bryant, M. P. and I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. **84**: 605-614.

Bull, R. C., D. O. Everson, D. P. Olson, K. W. Kelley, S. Curtis and G. Tzou. 1991. Concentrations of serum constituents in cold-stressed calves from heifers fed inadequate protein and (or) energy. J. Anim. Sci. **69**: 853-863.

Bunting, L., P. R. Leavitt, B. Wissel, K. R. Laird, B. F. Cumming, A. St. Amand and D. R. Engstrom. 2011. Sudden ecosystem change in Lake Winnipeg, Canada, caused by eutrophication arising from crop and livestock production during the 20th century. Government of Manitoba. [Online] Available:http://www.gov.mb.ca/waterstewardship/water_quality/lake_winnipeg/pdf/report_lake_wpg_paleolimnology_2011.pdf. 27 June 2011.

Campbell, K. L. and R. A. MacArthur. 1997. Urea recycling in muskrats (Ondatra zibethicus): a potential nitrogen-conserving tactic? Physiol. Zoo. **70**: 222-229.

Casper, D. P., H. A. Maiga, M. J. Brouk and D. J. Schingoethe. 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J. Dairy. Sci. **82**: 1779-1790.

CCAC, 1993. Guide to the care and use of experimental animals. 2nd ed. Vol. 1. E. D. Olfert, B.M. Cross and A.A. McWilliam, ed. Canadian Council on Animal Care, Ottawa, Canada.

Chambers, P. A., M. Guy, E. Roberts, M. N. Charlton, R. Kent, C. Gagnon, G. Grove, N. Foster, C. DeKimpe and M. Giddings. 2008. Threats to sources of drinking water and aquatic ecosystem health in Canada. 6. Nutrients – nitrogen and phosphorus. [Online] Available: http://ec.gc.ca/INRE-NWRI/default.asp?lang=En&n=235D11EB-1&offset=7&toc=show. 5 Aug 2011.

Chantiratikul, A., S. Chumpawadee and P. Chantiratikul. 2009. Effect of dietary phosphorus on nutrient and phosphorus digestibility in Thai-Indigenous x Brahman crossbred cattle. J. Anim. Vet. Adv. **8**: 1558-1562.

Chaves, A. V., L. C. Thompson, A. D. Iwaasa, S. L. Scott, M. E. Olson, C. Benchaar, D. M. Veira and T. A. McAllister. 2006. Effect of pasture type (alfalfa vs. grass) on methane and carbon dioxide production by yearling beef heifers. Can. J. Anim. Sci. **86**: 409–418.

Cheeke P. R. 2005. Applied animal nutrition: Feeds and feeding. 3rd ed. Pearson Prentice Hall, Upper Saddle River, NJ.

Chen, X. B. and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of the technical details. Int. Feed Resources Unit. Rowett Res. Inst. Aberdeen, UK.

Chimonyo, M., H. Hamudikuwana, N. T. Kusina and I. Ncube. 2002. Changes in stress-related plasma metabolite concentrations in working Mashona cows on dietary supplementation. Livest. Prod. Sci. **73**: 165-173.

Christophersen, C. T., A-D. G. Wright and P. E. Vercoe. 2008. In vitro methane emission and acetate:propionate ratio are decreased when artificial stimulation of the rumen wall is combined with increasing grain diets in sheep. J. Anim. Sci. **86**: 384-389.

Christopherson R. J. 1976. Effects of prolonged cold and the outdoor winter environment on apparent digestibility in sheep and cattle. Can. J. Anim. Sci. **56**: 201-212.

Christopherson, R. J. and P. M. Kennedy. 1983. Effect of the thermal environment on digestion in ruminants. Can. J. Anim. Sci. **63**: 477-496.

Cirio, A. and R. Boivin, 1990. Urea recycling from the renal pelvis in sheep: A study with [14C] urea. Am. J. Physiol. **258**: F1196-F1202.

Clark, H., C. Pinares and C. de Klein. 2005. Methane and nitrous oxide emissions from grazed grasslands. In: Grassland. A Global Resource, D. McGilloway (ed.), Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 279-293.

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.

Dauncey, M. J. 1990. Thyroid hormones and thermogenesis. Proc. Nutr, Soc. **49**: 203-215.

Dauncey, M. J. and A. Morovat. 1989. Cold-induced changes in nuclear 3,5,3'-triiodothyronine receptors of porcine skeletal muscle. J. Physiol. **418**: 174P.

Degen, A. A. and Young, B. A. 1980. Effects of cold exposure on liveweight and body fluid compartments of sheep. Can. J. Anim. Sci. **60**: 3341.

Del Curto, T., R.C. Cochran, D.L. Harmon, A.A. Beharka, K.A. Jacques, G. Towne and E.S. Vanzant. 1990. Supplementation of dormant tallgrass-prairie forage: I. Influence of varying supplemental protein and (or) energy levels on forage utilization characteristics of beef steers in confinement. J. Anim. Sci. **68**: 515-531.

Delfino, J. G. and G. W. Mathison. 1991. Effects of cold environment and intake level on the energetic efficiency of feedlot steers. J. Anim. Sci. **69**: 4577-4587.

DeRamus, H. A., T. C. Clement, D. D. Giampola and P. C. Dickison. 2003. Methane emissions of beef cattle on forages: Efficiency of grazing management systems. J. Environ. Qual. **32**: 269-277.

Devendra, C. 1976. Studies on the utilization of rice straw by sheep. Part 3: Water metabolism. Malays Agr. J. **50**: 340-357.

Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy. Sci. **87**: 59-66.

Eckles, C. H. and T. W. Gullickson. 1927. The relation of phosphorus deficiency to the utilization of feed. Amer. Soc. Animal Prod. Proc. pp 16-21.

Eckles, C. H., R. B. Becker and L. S. Palmer. 1926. A mineral deficiency in the rations of cattle. Minn. Agr. Exp. Sta. Bul. **229**: 1-49.

Ekpe, E. D. and R. J. Christopherson. 1999. Metabolic and endocrine responses to cold and feed restriction in ruminants. Can. J. Anim. Sci. **80**: 87-95.

Encyclopaedia Britannica. 2011. Acclimatization. [Online] Available: http://www.britannica.com/EBchecked/topic/2983/acclimatization. 26 June 2011.

Engels, E. A. N. 1981. Mineral status and profiles (blood, bone and milk) of the grazing ruminant with special reference to calcium, phosphorus and magnesium. S. Afr. J. Anim. Sci. **11**: 17-182.

Environment Canada. 2010. National inventory report 1990-2008: Greenhouse gas sources and sinks in Canada. The Canadian Government's Submission to the UN Framework Convention on Climate Change. Library and Archives Canada. Ottawa, ON.

Ergene, N. and E. C. Pickering. 1978. The effects of reducing dietary nitrogen and of increasing sodium chloride intake on urea excretion and reabsorption and on urine osmolality in sheep. Quarterly J. Exp. Phys. **63**: 67-76.

Erwin, E. S., G. J. Marco and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. **44**: 1768-1771.

Estermann, B. L., F. Sutter, P. O. Schlegel, D. Erdin, H. R. Wettstein and M. Kreuzer. 2002. Effect of calf age and dam breed on intake, energy expenditure, and excretion of nitrogen, phosphorus and methane of beef cows with calves. J. Anim. Sci. **80**: 1124-1134.

Eun, J. S. 2002. Characterizing microbial dynamics in continuous cultures and lactation performance of cows fed gamagrass. North Carolina State University. [Online] Available: http://repository.lib.ncsu.edu/ir/bitstream/1840.16/3242/1/etd.pdf. 24 Aug 2011.

Evans, S. E. and D. L. Ingram. 1974. The significance of deep body temperature in regulating the concentration of thyroxine in the plasma of the pig. J. Phys. **236**: 159-170.

Evans, S. E. and D. L. Ingram. 1977. The effect of ambient temperature upon the secretion of thyroxine in the young pig. J. Physiol. **264**: 511-521.

Faverdin, P. 1999. The effect of nutrients on feed intake in ruminants. Proceedings of the Nutrition Society. **58**: 523-531.

Finlay, B. J., G. Esteban, K. J. Clarke, A. G. Williams, T. M. Embley and R. P. Hirt. 1994. Some rumen ciliates have endosymbiotic methanogens. FEMS Microbiol. Lett. **117**: 157-161.

Fonnesbeck, P. V., L. E. Harris and C. W. Cook. 1970. Influence of protein and phosphorus on the composition of the rumen ingesta of sheep. J. Anim. Sci. **30**: 283-290.

Freeman, A. S., M. L. Galyean and J. S. Caton. 1992. Effects of supplemental protein percentage and feeding level on intake, ruminal fermentation, and digesta passage in beef steers fed prairie hay. J. Anim. Sci. **70**: 1562-1572.

Funston, R. N., J. L. Martin, D. C. Adams and D. M. Larson. 2008. Effects of winter grazing system and supplementation during late gestation on performance of beef cows and progeny. Proc. West. Sec. Am. Soc. Anim. Sci. **59**: 102-105.

Gabler, M. T. and A. J. Heinrichs. 2003a. Altering soluble and potentially rumen degradable protein for prepubertal Holstien heifers. **86**: 2122-2130.

Gabler, M.T. and A.J. Heinrichs. 2003b. Dietary protein to metabolizable energy ratios on feed efficiency and structural growth of prepubertal Holstein heifers. J. Dairy. Sci. **86**: 268 – 274.

Gabler, M. T. and A. J. Heinrichs. 2003c. Effects of increasing dietary protein on nutrient utilization in heifers. J. Dairy. Sci. **86**: 2170-2177.

Giesecke, D., L. Ehrentreich and M. Stangassinger. 1994. Mammary and renal excretion of purine metabolites in relation to energy intake and milk yield in dairy cows. J. Dairy Sci. 77: 2376-2381.

Gilbery, T. C., G. P. Lardy, S. A. Soto-Navarro, M. L. Bauer and J. S. Caton. 2006. Effects of corn condensed distillers solubles supplementation on ruminal fermentation, digestion and in situ disappearance in steers consuming low-quality hay. J. Anim. Sci. **84**: 1468-1480.

Godwin, I. R. and V. J. Williams. 1984. Renal control of plasma urea level in sheep: the diuretic effect of urea, potassium and sodium chloride. Q. J. Exp. Physiol. **69**: 49-59.

Gonyou, H. W., R. J. Christopherson and B. A. Young. 1979. Effects of cold temperature and winter conditions on some aspects of behaviour of feedlot cattle. Appl. Anim. Ethol. **5**: 113-124.

Graham, N. M., F. W. Wainman, K. L. Blaxter and D. G. Armstrong. 1959. Environmental temperature, energy metabolism and heat regulation in sheep. 1. Energy metabolism in closely clipped sheep. J. Agric. Sci. **52**: 13-24.

Guan, H., K. M. Wittenberg, K. H. Ominski and D. O. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. J. Anim. Sci. **84**: 1896-1906.

Guthrie, M. J. and D. G. Wagner. 1988. Influence of protein or grain supplementation and increasing levels of soybean meal on intake, utilization and passage rate of prairie hay in beef steers and heifers. J. Anim. Sci. **66**: 1529-1537.

Ham, G. A., R. A. Stock, T. J. Klopfenstein, E. M. Larson, D. H. Shain and R. P. Huffman. 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. **72**: 3246-3257.

Hammond, A. C. 1997. Update on BUN and MUN as a guide for protein supplementation in cattle. U. S. Department of Agriculture, Agricultural Research Service. [Online] Available: http://dairy. ifas. ufl. edu/rns/1997/frns1997. pdf. 27 June 2011.

Harlow, H. J. 1987. Urea-hydrolysis in euthermic hibernators and non-hibernators during periods of food availability and deprivation. J. Therm. Biol. **12**:149-154.

Harrison, D. G., D. E. Beever, D. J. Thomson and D. F. Osbourn. 1976. Manipulation of fermentation in the rumen. J. Sci. Food Agric. **27**: 617-620.

Hastings, M. M. and F. J. Zeman. 1979. Production and metabolism of thyroid hormones in protein-deficient and food-restricted pregnant rats. J. Nutr. **109**: 1925-1933.

Hemsley, J. A. and R. J. Moir. 1963. The influence of higher volatile fatty acids on the intake of urea-supplemented low quality cereal hay by sheep (Abstract). Australian J. Agr. Res. **14**: 509-517.

Hicks, R. B., F. N. Owens, D. R. Gill, J. W. Oltjen and R. P. Lake. 1990. Daily dry matter intake by feedlot cattle: influence of breed and gender. J. Anim. Sci. **68**: 245-253.

Hoover, W. H. and S. R. Stokes. 1991. Balancing carbohydrate and protein for optimum rumen microbial yield. J. Dairy. Sci. **74**: 3630-3644.

Horn G. W. and W. M. Beeson. 1969. Effects of corn distillers dried grains with solubles and dehydrated alfalfa meal on the utilization of urea nitrogen in beef cattle. J. Anim. Sci. **28**: 412-417.

Hristov, A. and E. Pfeffer. 2005. Nitrogen and phosphorus nutrition in cattle: reducing the environmental impact of cattle operations. CABI Publishing, Cambridge, M. A.

Hume, I. D., R. J. Moir and M. Somers. 1970. Synthesis of microbial proteins in the rumen. I. Influence of nitrogen intake. Australian J. Agr. Res. **21**: 283-296.

Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York, N. Y.

Huntington, G. B. and S. L. Archibeque. 1999. Practical aspects of urea and ammonia metabolism in ruminants. Proceedings of the American Society of Animal Science. pp 1-11.

Huntington, G. B., K. Magee, A. Matthews, M. Poore and J. Burns. 2009. Urea metabolism in beef steers fed tall fescue, orchardgrass, or gammagrass hays. J. Anim. Sci. **87**: 1346-1353.

Hurley, W. L., E. M. Convey, K. Leung, L. A. Edgerton and R. W. Hemken. 1980. Bovine prolactin, TSH, T_4 and T_3 concentrations as affected by tall fescue summer toxicosis and temperature. J. Anim. Sci. **51**: 374-379.

International Atomic Energy Agency (IAEA). 2002. Development and field evaluation of animal feed supplementation packages. In: Proceedings of the Final Review Meeting of an IAEA Technical Co-operation Regional AFRA Project. pp 1-163. Cairo, Egypt, 25-29 Nov 2000.

Intergovernmental Panel on Climate Change. 1996. Agriculture. In: Revised guidelines for national greenhouse gas inventories: Reference manual. Cambridge University Press, Cambridge, UK.

Intergovernmental Panel on Climate Change. 2006. Emissions from livestock and manure management. In: Guidelines for National Greenhouse Gas Inventories; Volume 4: Agriculture, Forestry and Other Land Use. [Online] Available: http://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/4_Volume4/V4_10_Ch10_Livestock.pdf. 27 June 2011.

Intergovernmental Panel on Climate Change. 2007a. Agriculture. In: Climate Change 2007: Mitigation of Climate Change. Contribution of working group III to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Intergovernmental Panel on Climate Change. 2007b. Changes in atmospheric constituents and in radiative forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Intergovernmental Panel on Climate Change. 2007c. Summary for policy makers. In: Climate Change 2007: The Physical Science Basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY.

Intergovernmental Panel on Climate Change. 2007d. Technical summary. In: Climate Change 2007: Mitigation of Climate Change. Contribution of working group III to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Janssen, P. H. and M. Kirs. 2008. Structure of the archaeal community of the rumen. Appl. Environ. Microbiol. **74**: 3619-3625.

Joblin, K. N. 2005. Methanogenic archaea. In: Makkar H.P.S., McSweeney C.S. (eds.). Methods in gut microbial ecology for ruminants. pp 47-53. Springer, Dordrecht, Netherlands.

Johnson, K. A. and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. **73**: 2483-2492.

Johnston, A. M. and T. L. Roberts. 2001. High soil phosphorous – is it a problem in Manitoba? In: Proceedings of the Second Annual Manitoba Agronomists Conference. pp 74 – 82. Winnipeg, MB.

Kay, R. N. B. and A. T. Phillipson. 1964. The influence of urea and other dietary supplements on the nitrogen content of the digesta passing to the duodenum of hay-fed sheep (Abstract). Proceedings of the Nutrition Society. 23: xlvi.

Kennedy, P. M. 1985. Influences of cold exposure on digestion of organic matter, rates of passage of digesta in the gastrointestinal tract, and feeding and rumination behaviour in sheep given four forage diets in the chopped, or ground and pelleted form. Br. J. Nutr. **53**: 159-173.

Kennedy, P. M. and L. P. Milligan. 1978. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformation in sheep. Br. J. Nutr. **39**: 105-117.

Kennedy, P. M., B. A. Young and R. J. Christopherson. 1977. Studies on the relationship between thyroid function, cold acclimation and retention time of digesta in sheep. J. Anim. Sci. **45**: 1084-1090.

Kennedy, P. M., R. J. Christopherson and L. P. Milligan. 1976. The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis. Br. J. Nutr. **36**: 231-242.

Kennedy, P. M., R. J. Christopherson and L. P. Milligan. 1982. Effects of cold exposure on feed protein degradation, MP synthesis and transfer of plasma urea to the rumen of sheep. Br. J. Nutr. **47**: 521-535.

Kennedy, P. M., R. J. Christopherson and L. P. Milligan. 1986. Digestive responses to cold. In: Control of Digestion and Metabolism in Ruminants, L. P. Milligan, W. L. Grovum and A. Dobson, eds. pp. 285 – 306. Prentice-Hall. Englewood Cliffs, N. J.

Kleiber, M., A. H. Smith and N. P. Ralston. 1950. Mixing rate of phosphate between plasma and interstitial body fluid of cows. J. Gen. Physiol. **33**: 525-534.

Klopfenstein, T. J. 2001. Distillers grains for beef cattle. In: Proceedings of the National Corn Growers Association Ethanol Co-Products Workshop "DDGS: Issues to Opportunities". pp 1–9. Lincoln, NE, Nov. 7.

Kohn, R. A., M. M. Dinneen and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs and rats. J. Anim. Sci. **83**: 879-889.

Köster, H. H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanznat, I. Abdelgadir and G. St-Jean. 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. J. Anim. Sci. **74**: 2473-2481.

Krebs, G. and R. A. Leng. 1984. The effect of supplementation with molasses/urea blocks on ruminal digestion. Proceedings of the Australian Society of Animal Production **15**: 704-711.

Krysl, L. J., M. E. Branine, M. L. Galyean, R. E. Estell and W. C. Hoefler. 1987. Influence of cottonseed meal supplementation on voluntary intake, ruminal and cecal fermentation, digesta kinetics and serum insulin and growth hormone in mature ewes fed prairie hay. J. Anim. Sci. **64**: 1178-1188.

Kumar, S., A. K. Puniya, M. Puniya, S. S. Dagar, S. K. Sirohi, K. Singh and G. W. Griffith. 2009. Factors affecting rumen methanogens and methane mitigation strategies. World J. Microbiol. Biotechnol. **25**: 1557-1566.

Lardy, G. P., K. K. Karges, M. L. Gibson and J. S. Caton. 2009. Effects of increasing levels of corn distillers dried grains with solubles to steers offered moderate-quality forage. J. Anim. Sci. **87**: 4064-4072.

Larson, D. M., J. L. Martin, D. C. Adams and R. N. Funston. 2009. Winter grazing system and supplementation during late gestation influence performance of beef cows and steer progeny. J. Anim. Sci. **87**: 1147-1155.

Leng, R. A. 1990. Factors affecting the utilization of 'poor-quality' forages by ruminants particularly under tropical conditions. Nutr. Res. Rev. **3**: 277-303.

Leng, R. A. 1991. Fermentative efficiency in the rumen. In: Application of Biotechnology to Nutrition of Animals in Developing Countries. [Online] Available: http://www.fao.org/DOCREP/004/T0423E/T0423E03.htm#ch3.2. 24 Aug 2011.

Leng, R. A. 1991. Improving ruminant production and reducing methane emissions from ruminants by strategic supplementation. U.S. Environmental Protection Agency. Washington, D.C.

Leupp, J. L., G. P. Lardy, K. K. Karges, M. L. Gibson and J. S. Caton. 2009. Effects of increasing level of corn distillers dried grains with solubles on intake, digestion and ruminal fermentation in steers fed seventy percent concentrate diets. J. Anim. Sci. **87**: 2906-2912.

Li, Y. L., T. A. McAllister, K. A. Beauchemin, M. L. He, J. J. McKinnon and W. Z. Yang. 2011. Substitution of wheat dried distillers grains with solubles for barley grain or barley silage in feedlot cattle diets: Intake, digestibility and ruminal fermentation. J. Anim. Sci. **89**: 2491-2501.

Lobely, G. E., A. Connell, M. A. Lomax, D. S. Brown, E. Milne, A. G. Calder and D. A. H. Farningham. 1995. Hepatic detoxification of ammonia in the ovine liver: possible consequences of amino acid catabolism. Br. J. Nutr. **73**: 667-685.

Lomba, F., R. Paquay, V. Bienfet and A. Lousse. 1969. Statistical research on the fate of dietary mineral elements in dry and lactating cows III. Phosphorus. J. Agri. Sci. **73**: 215-222.

Macari, M., M. J. Dauncey, D. B. Ramsden and D. L. Ingram. 1983. Thyroid hormone metabolism after acclimatization and warm or cold temperature under conditions of high or low energy intake. Q. J. Exp. Phys. **68**: 709-718.

Mader, T. L. 2003. Environmental stress in confined beef cattle. J. Anim. Sci. **81**: E110-119.

Maeng, W. J., C. J. Van Nevel, R. L. Baldwin and J. G. Morris. 1976. Rumen microbial growth rates and yields: Effect of amino acids and protein. J. Dairy Sci. **59**: 68-79.

Manninen, M., S. Sankari, L. Jauhiainen, T. Kivinen and T. Soveri. 2007. Insulated, uninsulated and outdoor housing for replacement heifers on restricted grass silage-based diet in a cold environment. Livestock Sci. **107**: 113-125.

Marston, T. T., D. A. Blasi, F. K. Brazle and G. L. Kuhl. 1998. Beef cow nutrition guide. Kansas State University. [Online] Available: http://www.ksre.ksu.edu/library/lvstk2/c735.pdf. 27 June 2011.

Marston, T. T., K. S. Lusby, R. P. Wettemann and H. T. Purvis. 1995. Effects of feeding energy or protein supplements before or after calving on performance of spring-calving cows grazing native range. J. Anim. Sci. **73**: 657-664.

Matejovsky, K. M. and D. W. Swanson. 1995. Intake and digestion of low-, medium-, and high-quality grass hays by lambs receiving increasing levels of corn supplementation. J. Anim. Sci. **73**: 2156-2163.

Mathison, G. W., E. K. Okine, T. A. McAllister, Y. Dong, J. Galbraith and O. I. N. Dmytruk. 1998. Reducing methane emissions from ruminant animals. J. Appl. Anim. Res. **14**: 1-28.

Mawuenyegah, P. O., M. N. Shem, L. Warly and T. Fujihara. 1997. Effect of supplementary feeding with protein and energy on digestion and rumination behaviour of sheep consuming straw diets. J. Agr. Sci. **129**: 479-484.

McCaughey, W. P., K. Wittenberg and D. Corrigan. 1999. Impact of pasture type on methane production by lactating beef cows. Can. J. Anim. Sci. **79**: 221-226.

McCollum, F. T. and M. L. Galyean. 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. J. Anim. Sci. **60**: 570-577.

McIntyre, K. H. 1970. The effects of increased nitrogen intakes on plasma urea nitrogen and rumen ammonia levels in sheep. Aus. J. Agr. Res. **21**: 501-507.

McSweeney, C. S., S. E. Denman, A. D. G. Wright and Z. Yu. 2007. Application of recent DNA/RNA-based techniques in rumen ecology. Asian-Aust. J. Anim. Sci. **20**: 283-294.

Merck and Company Inc. 2011. Beef cattle: Nutritional requirements. In: The Merck Veterinary Manual. [Online] Available:http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/182303.htm. 27 June 2011.

Merck Veterinary Manual, 2011. Chronic phosphorus deficiency/ hypophosphatemia. [Online] Available: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/80501.htm. 24 July 2011.

Mertens, D. L. 1977. Dietary fiber components: Relationship to the rate and extent of ruminal digestion. Fed. Proc. **36**: 187-192.

Miaron, J. O. and R. J. Christopherson. 1992. Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and –particulate passage-rate constants, and apparent digestibility in steers. Can. J. Anim. Sci. **72**: 809-819.

Montgomery, S. P., J. S. Drouillard, E. C. Titgemeyer, J. J. Sindt, T. B. Farran, J. N. Pike, C. M. Coetzer, A. M. Trater and J. J. Higgins. 2004. Effects of wet corn gluten feed and intake level on diet digestibility and ruminal passage rate in steers. J. Anim. Sci. **82**: 3526-3536.

Moorby, J. M., R. J. Dewhurst, R. T. Evans and J. L. Danelon. 2006. Effects of dairy cow diet forage proportion on duodenal nutrient supply and urinary purine derivative excretion. J. Dairy. Sci. **89**: 3552-3562.

Moore, J. A., K. R. Pond, M. H. Poore and T. G. Goodwin. 1992. Influence of model and marker on digesta kinetic estimates for sheep. J. Anim. Sci. **70**: 3528-3540.

Moreira, V. R., L. K. Zeringue, C. C. Williams, C. Leonardi and M. E. McCormick. 2009. Influence of calcium and phosphorus feeding on markers of bone metabolism in transition cows. J. Dairy. Sci. **92**: 5189-5198.

Moss, A. R., J. P. Jouany and J. Newbold. 2000. Methane production by ruminants: Its contribution to global warming. Ann. Zootechnol. **49**: 231-253.

Müller, H. L., J. Von Sax and M. Kirchgessner. 1980. Energieverluste uber kot, harn und methan durch unterschiedliche haufigkeit der futterrumg bei nichtlaktierenden und laktierenden kuhen. Journal of Animal Physiology and Animal Nutrition. Tierphysiol., Tierernahrg. U Futtenrmittelkde. **44**: 181-189.

Murray, R. M., A. M. Bryant and R. A. Leng. 1976. Rates of production of methane in the rumen and large intestine of sheep. Br. J. Nutr. **36**: 1-14.

National Research Council (NRC), 1981. Effect of environment on nutrient requirements of domestic animals. National Academy Press, Washington, D. C.

Ndlovu, T., M. Chimonyo, A. I. Okoh, V. Muchenje, K. Dzama and J. G. Raats. 2007. Review: Assessing the nutritional status of beef cattle: current practices and future prospects. Afr. J. Biotechnol. **6**: 2727-2734.

Nelson, R. A., J. D. Jones, H. W. Wahner, D. B. McGill and C. F. Code. 1975. Nitrogen metabolism in bears: Urea metabolism in summer starvation and in winter sleep and role of urinary bladder in water and nitrogen conservation. Mayo. Clin. Proc. **50**: 141-146.

Nisa, M. U., M. Sarwar, Q. Bilal and M. A. Feroz. 1999. Review: Effect of temperature stress on nutrient utilization and different physiological functions of ruminant animals. Int. J. Agri. Biol. **1**: 174-178.

Novozamsky, I., R. Van Eck, J. C. H. Showenburg and F. Walinga. 1974. Total nitrogen determination in plant material by means of the indole-phenol blue method. Neth. J. Agric. Sci. **22**: 3-5.

NRC. 1984. Nutrient requirements of beef cattle. 6th rev. ed. National Academy Press, Washington, D.C.

NRC. 1985a. Nutrient requirements of sheep. 6th rev. ed. National Academy Press, Washington, D.C.

NRC. 1985b. Ruminant nitrogen usage. National Academy Press, Washington, D. C.

NRC. 1987. Predicting feed intake of food-producing animals. National Academy Press, Washington, D. C.

NRC. 1996. Nutrient requirements of beef cattle. 7th rev. ed. National Academy Press, Washington, D.C.

NRC. 2000a. Nutrient requirements of beef cattle. 7th rev. ed. National Academy Press, Washington, D.C.

NRC. 2000b. Protein. 7th rev. ed. National Academy Press, Washington, D.C.

NRC, 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy Press, Washington, D.C.

Oh, H. K., W. M. Longhurst and M.B. Jones. 1969. Relation of nitrogen intake to rumen microbial activity and consumption of low-quality roughage by sheep. J. Anim. Sci. **28**: 272-278.

Okine, E. K., G. W. Mathison and R. T. Hardin. 1989. Effects of changes in frequency of reticular contractions on fluid and particulate passage rates in cattle. J. Anim. Sci. **67**: 3388-3396.

Oldham, J. D. 1984. Protein-energy interrelationships in dairy cows. J. Dairy Sci. **67**: 1090-1114.

Ominski, K. H., D. A. Boadi and K. M. Wittenberg. 2006. Enteric methane emissions from backgrounded cattle consuming all-forage diets. Can. J. Anim. Sci. **86**: 393-400.

Ominski, K. H., D. A. Boadi, K. M. Wittenberg, D. L. Fulawka and J. A. Basarab. 2007. Estimates of enteric methane emissions from cattle in Canada using the IPCC Tier-2 methodology. Can. J. Anim. Sci. **87**: 459-467.

Ørskov, E. R., C. Fraser and I. McDonald. 1972. Digestion of concentrate in sheep. 4. The effect of urea on digestion, nitrogen retention and growth in young lambs. Br. J. Nutr. **27**: 491-501.

Ortho Clinical Diagnostics. 1993. Veterinary reference guide. Eastman Kodak Company, Rochester, N.Y.

Säkkinen, H. 2005. Variation in the blood chemical constituents of reindeer: Significance of season, nutrition and other extrinsic and intrinsic factors. Acta Univesitatis Ouluensis, Scientiae Rerum Naturalium, 1-61.

Palmblad, J., L. Levi, A. Burger, A. Melander, U. Westgren, H. von Schenk and G. Skude. 1977. Effects of total energy withdrawal (fasting) on the levels of growth hormone, thyrotropin, cortisol, adrenaline, noradrenaline, T_4, T_3 and rT_3 in healthy males. Acta Med. Scand. **201**: 15-22.

Parker, D. S., M. A. Lomax, C. J. Seal and J. C. Wilton. 1995. Metabolic implications of ammonia production in the ruminant. Proc. Nutr. Soc. **54**: 549-563.

Patterson, J. A. and R. B. Hespell. 1979. Trimethylamine and methylamine as growth substrates for rumen bacteria and *Methanosarcina barkeri*. Curr. Microbiol. **3**: 79-83.

Perdok, H. B., R. A. Leng, S. H. Bird, G. Habib and M. Van Houtert. 1988. Improving livestock production from straw-based diets. In: Increasing Small Ruminant Productivity in Semi-Arid Areas, pp. 81-91. E. F. Thomson and F. S. Thomson, ed. International Center for Agricultural Research in Dry Areas. Aleppo, Syria.

Pfeiffer, E. W. 1968. Comparative anatomical observations of the mammalian renal pelvis and medulla. J. Anat. **102**: 321-331.

Phillipson, A. T. 1977. Ruminant digestion. In: Swenson, ed. Dukes Physiology of Domestic Animals. 9th ed. Cornell University Press. London, England.

Pina, D. S., S. C. Valadares Filho, L. O. Tedeschi, A. M. Barbosa and R. F. D. Valadares. 2009. Influence of different levels of concentrate and ruminally undegraded protein on digestive variables in beef heifers. J. Anim. Sci. **87**: 1058-1067.

Pond, K. R., W. C. Ellis, J. H. Matis, H. M. Ferreiro and J. D. Sutton. 1988. Compartment models for estimating attributes of digesta flow in cattle. Br. J. Nutr. **60**: 571-595.

Pontius, B. E., R. H. Carr and L. P. Doyle. 1932. The relation of atmospheric temperature to water consumption and volume of urine in sheep. J. Anim. Sci. **1932**: 210-215.

Poore, M. H., M. E. Scott and J. T. Green, Jr. 2006. Performance of beef heifers grazing stockpiled fescue as influenced by supplemental whole cottonseed. J. Anim. Sci. **84**: 1613-1625.

Preston, R. L., D. D. Schnakenberg and W. H. Pfander. 1965. Protein utilization in ruminants: I. Blood urea nitrogen as affected by protein intake. J. Nutr. **86**: 281-288.

Reed, J. J., G. P. Lardy, M. L. Bauer, M. Gibson and J. S. Caton. 2006. Effects of season and inclusion of corn distillers dried grains with solubles in creep feed on intake, microbial protein synthesis and efficiency, ruminal fermentation, digestion and performance of nursing calves grazing native range in southeastern North Dakota. J. Anim. Sci. **84**: 2200-2212.

Reichlin, S., J.B. Martin, M.A. Mitnich, R.L. Boshans, Y. Grimm, J. Bollinger, J. Gordon and J. Malacara. 1972. The hypothalamus in pituitary thyroid regulation. Recent Prog. Horm. Res. **28**: 229-286.

Reynolds, C. K. 1992. Metabolism of nitrogenous compounds by ruminant liver. J. Nutr. **122**: 850-854.

Reynolds, C. K. and N. B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. J. Anim. Sci. **86**: E293-E305.

Robles, A. Y., R. L. Belyea and F. A. Martz. 1981. Intake, digestibility, ruminal characteristics and rate of passage of alfalfa diets fed to sheep. J. Anim. Sci. **53**: 774-779.

Russell, J. B., J. K. 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.

Sano, H., S. Nakamura, S. Kobayashi, H. Takahashi and Y. Terashima. 1995. Effect of cold exposure on profiles of metabolic and endocrine responses and on responses to feeding and arginine injection in sheep. J. Anim. Sci. **73**: 2054-2062.

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

Schaefer, A. L. and B. A. Young. 1980. Gastrointestinal blood flow distribution in cold-exposed sheep. Can. J. Anim. Sci. 60: 677-681.

Schauer, C. S., D. W. Bohnert, D. C. Ganskopp, C. J. Richards and S. J. Falck. 2005. Influence of protein supplementation frequency on cows consuming low-quality forage: performance, grazing behavior and variation in supplement intake. J. Anim. Sci. **83**:1715-1725.

Schingoethe, D. J. 2006. Utilization of DDGS by cattle. Proc. 27th Western Nutrition Conf. Winnipeg, MB. Sept. 19-20. pp. 61-74.

Schmidt-Nielsen, B. 1958. Urea excretion in mammals. Physiol. Rev. **38**: 139-168.

Sharpley, A. N., J. L. Weld and P. J. A. Kleinman. 2005. Assessment of best management practices to minimize the runoff of manure-bourne phosphorus in the United States. N. Z. J. Agr. Res. **47**: 461-477.

Silvestri, E., L. Schiavo, A. Lombardi and F. Goglia. 2005. Review: Thyroid hormones as molecular determinants of thermogenesis. Acta Physiol. Scand. **184**: 265-283.

Simpson, T. W., A. N. Sharpley, R. W. Howarth, H. W. Paerl and K. R. Mankin. 2008. The new gold rush: Fueling ethanol production while protecting water quality. J. Environ. Qual. **37**: 318-324.

Slyter, L. L., L. D. Satter and D. A. Dinius. 1979. Effect of ruminal ammonia concentration on nitrogen utilization by steers. J. Anim. Sci. **48**: 906-912.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, R. J. Scholes, O. Sirotenko, M. Howden, T. McAllister, G. Pan, V. Romanenkov, U. Schneider, S. Towprayoon, M. Wattenback and J.U. Smith. 2008. Greenhouse gas mitigation in agriculture. Phil. Trans. R. Soc. B. **363**: 789-813.

Sniffen, C. J. and P. H. Robinson. 1987. Microbial growth and flow as influenced by dietary manipulations. J. Dairy. Sci. **70**: 425-441.

Song, M. K. and J. J. Kennelly. 1989. In situ degradation of feed ingredients, fermentation pattern and microbial populations as influenced by ruminal ammonia concentration. Can. J. Anim. Sci. **69**: 999-1006.

Spiehs, M. J., M. H. Whitney and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnedosa and South Dakota. J. Anim. Sci. **80**: 2639-2645.

Stanier, G. and A. Davies. 1981. Effects of the antibiotic monensin and an inhibitor of methanogenesis on in vitro continuous tureen fermentation. Br. J. Nutr. **45**: 567-578.

Statistics Canada. 2011a. Cattle inventories, by province (Canada). [Online] Available: http://www40.statcan.gc.ca/l01/cst01/PRIM50A-eng.htm. 27 June 2011.

Statistics Canada. 2011b. Livestock estimates. Table 1: Cattle inventories. [Online] Available: http://www.statcan.gc.ca/daily-quotidien/110217/t110217c1-eng.htm. 27 June 2011.

Stewart, C. S. and Bryant, M. P. 1988. The rumen bacteria. In: P.N. Hobson (ed.). The rumen microbial ecosystem. pp 21-76. Elsevier Appl. Sci., New York.

Storm, A. C., M. D. Hanigan and N. B. Kristensen. 2011. Effects of ruminal ammonia and butyrate concentrations on reticuloruminal epithelial blood flow and volatile fatty acid absorption kinetics under washed reticulorumen conditions in lactating dairy cows. J. Dairy Sci. **94**: 3980-3994.

Sutton, J. D., I. C. Hart, W. H. Broster, R. J. Elliott and E. Schuller. 1986. Feeding frequency for lactating dairy cows: Effects of rumen fermentation and blood metabolites and hormones. Br. J. Nutr. **56**: 181-192.

Sykes, A. R. and J. Slee. 1968. Acclimatization of Scottish Blackface sheep to cold. 2. Skin temperature, heart rate, respiration rate, shivering intensity and skinfold thickness. Anim. Prod. **10**: 17-35.

Sykes A. R., A. C. Field and J. Slee. 1969. Cold exposure of Southdown and Welsh Mountain sheep. 3. Changes in plasma calcium, phosphorus, magnesium, sodium and potassium levels. Anim. Prod. **11**: 91-99.

Symonds, H. W., D. L. Mather and K. A. Collis. 1981. The maximum capacity of the liver of the adult dairy cow to metabolize ammonia. Br. J. Nutr. **46**: 481-486.

Tarr, B. 2007. Cold stress in cows. Ontario Ministry of Agriculture, Food and Rural Affairs. [Online] Available: http://www.omafra.gov.on.ca/english/livestock/beef/facts/07-001.htm. 21 Aug 2011.

Tas, B. M. and A. Susenbeth. 2007. Urinary purine derivatives excretion as an indicator of in vivo microbial N flow in cattle: A review. Livest. Sci. **111**: 181-192.

Thompson, G. E. 1977. Physiological effects of cold exposure. In: D. Robertshaw, ed. International Physiology II. Vol. 15. University Park Press, Baltimore, MD.

Thompson, G. E. and D. P. Clough. 1972. The effect of cold exposure on plasma lipids of the new-born and adult ox. Q. J. Exp. Physiol. **57**: 192-198.

Tomas, F. M. 1974. Phosphorus homeostasis in sheep. III. Relationship between the amount of salivary phosphorus secreted and the quantities of phosphorus excreted via the urine and faeces. Aust. J. Agric. Res. **25**: 495-507.

Uden, 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**: 632-635.

Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy. Sci. **82**: 2686-2696.

Valtonen, M. 1979. Renal responses of reindeer to high and low protein diet and sodium supplement. J. Scient. Agrig. Soc. Finl. **51**: 381-419.

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.

Van Kessel, J. A. S. and J. B. Russell. 1996. The effect of pH on ruminal methanogenesis. FEMS Microbiol Ecol. **20**: 205-210.

Van Nolte, J., A. V. Ferreira and H. H. Köster. 2003. Effect of amount of rumen degradable protein on the utilization of wheat straw by Dohne Merino wethers. Anim. Sci. **76**: 319-326.

Vasconcelos, J. T., L. W. Greene, N. A. Cole, M. S. Brown, F. T. McCollum III and L. O. Tedeschi. 2006. Effects of phase feeding of protein on performance, blood urea nitrogen concentration, manure nitrogen:phosphorus ratio and carcass characteristics of feedlot cattle. J. Anim. Sci. **84**: 3032-3038.

Villalobos, G. T. J. Klopfenstein, D.C. Adams, D. Shain and T. E. Long. 1997. Grass hay as a supplement for grazing cattle II. Ruminal digesta kinetics. J. Range Mgmt. **50**: 357-360.

Von Keyserlingk, G. E. and G. W. Mathison. 1993. The effect of ruminal escape protein and ambient temperature on the efficiency of utilization of metabolizable energy by lambs. J. Anim. Sci. **71**: 2206-2217.

Wainman, F. W., P. J. S. Dewey and A. C. Brewer. 1984. Feedstuffs evaluation unit. Fourth Report. Rowett Research Institute, Aberdeen, Scotland.

Walker, D. G., A. R. Egan, C. J. Nader, M. J. Ulyatt and G. B. Storer. 1975. Rumen microbial protein synthesis and proportions of microbial and non-microbial nitrogen flowing to the intestines of sheep (Abstract). Australian J. Agr. Res. **26**: 699-708.

Walker, D. J. 1968. Energy utilization for polysaccharide synthesis by mixed rumen organisms fermenting soluble carbohydrates. Appl. Microbiol. **16**: 1672-1677.

Warren, R. J., R. L. Kirkpatrick, A. Oelschlaeger, P. F. Scanlon, K. E. Webb Jr. And J. B. Whelan. 1982. Energy, protein and seasonal influences on white-tailed deer fawn nutritional indices. J. Wildl. Manage. **46**: 302-312.

West, J. B. 1985. Best and Taylor's Physiological Basis of Medical Practice. 11th ed. Williams and Wilkens, Baltimor, M. D.

Westra, R. and R. J. Christopherson. 1976. Effects of cold on digestibility, retention time of digesta, reticulum motility and thyroid hormones in sheep. Can. J. Anim. Sci. **56**:699-708.

Westra, R. and R. J. Hudson. 1981. Digestive function of Wapiti calves. J. Wildl. Manage. **45**: 148-155.

Whitelaw, F. G., J. Margaret Eadie, L. A. Bruce and W. J. Shand. 1984. Methane formation in faunated and ciliate-free cattle and its relationship with rumen volatile fatty acid proportions. Br. J. Nutr. **52**: 261-275.

Williams, C. H., D. J. David and O. Iismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. **59**: 381-385.

Williams, S. N., L. R. McDowell, A. C. Warnick, N. S. Wilkinson and L. A. Lawrence. 1991. Phosphorus concentrations in blood, milk, feces, bone and selected fluids and tissues of growing heifers as affected by dietary phosphorus. Livestock Res. Rural Dev. **3**: 67-80.

Witt, K. E. and F. N. Owens. 1983. Phosphorus: ruminal availability and effects on digestion. J. Anim. Sci. 56: 930-937.

Wolin, M. J. 1960. A theoretical rumen fermentation balance. J. Dairy. Sci. **43**: 1452-1459.

Wolkers, H., T. Wensing and J. T. Schonewille. 1994. Effect of undernutrition on haematological and serum biochemical characteristics in red deer (*Cervus elaphus*). Can. J. Zool. **72**: 1291-1296.

Wright, A. D. G., A. J. Williams, B. Winder, C. T. Christopherson, S. L. Rodgers and K. D. Smith. 2004. Molecular diversity of rumen methanogens from sheep in Western Australia. Appl. Environ. Microbiol. **70**: 1263-1270.

Wright, A. D. G., C. H. Auckland and D. H. Lynn. 2007. Molecular diversity of methanogens in feedlot cattle from Ontario and Prince Edward Island, Canada. Appl. Environ. Microbiol. **73**: 4206-4210.

Yen, P. M. 2001. Physiological and molecular basis of thyroid hormone action. Physiol. Rev. **81**: 1097-1142.

Young, B. A. 1975. Temperature-induced changes in metabolism and body weight of cattle (*Bos taurus*). Can. J. Physiol. Pharmacol. **53**: 947-953.

Young, B. A. 1981. Cold stress as it affects animal production. J. Anim. Sci. **52**: 154-163.

Young, B. A. 1986. Food intake of cattle in cold climates. In: Symposium Proceedings: Feed intake by beef cattle. Annual Report – Oklahoma Agricultural Experimental Station. pp. 328-340.

Yousef, M. K., H. H. Kibler and H. D. Johnson. 1967. Thyroid activity and heat production in cattle following sudden ambient temperature changes. J. Anim. Sci. **26**: 142-148.

Zebeli, Q., M. Tafaj, I. Weber, J. Dijkstra, H. Steingass and W. Drochner. 2007. Effects of varying dietary forage particle size in two concentrate levels of chewing activity, ruminal mat characteristics and passage in dairy cows. J. Dairy Sci. **90**: 1929-1942.

8. APPENDIX

Appendix 1: Trace Mineralized Stock Salt Guaranteed Analysis[1]

Salt (minimum)	96.5%
Zinc (actual)	4000 mg/kg
Iron (actual)	1600 mg/kg
Manganese (actual)	1200 mg/kg
Copper (actual)	330 mg/kg
Iodine (actual)	100 mg/kg
Cobalt (actual)	40 mg/kg

[1] Sifto Canada Corporation, Mississauga, ON

Appendix 2: Biofos/Dicalcium Erco Phosphate (Fine) Guaranteed Analysis[1]

Phosphorus (minimum)	21%
Calcium (minimum)	17%

[1] Feed Rite, Winnipeg, MB

Appendix 3: Forage Analysis Energy Calculations, DM Basis[1]

DE = 3.44 – (0.022 * [ADF])

ME = ([DE] * 0.82)

NE_m = ((1.37 * [ME]) – (0.138 * [ME] * [ME]) +
(0.0105 * [ME] * [ME] * [ME]) – 1.12)

TDN = ([DE] / 4.4)*100

[1] Central Testing Laboratory Ltd., Winnipeg, MB

Appendix 4: Calculations for Determination of CO_2 Equivalents and Net GHG Emissions from Total Nitrogen Excretion and Enteric CH_4 Emissions

1. Total N excretion (Fecal N excretion + Urine N excretion)

- CON: 59.6 g N d^{-1}
- 10%DDGS: 109.2 g N d^{-1}
- 20%DDGS: 153.9 g N d^{-1}
- Fall: 98.4 g N d^{-1}
- Winter: 116.8 g N d^{-1}

2. Conversion of total N excretion to N_2O using 2% N_2O conversion factor (IPCC, 1996).

- CON: 59.6 g N d^{-1} x 0.02 = 1.2 g N_2O d^{-1}
- 10%DDGS: 109.2 g N d^{-1} x 0.02 = 2.2 g N_2O d^{-1}
- 20%DDGS: 153.9 g N d^{-1} x 0.02 = 3.1 g N_2O d^{-1}
- Fall: 98.4 g N d^{-1} x 0.02 = 2.0 g N_2O d^{-1}
- Winter: 116.8 g N d^{-1} x 0.02 = 2.3 g N_2O d^{-1}

3. Conversion of enteric CH_4 emissions from L d^{-1} to g d^{-1}

   Given that:

   1 mol CH_4 = 16.04 g CH_4

   1 mol CH_4 = 22.4 L CH_4 at STP

   22.4 L CH_4 = 16.04 g CH_4

- CON: (294.2 L d^{-1} * 16.04) / 22.4 = 210.7 g CH_4 d^{-1}
- 10%DDGS: (311.9 L d^{-1} * 16.04) / 22.4 = 223.3 g CH_4 d^{-1}
- 20%DDGS: (280.9 L d^{-1} * 16.04) / 22.4 = 201.1 g CH_4 d^{-1}
- Fall: (334.7 L d^{-1} * 16.04) / 22.4 = 239.7 g CH_4 d^{-1}
- Winter: (256.7 L d^{-1} * 16.04) / 22.4 = 183.8 g CH_4 d^{-1}

4. Conversion of CH_4 and N_20 to CO_2 eq

   Given that:

   CH_4 GWP = 25; N_2O GWP = 298 (IPCC, 2007b)

- <u>Fecal and urine N_2O emissions:</u>
  - CON: 1.2 g N_2O d^{-1}* 298 = 357.6 g CO_2 eq d^{-1}
  - 10%DDGS: 2.2 g N_2O d^{-1} * 298 = 655.6 g CO_2 eq d^{-1}
  - 20%DDGS: 3.1 g N_2O d^{-1} * 298 = 923.8 g CO_2 eq d^{-1}
  - Fall: 2.0 g N_2O d^{-1} * 298 = 596.0 g CO_2 eq d^{-1}
  - Winter: 2.3 g N_2O d^{-1} * 298 = 685.4 g CO_2 eq d^{-1}

- <u>Enteric CH_4 emissions:</u>
  - CON: 210.7 g CH_4 d^{-1} * 25 = 5267.5 g CO_2 eq d^{-1}
  - 10%DDGS: 223.3 g CH_4 d^{-1} * 25 = 5582.5 g CO_2 eq d^{-1}
  - 20%DDGS: 201.1 g CH_4 d^{-1} * 25 = 5027.5 g CO_2 eq d^{-1}
  - Fall: 239.7 g CH_4 d^{-1} * 25 = 5992.5 g CO_2 eq d^{-1}
  - Winter: 183.8 g CH_4 d^{-1} * 25 = 4595.0 g CO_2 eq d^{-1}

5. Net GHG emission estimates (CO_2 eq for fecal and urine N_2O emissions + CO_2 eq for enteric CH_4 emissions)
    - CON: 5267.5 g CO_2 eq + 357.6 g CO_2 eq = 5625.1 g CO_2 eq d^{-1}
    - 10%DDGS: 5582.5 g CO_2 eq + 655.6 g CO_2 eq = 6238.1 g CO_2 eq d^{-1}
    - 20%DDGS: 5027.5 g CO_2 eq + 923.8 g CO_2 eq = 5951.3 g CO_2 eq d^{-1}
    - Fall: 5992.5 g CO_2 eq + 596.0 g CO_2 eq = 6588.5 g CO_2 eq d^{-1}
    - Winter: 4595.0 g CO_2 eq + 685.4 g CO_2 eq = 5280.4 g CO_2 eq d^{-1}