NUTRIENT EXCRETION AND SOIL GREENHOUSE EMISSION FROM EXCRETA OF OVERWINTERING BEEF COWS FED FORAGE-BASED DIETS SUPPLEMENTED WITH DRIED DISTILLERS' GRAINS WITH SOLUBLES

BY

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ABSTRACT

Donohoe, Gwendolyn R. M.Sc., University of Manitoba, September, 2010. Nutrient excretion and soil greenhouse emission from excreta of overwintering beef cows fed forage-based diets supplemented with dried distillers' grains with solubles. Major Professor: Dr. Mario Tenuta.

A study was conducted to examine the impact of diet and cold weather on the excretion of nitrogen (N) and phosphorus (P) from beef cows, and the potential for these nutrients to be lost to waterways or as greenhouse gases (GHG). Feces and urine were collected from mature cows fed low-quality forage supplemented with DDGS to 0%, 10%, and 20% ww^{-1} in the fall of 2008 and winter of 2009. A detailed nutrient analysis was performed to determine forms of N and fractions of P in excreta. Feces, urine, and a simulated bedding pack were then applied to grassland to determine soil GHG emission. Cattle receiving DDGS supplementation excreted greater proportions of labile P in feces and greater concentrations of P in urine. The 20% DDGS diets had greater nitrous oxide emission from urine patches and greater proportions of available N in urine and feces.

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TABLE OF CONTENTS

ABSTRACT I
ACKNOWLEDGEMENTS II
TABLE OF CONTENTS III
LIST OF FIGURES VII
LIST OF TABLES IX

1.0 INTRODUCTION 1
1.1 References 5

2. DIET AND COLD ACCLIMIZATION EFFECTS ON FORMS OF NITROGEN AND FRACTIONS OF PHOSPHORUS IN EXCRETA OF BEEF CATTLE 6
2.1 Abstract 6
2.2 Introduction 8
2.3 Materials and Methods 13
2.3.1. Feces and urine collection 13
2.3.2. Feces and urine analysis 17
2.3.3. Data analysis 19
2.3.4. Statistical analysis 20
2.4 Results 21
2.4.1. Mass of nitrogen and phosphorus excreted 21
2.4.2. Proportions of nitrogen and phosphorus excreted via feces and urine 23
2.4.3. Excreta nitrogen concentrations 25
2.4.4. Excreta phosphorus concentrations 29
2.4.5. Ratios of nitrogen and phosphorus excreted 33
2.5 Discussion 35
2.5.1. Relationships between diet and excretion of nitrogen and phosphorus 35
2.5.1.1. Nitrogen 35
2.5.1.1.1. Nitrogen intake and excretion 35
2.5.1.1.2. Protein metabolism and relationship to forms of nitrogen in excreta 39

2.5.1.1.3. Minimizing excretion of available nitrogen 44
2.5.1.2. Phosphorus 45
2.5.1.2.1. Phosphorus intakes and metabolism 45
2.5.1.2.3. Phosphorus excretion and forms of phosphorus 47
2.5.1.2.4. Relationship between feces total phosphorus and labile phosphorus 49
2.5.1.2.5. Effect of diet on labile P 50
2.5.2. Effect of season on excretion of nitrogen and phosphorus 53
2.5.3. Nitrogen to phosphorus ratios 56
2.6 Conclusions 59
2.7 References 61

3. SOIL GHG EMISSION FROM EXCRETA COLLECTED FROM OVERWINTERING BEEF CATTLE FED DIETS OF LOW-QUALITY FORAGE SUPPLEMENTED WITH DDGS DEPOSITED ON GRASSLAND FOLLOWING SNOW MELT 66
3.1 Abstract 66
3.2 Introduction 68
3.3 Materials and Methods 71
3.3.1. Excreta Collection 71
3.3.2. Site Description and Study Design 73
3.3.4. Measurements 76
3.3.4.1. Gas Sampling 76
3.3.4.2 Soil Sampling 77
3.3.4.3. Meteorological Data 78
3.3.5 Analysis 78
3.3.5.1. Nutrient Analysis 78
3.3.5.2. Flux Determination 79
3.3.6. Statistics 80
3.4 Results 80
3.4.1. Meteorological conditions 80
3.4.2 Nitrous Oxide Emissions 81
3.4.3 Methane Emissions 86

3.4.5. Spearman Rank Correlation Coefficients 87
3.4.6. Soil Conditions 90
3.4.7. Carbon Dioxide Emission 94
3.5 Discussion 96
3.5.1. Limitations 96
3.5.2. N_2O Emission 96
3.5.2.1. Early Season N_2O Emission from Urine Patches 97
3.5.2.1.1. Soil Available-N and Soil Texture 97
3.5.2.1.2. Soil Moisture 99
3.5.2.1.3. Nitrification 100
3.5.2.1.4. Denitrification 102
3.5.2.2. Growing Season N_2O Emission from Urine Patches 103
3.5.2.2.1. Temperature 104
3.5.2.2.2. Microbial Health 105
3.5.2.2.3. Mid Season N_2O Peak 106
3.5.2.3. N_2O Emission from Feces 109
3.5.2.4. Diet effect on N_2O emission 110
3.5.2.5. N Emission Factors 113
3.5.3 Methane emission 115
3.5.4. Extrapolation of Effect of Diet on Excretion Rates and Relationship to GHG Emission 117
3.6 Conclusions 119
3.7 References 122

4.0 SYNTHESIS 126
4.1 References 132

APPENDIX A 135
APPENDIX B 138
APPENDIX C 139
APPENDIX D 140

APPENDIX E 141
APPENDIX F..... 142

LIST OF FIGURES

Figure **Page**

Figure. 3.1. Location of the study site and randomize complete block design at the "Point" Field Research Laboratory on the University of Manitoba campus. 76

Figure 3.2. N_2O flux from patches of urine, feces, and simulated bedding pack, from three diet treatment groups, and grassland background emission, deposited on grassland in April 2009 and measured until September 2009. Average values (n=6) +1 standard error of the average is shown as bars. 84

Figure 3.3. Urine patch soil NH_4^+-N, NO_3^--N, and NO_2^--N, mean temperature and precipitation events for urine, feces, bedding pack and background patches during 2009 sampling period. Average values (n=6) +1 standard error of the average is shown as bars. 92

Figure 3.4. Mean temperature and total daily precipitation and mean 0-5 cm soil temperature and gravimetric moisture content (GMC) for urine, feces, bedding pack and background patches during 2009 sampling period. Average values (n=6) +1 standard error of the average is shown as bars. 93

Figure 3.5. CO_2 flux from patches of urine, feces, and simulated bedding pack, from three diet treatment groups, and grassland background emission, deposited on grassland in April 2009 and measured until September 2009. Average values (n=6) +1 standard error of the average is shown as bars. 95

Figure D.1. Methane emissions from urine, fecal, simulated bedding pack, and background patches over 2009 growing season. Average values (n=6) +1 standard error of the average is shown as bars. 140

Figure E.1. Carbon dioxide emissions from urine, feces, and simulated bedding pack patches with background emissions subtracted over 2009 growing season. Average values (n=6) +1 standard error of the average is shown as bars. 141

Figure F.1. Spearman Rank Correlation Analysis Scatter Plots 142

LIST OF TABLES

Table **Page**

Table 2.1. Means[†] and standard deviation of dry matter (DM) intake, crude protein (CP), total N[§], and total P, and ratios of N to P, fed per cow per day in the diet trial during fall and winter collection periods. 15

Table 2.2. Techniques and labs used to conduct feces nutrient analyses. 18

Table 2.3. Techniques and labs used to conduct urine nutrient analyses. 18

Table 2.4. Means[†] of total excreta, total nitrogen (N_T), and total phosphorus (P_T) produced per cow during the 8-hour collection period. 23

Table 2.5. Percentage[†] of total nitrogen (N_T) and total phosphorus (P_T) excreted via urine and feces per cow during the 8-hour collection period. 25

Table 2.6. Mean[†] concentrations of total N (N_T), organic N (N_{Org}) and ammonium N (N_{NH4+}) in feces expressed as g kg^{-1} and as a percentage of total N during an 8-hour collection period. 27

Table 2.8. Mean total P (P_T) concentrations of feces and urine for the 8-hour collection period. 31

Table 2.9. Mean[†] proportions of concentrations of water (H_2O-P) and sodium-bicarbonate ($NaHCO_3$-P) extractable P, residual P, and labile P[§] (P_L) in fresh feces as determined by modified Hedley sequential fractionation. 32

Table 2.10. Mean[†] ratios of nitrogen to phosphorus excreted per cow during 8 hour collection period in total excreta, feces, and urine. 34

Table 3.1. Forms and quantity of N in urine added to soil. 74

Table 3.2. Moisture and forms and quantity of N in feces added to soil. 74

Table 3.3. Composition of simulated bedding pack, composed from a mixture of feces and urine, added according to the ratio of kg feces to L urine produced by beef cows, and straw[†]. 75

Table 3.5. Means[†] of cumulative N_2O emission above background levels and associated N_2O emission factors for patches of urine, feces, and simulated bedding pack from three diet treatments deposited on grassland in April 2009 and gas emissions measured until September 2009. 85

Table 3.6. Means[†] of cumulative CH_4 emission above background levels for patches of urine, feces, and simulated bedding pack from three diet treatments deposited on grassland in April 2009 and gas emissions measured until September 2009. 86

Table 3.7. Spearman rank correlation coefficients for the associations between N_2O emission from urine, feces, bedding pack patches and measured soil variables over the growing season. 89

Table B.1. Fecal P fraction concentrations and standard deviation determined by modified Hedley sequential fractionation (dry matter basis). 138

Table C.1. Ion analysis of urine using Ion Chromatography. 139

1.0 INTRODUCTION

In Canada, 8% of anthropogenic greenhouse gas (GHG) emissions are attributed to agriculture (Gregorich et al. 2005). According to Cole et al. (1997), agriculture contributes 70% and 50% of the total anthropogenic emission of nitrous oxide (N_2O) and methane (CH_4) globally, respectively. The livestock industry generates a large portion of this, responsible for 48% of Canada agriculture's GHG emissions with the majority of these emissions in the forms of N_2O and CH_4 (Ominski et al. 2007). Of Canada's total agricultural GHG emission, 31% is contributed from N_2O (Desjardins et al. 2001), 45% of which is estimated to be from livestock manure sources, including storage, application, and handling (Gregorich et al. 2005). Methane contributes 43% of Canada's total agricultural GHG emissions (Desjardins et al. 2001) of which 72% is derived from enteric methane emission with the remainder derived from manures and soils (Ominski et al. 2007). Within Manitoba's portion of Lake Winnipeg's watershed, 33% of the phosphorus (P) loading and 11% of the nitrogen (N) loading to the lake has been attributed to agriculture (LWSB 2006). Livestock operations contribute a significant portion of this loading, particularly in terms of P which is often over-applied to land in cattle and hog operations due to economic limitations of manure transportation (LSWB 2006).

As such, much pressure has been put on producers to adopt management practices that reduce contributions of livestock production to GHG emissions and nutrient loading of surface waterways. As soils can be both sources and sinks of N_2O and CH_4, management practices can be developed to reduce agricultures emission of N_2O and CH_4,

however, these practices are not always cost-effective for producers in the short term. For example, regulations have been set in place to achieve reductions in nutrient losses to waterways in Manitoba, regulating timing of nutrient applications and thresholds for soil nutrient concentrations of N and P. Some incentive programs to encourage producers to implement these practices, despite incurring a higher cost of production, have been developed.

New management practices in the livestock industry in Manitoba have been developed in recent years to achieve reductions in cost of production. In particular, the beef industry in Manitoba, consisting primarily of cow-calf producers, has adopted practices to attempt to decrease cost of production during winter months where the highest costs of production are incurred. Winter on the Canadian prairies requires supplemental feed to be supplied to livestock. Traditionally, over the winter period cow-calf producers delivered feed in drylots, where bedding was supplied to animals resulting in manure pack build-up. In an attempt to reduce labour and manure handling costs from these drylots, producers have begun feeding on pasture. Cow-calf producers may also reduce overwintering costs through feed, by using lower-quality forages topped-up with protein and energy supplements to meet animal nutritional requirements, as beef-cow nutritional requirements are low compared to high production dairy cows or beef finishing animals. Dried distillers' grains with solubles (DDGS), a by-product of the ethanol industry, is becoming readily available as a low-cost, protein and energy supplement for beef producers.

As these overwintering management practices are relatively new, little to no research has been done on their environmental implications. Most of the research done on

dairy and beef finishing animals is not applicable to beef cows due to the extreme differences in diet formulation. One study in Western Canada has looked at the effect of wheat- based DDGS supplementation on nutrient excretion by beef animals, but this study used finishing animals receiving high protein and energy diets (Hao et al. 2009). Researchers at the University of Saskatchewan have looked at the effects of new overwintering management practices on animal and pasture productivity, soil nutrient status, and economics of production (Jungnitsch 2008; Kelln 2010) but have not looked at their impacts on soil GHG emissions or the effect of diet on nutrient excretion. More information is needed to ensure that recommended management practices that reduce overwintering cost of production for beef producers are also environmentally beneficial. This information is also necessary to develop accurate emission factors and nutrient loss potentials from cow-calf operations, in order for both producers and policy makers to develop an accurate description of the impact of the beef industry on the environment. From this information, programs to assist producers adopt environmentally sustainable management practices can be developed.

The University of Manitoba has developed a multidisciplinary project to address these gaps in knowledge for Western Canadian beef producers. Through the departments of Animal Science and Soil Science, the impacts of overwintering beef cattle management practices in terms of animal productivity, enteric methane emission, soil GHG emission, and soil nutrient implications will be studied on beef cows fed low-quality forages with and without protein and energy supplementation. This thesis will discuss the soil GHG emission and soil nutrient implications from the first phase of the project.

The objectives of this thesis were to:

1. Examine the environmental implications of overwintering beef cows fed a low-quality forage diet with and without supplementation with DDGS in terms of the partitioning of nutrients in excreta and the potential of these nutrients to be lost to the environment.
2. Examine the environmental implications of overwintering beef cattle on pasture in terms soil GHG emissions from excreta.
3. Develop emission factors and nutrient loss potentials for modelling the environmental impacts of overwintering beef cattle production practices in Western Canada.

This thesis consists of four chapters. The first is this introductory chapter, which justifies the research area. Two data chapters are as follows: the first chapter discusses the effect of low-quality forage diets supplemented with DDGS and cold acclimatization on partitioning of the nutrients N and P to excreta and the potential environmental implications, while the second chapter looks at the soil GHG emissions generated from the excreta when it is deposited on grassland. The final chapter consists of a discussion that ties together the two data chapters. It examines potential beneficial management practices based on soil nutrient status and soil GHG emissions, and places the results into the context of whole system research.

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2. DIET AND COLD ACCLIMIZATION EFFECTS ON FORMS OF NITROGEN AND FRACTIONS OF PHOSPHORUS IN EXCRETA OF BEEF CATTLE

2.1 Abstract

Donohoe, Gwendolyn R. M.Sc., University of Manitoba, September, 2010. Diet and cold acclimatization effects on forms of nitrogen and fractions of phosphorus in excreta of beef cattle. Major Professor: Dr. Mario Tenuta.

To minimize cost of production, Canadian beef cattle producers feed low-quality forage rations during winter months, adding protein and energy supplements to meet animal nutrition requirements, if needed. Dried distillers' grains with solubles (DDGS) is a low-cost and readily available protein and energy supplement becoming popular with livestock producers, and is known to have high concentrations of nitrogen (N) and phosphorus (P). As environmentally sustainable management practices are becoming necessary for producers, the environmental implications of this overwintering production practice need to be determined. The objective of this study was to examine the effect of DDGS-supplemented, low-quality forage diets and cold weather on the partitioning of N and P to urine and feces of beef cows and further to examine the potential loss of these nutrients to the environment through gaseous losses or by water movement.

Thirty open, mature, dry beef cows were divided into three treatment groups and fed diets of forage having 6% crude protein (CP; Control), the forage supplemented with dried distillers grains with solubles (DDGS) to 0% w w^{-1} (N deficient), 10% w w^{-1} (borderline sufficient N, 8.7% CP), and 20% w w^{-1} (excess N, 11.5% CP). The trial was

conducted in fall 2008 and again in winter 2009 to determine the effects of cold acclimatization. Feces were analyzed for forms of N, including ammonium N, organic N, and total N, and fractions of P, including total P and labile P. Urine was analyzed for total N, ammonium N, urea N, and total P.

Volume of urine and urine ammonium, urea, and organic N concentrations increased significantly with DDGS supplementation. The proportions of urine ammonium N and urea N increased significantly with 20% DDGS supplementation, with urea N accounting for 30% and 54% of the total urine N excreted in the 0% and 20% DDGS diets, respectively. Concentrations of total N, ammonium N, and organic N in feces increased with increasing DDGS supplementation and decreased during the winter sampling period. However, the mass of feces excreted and proportion of organic and ammonium N of total fecal N was not affected by DDGS supplementation. Total P concentrations in urine and feces increased with increasing DDGS supplementation, with lower feces P concentrations and higher urine P concentrations in the winter compared to fall trial. The addition of DDGS to the diets increased the proportion of P excreted in urine, having increased from 1% in the 0% DDGS diet to 18% in the 20% DDGS diet. The proportion of labile P in feces was 66% of total P for 0% DDGS diets, and 77% for both the 10% and 20% DDGS diets. Total N to P ratios of excreta produced increased from 7.3 to 8.3 with increasing DDGS supplementation. Available N, being the sum of ammonium and urea N, to total P ratios ranged from 1.5 to 2.9, from the 0 and 20% DDGS diets, respectively. The addition of DDGS to cattle diets resulted in increased excretion of both labile forms of N and fractions of P. These results will be used to

further the development of best management practices for overwintering beef cattle in Manitoba.

2.2 Introduction

Nutrient management regulations for livestock producers in Manitoba have made it increasingly important for producers and agricultural professionals to understand the impact of management practices on nutrient excretion by livestock. Beef cattle (*Bos primigenius taurus* L.) producers in Manitoba, the majority of which are of cow-calf operations, incur their highest cost of production during winter months, due to feeding and manure handling costs. As a result, in recent years producers have been adopting new management practices to decrease these winter production costs, including feeding cheaper sources of feed and using winter grazing systems. These new, low-cost production practices have not been studied extensively in terms of the environmental implications of loss of nitrogen (N) and phosphorus (P) to the environment.

One of these low-cost feed sources is dried distillers' grains with solubles (DDGS), a by-product of the ethanol industry. Distillers' grains are known to provide a source of protein and energy for livestock (Gibb et al. 2008). As ethanol production is predicted to increase in the future, the availability of DDGS as a low-cost protein and energy supplement for livestock production is predicted to increase (Gibb et al. 2008; Simpson et al. 2008). In Western Canada, wheat is commonly used in ethanol production, while in the United States corn is the main crop used. As a result, most studies that have

examined the use of DDGS in cattle production have used corn-based DDGS (Klopfenstein et al. 2008). The few studies that have used wheat-based DDGS have used it as a supplement for feedlot cattle in back grounding and finishing rations (Gibb et al. 2008; Hao et al. 2009). As the nutrient requirements of mature beef cows are much lower than that of growing finishing animals, cow-calf producers generally feed lower-quality forages in winter months and then use protein supplements to meet animal nutritional requirements. The use of DDGS as a supplement to these overwintering forage-based diets for mature beef cows has not been studied in the literature. As well, most studies looking at the forms of N and fractions of P in excreta as affected by diet use dairy cattle or finishing animals receiving high-quality feeds such as alfalfa and soybean meal (Bristow et al. 1992; Erickson et al. 2000; Dou et al. 2002; Broderick 2003; Chapuis-Lardy et al. 2004; He et al. 2004; Kebreab et al. 2005; Kincaid et al. 2005; Meyer et al. 2006; Powell et al. 2006; Powell et al. 2008; Powell et al. 2009). As such, determining the effect of DDGS and low-quality forages on the forms of N and fractions of P in beef cow excreta will be of greater informative value to Manitoba beef producers.

Nitrogen excretion by cattle is influenced by protein intake (Satter et al. 2002). The National Research Council (NRC) uses a factor of 6.25 divided by CP intake to estimate N intakes (NRC 2001). Nitrogen excreted by cattle can be in either organic or inorganic form, although beef cattle manure is generally considered to consist of primarily organic N (MAFRI 2004). Inorganic N in manure is often termed ammoniacal N, which includes ammonium N and urea N. Although urea N is an organic N form, it is quickly hydrolyzed to ammonium by microorganisms. Ammoniacal N excreted by cattle is considered plant available but it may also be to be lost to the environment through

gaseous losses or in water as it is subjected to the processes of nitrification, denitrification, and ammonia volatilization. Gaseous losses of ammonium through ammonia volatilization can account for 10% to 70% of N applied in feces, urine, or manure and can cause air contamination and secondary losses of N as nitrous oxide (Bussink et al. 1998). Nitrous oxide, a potent greenhouse gas (GHG), has been estimated to have an emission factor of 2% of applied N in solid livestock manures that is either spread, stored in drylots, or deposited in grazing systems, by the International Panel on Climate Change (IPCC 1996). Nitrogen loss to waterways is of concern to aquatic ecosystems and can contaminate drinking water (LWSB 2006). Literature suggests that diet can alter the proportions of the different forms of N excreted by cattle, thereby potentially affecting the environmental fate of N (Bristow et al. 1992; Broderick 2003). Although beef cattle producers generally do not feed excessive amounts of N, as DDGS becomes more popular as a low-cost and readily available protein and energy supplement, its effect on excretion of environmentally available forms of N needs to be understood.

Phosphorus loss to waterways is becoming an increasing concern due to its role in eutrophication of surface water bodies (Sharpley et al. 2001). Therefore, reducing P excretion by cattle is becoming an important environmental best management practice, especially since it has been found that P excreted from cattle receiving reduced P diets is less susceptible to loss in water (Satter et al. 2002). Phosphorus in manure can exist in either inorganic or organic forms, although inorganic P is considered the most abundant form of P in livestock manure, ranging from 63-92% (Sharpley and Moyer 2000). Unlike N, however, both organic and inorganic P forms can be considered plant-available and labile in water. Although plants take up P in an inorganic form as orthophosphate, some

forms of organic P are readily soluble and can quickly be mineralized to replenish soil solution P concentrations. Organic P forms have also been found to be highly mobile and found in runoff and leachate (Condron et al. 2005).

As a result, the potential of P to be lost in water is often determined by determining the labile fraction of P instead of determining specific forms of P. The potential of P to be lost due to water movement or used by plants therefore depends more on the fractions of P excreted by the animal than total P (Sharpley and Moyer 2000). The P fractionation procedure developed by Hedley et al. (1982) uses increasing strengths of chemical extractants to remove P from soil. Fractions of P removed in weak extractants, such as deionised water and sodium bicarbonate, have a high potential to be lost in water and are considered the labile fraction. This procedure has been modified to be used on livestock excreta to determine the potential fractions of labile P added to soil through excreta and manure deposition (Sharpley and Moyer 2000; Dou et al. 2000; Ajiboye et al. 2004). High correlations have been found between labile P fractions determined in manure and P loss in water following application (Sharpley and Moyer 2000; Dou et al. 2000). These labile fractions of P have been found to contain both organic and inorganic P sources, and the percentage of labile P found in cattle manures has been found to be highly variable depending on diet and production practices (Dou et al. 2000; He et al. 2004; Ajiboye et al. 2007; Mamo et al. 2007).

Often, protein and energy supplements contain high concentrations of P, resulting in P being fed in excess in order to meet CP and energy requirements (Powell et al. 2002; Satter et al. 2002; Buckley and Penn, 2003; Vasconcelos et al. 2009). This can result in farm scale surpluses of P (Powell et al. 2002; Rotz et al. 2002; Satter et al. 2002). Diet

can play a considerable role in the composition of labile fractions of manure P (Dou et al. 2000; Satter et al. 2002; He et al. 2004; Hao et al. 2009). For example, Hao et al. (2009) found that concentrations of water soluble reactive P in feces from finishing beef animals increased with distiller's grain supplementation fed at 40% and 60% w w^{-1} compared to the control. Cow-calf producers generally do not import excess amounts of P onto farms; however, the high availability and low-cost of DDGS may change this (Simpson et al. 2008) as DDGS is known to be a high source of P (Buckley and Penn 2003; Simpson et al. 2008; Hao et al. 2009).

Protein and energy supplementation on cow-calf operations generally occurs during winter months when lower quality forages are fed. Cattle are traditionally housed in drylots where manure packs, a combination of straw, feces and urine, are formed and later spread on fields during the following growing season. The new practice of winter grazing, where cattle are fed on pasture to avoid the formation of manure packs, results in most urine and feces being deposited on snow pack or frozen soil. Application of manure or deposition of feces during winter months on Canadian prairies can pose a significant risk in terms of N and P loss to the environment, as plant uptake is not occurring and snow melt will transport available and labile forms of N and P to surface waterways. Extreme cold temperatures will also result in increased animal requirements for nutrients (NRC 2001). Metabolism of N and P may be altered as a result, as animals adapt and attempt to make more efficient use of intake nutrients, potentially altering forms and proportions of available N and P excreted as well.

The objectives of this study were to:

1. Determine if supplementation with DDGS to a low quality forage beef-cow ration results in:
    - Increased concentrations and altered partitioning of forms of N and fractions of P in urine and feces.
2. Determine if cold temperatures result in beef cattle excreting:
    - Increased concentrations and different proportions of forms of N and fractions of P in urine and feces.

These results will be used to help develop best overwintering management practices for beef producers in Manitoba.

2.3 Materials and Methods

2.3.1. Feces and urine collection

Feces and urine were collected from mature, non-lactating, non-pregnant beef cows during a diet trial at the University of Manitoba's Glenlea Research Station. This diet trial was part of a multidisciplinary study examining the metabolic responses and environmental implications of overwintering beef cattle fed low quality forage diets supplemented with an increasing proportion of protein and energy with wheat DDGS (Bernier 2010). All procedures were approved by the Animal Care and Use Committee at the University of Manitoba (2010).

Twenty-four Simmental and Gelbveih cross commercial beef cows, with an initial average body weight of 675.4 ± 51.8 kg, were divided into three diet treatment groups. Cows were considered replicates within each diet treatment group. All diets consisted of

a baseline low quality forage (6% crude protein; CP) and were supplemented with either no DDGS (0% DDGS), 10% DDGS w w^{-1} (borderline sufficient N, 8.7% CP), or 20% DDGS w w^{-1} (excess N, 11.5% CP). The DDGS used in the trial was from the Husky Energy Ltd. Ethanol plant located in Minnedosa, Manitoba, and was determined to have an average CP content of 38%, 93% P, 35% neutral detergent fibre (NDF), 18 % acid detergent fibre (ADF), on a dry matter (DM) basis.

The trial had two data collection periods. The first took place during the fall of 2008 (beginning in October) and the second during the winter of 2009 (beginning in January), in order to look at the difference of temperature on animal metabolism. Animals were fed the diets ad libitum in the drylot for a minimum of 21 days prior to the start of each data collection period. Table 2.1 shows the mean total DM intakes, CP, N, and P fed to cows within each diet, along with the ratio of N to P fed (Bernier 2010). Diets fed in fall and winter collection periods were not statistically different from each other (Table 2.1). Average temperature during the fall trial was 7.3^{o}C and during the winter trial was -17.7^{o}C.

Table 2.1. Means[†] and standard deviation of dry matter (DM) intake, crude protein (CP), total N[§], and total P, and ratios of N to P, fed per cow per day in the diet trial during fall and winter collection periods.

Diet	DM Intake[‡]	CP[‡]	N[‡]	P[‡]	N: P[‡]
	kg cow^{-1} day^{-1}	% DM intake	g fed cow^{-1} day^{-1}		
Fall					
0% DDGS	7.5 a (1.6)	5 (0.6)	63.1 (10.1)	8.2 d (1.0)	7.6 a (0.3)
10% DDGS	9.1 a (0.9)	8 (0.2)	120.2 (13.0)	17.5 bc (1.7)	6.9 b (0.4)
20% DDGS	10.4 a (1.7)	12 (0.4)	172.0 (57.9)	26.7 a (8.8)	6.4 c (0.1)
Winter					
0% DDGS	9.0 a (1.1)	6 (0.4)	83.6 (13.4)	10.8 cd (1.7)	7.7 a (0.1)
10% DDGS	9.7 a (2.0)	9 (0.1)	130.9 (26.8)	20.8 ab (4.6)	6.6 bc (0.2)
20% DDGS	9.0 a (1.7)	11 (0.7)	154.7 (31.1)	23.7 ab (2.8)	6.5 bc (0.2)
Season					
Fall	8.9	8	118.2	17.5	7.0
Winter	9.3	8	122.9	18.4	6.9
Diet					
0% DDGS	8.2	6 c	72.7 c	9.4	7.7
10% DDGS	9.4	9 b	125.3 b	19.2	6.7
20% DDGS	9.6	11 a	163.7 a	25.3	6.5
ANOVA					
Season	NS	NS	NS	NS	NS
Diet	NS	<0.0001	<0.0001	<0.0001	<0.0001
Season x Diet	0.0377	NS	NS	0.0415	0.0112

NS, not significant; Numbers in brackets are standard deviation of the mean.

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets (7 replicates used for all winter diets and the 10% DDGS fall diet; 6 replicates used for the 0% and 20% DDGS fall diets).

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

[§] N intake calculated as CP fed divided by 6.25 (NRC 2001).

In order to collect individual samples of feces and urine from cows and to monitor individual animal intakes, cows were brought in from the drylot in groups of 8 and

housed in tie-stalls for 9 days at a time in a metabolism unit. Average temperature in the metabolism unit during the fall collection was 14.8 +/- 2.1^{o}C and was 12.6 +/- 2.0^{o}C for the winter trial. Prior to the start of the trial, cows were trained to lead and stand with a halter. The first three days in the metabolism tie-stalls were considered an adaptation period, to allow animals to become accustomed to tie-stalls, urine collection apparatus, and collection activities. Cows were fed daily at 0600 and 1200 hours. A total feces and urine collection took place on days 4 through 8 (5 day collection period) for diet and metabolic studies (Bernier 2010). Feces and urine were then collected on the ninth day for use in detailed nutrient analysis and soil GHG studies.

Excreta collection on day 9 commenced at 0800 and ceased at 1600 hours (8 hour collection), in between feeding periods. Feces were collected in individual steel collection trays located under grates at the back of each tie stall, while urine was collected from each cow with a catheter and drained by gravity into polyethylene carboys. Feces were weighed in the collection trays in kilograms to two decimal places while urine was poured into a volumetric cylinder and measured in millilitres to one decimal place. Total feces and urine collection was performed over the 8 hours, and all materials collected were mixed thoroughly prior to storage in 1 L or 1kg containers at the end of the collection.

Feces and urine collected for detailed nutrient analysis and soil GHG studies were collected hourly and kept cool (< 4^{o}C), in order to minimize losses of nitrogen from ammonia volatilization and to prevent nutrient transformations. All collection and measuring materials were thoroughly rinsed with reverse osmosis (R.O.) water between hourly collections. All samples were frozen (-20^{o}C) at the end of the collection until

analysis or use in soil GHG emission studies. Urine collected during the 5-day collection period was acidified to help minimize ammonia volatilization and as a result this urine could not be used in soil GHG studies due to the acidic pH. Knowlton et al. (2010) also noted that acidifying urine during collection may not be an appropriate technique if urine urea-N determinations were needed. As well, feces and urine collected during the 5-day collection period were not collected frequently enough to minimize nutrient transformations and gaseous losses of N, as feces and urine collected during this 8 hour collection were intended to represent excreta that were freshly deposited by the animals.

2.3.2. Feces and urine analysis

Feces and urine samples collected from cows were analyzed individually to maintain true replicate samples within each diet treatment group. Feces were analyzed for concentrations of total N (N_T), ammonium N (N_{NH4+}), organic N (N_{Org}), total P (P_T), and labile P (P_L) while urine was analyzed for concentrations of N_T, urea N (N_U), N_{NH4+}, and N_{Org} and P_T (Tables 2.2 and 2.3). Labile P, also known as easily removable P, was considered to be the sum of total P extracted in sequential water (H_2O-P) and sodium bicarbonate ($NaHCO_3$-P) fractions (Ajiboye et al. 2004; Qian and Schoenau 2000). Labile P was determined using a modified Hedley fractionation (Hedley et al. 1982), following the methodology described in Ajiboye et al. (2004), however the sodium hydroxide (NaOH) and hydrochloric acid (HCl) extractions were not performed as they were not necessary to determine labile P. A detailed methodology is given in Appendix A. Environmentally labile and plant available forms of N were considered to be ammonium N and urea N. Although urea is an organic form of N, due to the rapid hydrolysis of urea

once applied to soil it was analysed for separately and was not included in the N_{Org} determinations.

Table 2.2. Techniques and labs used to conduct feces nutrient analyses.

Nutrient	Analysis	Lab	Reference
$\mathbf{N_{NH4+}}$	Colourimetric, 2M extractable ammonium-N (tested as received),	A&L	Keeney and Nelson (1982)
$\mathbf{N_T}$	Elemental analyzer combustion (tested as received)	A&L	LECO
$\mathbf{N_{Org}}$	Elemental analyzer combustion (tested as received)	A&L	LECO
DM	Low temperature oven drying, 60^{o}C for 48 hours	A&L	
$\mathbf{P_T}$	Inductively coupled plasma (ICP) spectrophotometry tested after drying and reported on dry weight)	A&L	AOAC-985.01
$\mathbf{P_L}$	Modified Hedley fractionation for Labile P and ICP analysis for total P (Appendix A)	Soil Ecology Lab, U of M	Ajiboiye et al. (2004)

Table 2.3. Techniques and labs used to conduct urine nutrient analyses.

Nutrient	Analysis	Lab	Reference
$\mathbf{N_{NH4+}}$	Colourimetric (2M extractable ammonium-N)	A&L	Keeney and Nelson (1982)
$\mathbf{N_T}$	Kjeldahl Total N with persulfate digestion and titration	A&L	Standard Methods for the Examination of Water and Wastewater, 21st ed. (2005)
$\mathbf{N_{Org}}$	By difference ($N_T - N_U$-N_{NH4+})		
$\mathbf{N_U}$	Enzymatic Distillation	A&L	Keeney and Bremner (1967)
$\mathbf{P_T}$	Inductively coupled plasma spectrophotometry	A&L	AOAC 985.01

2.3.3. Data analysis

Concentrations of forms of N and fractions of P in feces and urine were calculated as a proportion of the total N and P concentrations, in order to determine if the addition of DDGS supplementation or sampling period resulted in metabolic changes in digestion and subsequent excretion of environmentally labile nutrients. Concentrations were then combined with the 8-hour excreta collection masses of feces and volumes of urine, to determine total nutrient output during the 8-hour collection period. This data could then be used to determine the effect of sample collection period and diet on the combined effects of changing mass of feces and volume of urine with changing N and P concentrations. Total excretion of N and P in feces and urine over the 8 hours was then used to determine ratios of N to P excreted by animals, important for calculating manure application rates and risk of environmental losses of N and P. Available N was considered to be the sum of feces and urine N_{NH4+} and N_U and excreted per cow over the 8-hour collection period.

As the 8-hour collection was not a 24-hour collection, this data cannot be used to extrapolate 24-hour nutrient excretion (Powell et al. 2009). However, trends observed in feces and urine excretion and concentrations followed similar trends to the 24-hour excreta collection data (Bernier 2010) and can therefore be used to provide insight and reasonable estimates as to the effect of diet and season on excretion of environmentally labile forms of N and fractions of P from mature beef cows (Powell et al. 2009).

2.3.4. Statistical analysis

During the fall collection period, 2 cows with poor average daily intakes were removed from the data set according to Bernier (2010). Three cows were also removed from both the fall and winter collection data sets: two were found to be pregnant and the third due to health complications. This resulted in 6 replicates used in the fall 0% and 20% DDGS diet data sets, 7 replicates in the fall 10% DDGS diet data set, and 7 replicates for all three diets during the winter collection period.

All data were analysed using SAS version 9.2 (SAS Institute Inc. 2000). Cows were considered replicates within 3 diet treatment groups collected over 2 the sampling times (fall and winter), with cows considered a random effect within diet. All nutrient concentrations were compared using Proc Mixed for a two-way analysis of variance (ANOVA) to compare the 3 diet treatment groups and 2 time periods (seasons) and to determine diet by season interactions. A Bonferroni test for multiple comparison of means was used to determine significant differences ($P \leq 0.05$). Log transformation of data was performed and used to determine significant differences if tests for normality (Shapiro-Wilks and homogeneous distribution of residuals) failed. Log transformations were also used on all percentage data.

2.4 Results

2.4.1. Mass of Nitrogen and Phosphorus Excreted

The 8-hour excreta collection revealed that the mass of feces DM excreted per cow was not affected by adding DDGS supplementation to the diets, but was significantly affected by collection period (Table 2.4). Total feces DM ranged between 1386.0 and 1309.5 g DM cow^{-1} over the 8-hour collection period from cows receiving the 0% to 20% DDGS diets, respectively. Feces collected during the winter collection period had a significantly greater DM content than did feces collected in the fall collection period, although the total mass of feces excreted per cow over the 8 hours (as is basis) was not affected by season (data not shown).

Conversely, urine volumes increased with increasing DDGS supplementation and were not affected significantly by season (Table 2.4). Cows receiving the 0% DDGS diet excreted a significantly lower volume of urine over the 8-hours (2208 mL) compared to cows receiving the 20% DDGS diet (3692 mL), while those receiving the 10% diet excreted intermediate volumes of urine.

Mass of feces (DM basis) and volume of urine excreted per cow over the 8-hour collection and nutrient concentrations were used to determine the total mass of N and P excreted per cow (Table 2.4). The mass of N excreted in both feces and urine over the 8 hours was greatest from cows receiving the 20% DDGS supplementation, with 25.3 g and 43.3 g N excreted cow^{-1} $8h^{-1}$ in feces and urine, respectively. Cows receiving the 0% and 10% DDGS supplemented diets did not excrete significantly different amounts of N in feces, with 16.1 and 20.2 g N excreted cow^{-1} $8h^{-1}$, respectively. However, cows receiving

the 0% DDGS diet excreted a significantly lower amount of N in urine than those receiving the 10% diet over the 8 hours, with 12.0 and 29.5 g N excreted cow^{-1} $8h^{-1}$. Collection period did not significantly affect the total mass of N excreted in feces or urine.

The total mass of P excreted per cow during the 8 hour collection was affected by both diet and collection period (Table 2.4). Cows receiving the 10% and 20% DDGS supplementation diets excreted a significantly greater mass of P in feces, with 5.9 and 8.0 g P excreted cow^{-1} $8h^{-1}$, respectively, compared to the 3.5 g P excreted cow^{-1} $8h^{-1}$ from cows receiving no DDGS supplementation. The mass of P excreted in urine was much lower than that of feces. Cows receiving the 20% DDGS supplementation excreted a significantly greater mass of P in urine over the 8 hours, with 1.7 g P, compared to cows receiving the 0% DDGS diet, with 0.1 g P, while the 10% DDGS was intermediate to the other two diets. Total P excreted in urine was significantly greater in winter compared to fall collection periods.

Table 2.4. Means[†] of total excreta, total nitrogen (N_T), and total phosphorus (P_T) produced per cow during the 8-hour collection period.

Effect	Feces[‡]			Urine[‡]		
	Mass DM	N_T	P_T	Volume	N_T	P_T
	g cow^{-1} 8h^{-1}			mL cow^{-1} 8h^{-1}	g cow^{-1} 8h^{-1}	
Season						
Fall	1211 b	20.5	5.7	3015	28.4	0.6 b
Winter	1467 a	20.6	5.9	2787	28.1	1.0 a
Diet						
0% DDGS	1386	16.1 b	3.5 b	2208 b	12.0 c	0.1 b
10% DDGS	1322	20.2 b	5.9 a	2804 ab	29.5 b	0.7 ab
20% DDGS	1310	25.3 a	8.0 a	3692 a	43.3 a	1.7 a
ANOVA						
Season	0.0122	NS	NS	NS	NS	0.0126
Diet	NS	0.0002	0.0003	0.0024	<0.0001	0.0234
Season x Diet	NS	NS	NS	NS	NS	NS

NS, not significant.

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets (n=7 for all winter diets and the 10% DDGS fall diet; n=6 for the 0% and 20% DDGS fall diets).

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

2.4.2. Proportions of nitrogen and phosphorus excreted via feces and urine

The proportion of the total N excreted via feces and urine per cow during the 8-hour collection was significantly affected by diet (Table 2.5). Cows receiving 10% and 20% DDGS supplementation excreted a significantly greater mass of N via urine whereas cows receiving no DDGS supplementation excreted more N via feces than urine. Percentages of N excreted via urine ranged from 59% to 62% of total N excreted for

cows receiving 10% and 20% DDGS supplementation, respectively, while cows receiving no DDGS supplementation excreted 42% of the total N excreted via urine.

The proportion of the total P excreted via feces and urine per cow during the 8-hour collection was affected by both diet and season (Table 2.5). Unlike N, cows excreted the majority of the total P via feces. However, the percentage of P excreted via urine was significantly increased when cows received DDGS supplementation. The percentage of P excreted via urine was 1%, 9% and 18% from cows receiving 0%, 10% and 20% DDGS supplementation, respectively, with the 10% and 20% diets being significantly greater than the 0% diet, with an average loss via urine of 13.5%. The percentage of total P excreted via feces decreased during the winter collection period, whereas the percentage of total P excreted via urine increased during the winter collection period.

Table 2.5. Percentage[†] of total nitrogen (N_T) and total phosphorus (P_T) excreted via urine and feces per cow during the 8-hour collection period.

Effect	N_T		P_T	
	Feces[‡]	Urine[‡]	Feces[‡]	Urine[‡]
	% of Total N Excreted		% of Total P Excreted	
Season				
Fall	46	54	94 a	6 b
Winter	45	55	88 b	12 a
Diet				
0% DDGS	58 a	42 b	99 a	1 b
10% DDGS	41 b	59 a	91 b	9 a
20% DDGS	38 b	62 a	82 b	18 a
ANOVA				
Season	NS	NS	0.0023	0.0183
Diet	<0.0001	00002	0.0383	0.0003
Season x Diet	NS	NS	NS	NS

NS, not significant.

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets (n=7 for all winter diets and the 10% DDGS fall diet; n=6 for the 0% and 20% DDGS fall diets).

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

2.4.3. Excreta nitrogen concentrations

Concentrations of N in feces decreased in the winter collection period and increased with the addition of DDGS supplementation to the diets (Table 2.6). Total N concentrations in feces were 12.2, 15.5, and 19.5 g N kg^{-1} DM from cows receiving the 0, 10, and 20% DDGS diets, respectively. Similar trends were also observed in feces N_{Org} and N_{NH4+}. Organic N concentrations were the greatest, with mean concentrations ranging from 11.0 to 17.7 g N kg^{-1} DM compared to N_{NH4+} concentrations which ranged from 0.8 to 1.2 g N kg^{-1} DM, from cows receiving the 0% and 20% DDGS diets, respectively.

Despite the increase in concentrations of the forms of N in feces with supplementation, there were no significant differences in the proportions of N_{NH4+} and N_{Org} in feces, with 90% and averaging 6.7% of the total feces N concentration as N_{Org} and N_{NH4+}, respectively. The N which was unaccounted for was assumed to be nitrate N and experimental error.

Unlike the feces N concentrations, urine N concentrations were not affected by sampling period (Table 2.7). Urine N_T concentrations increased with DDGS supplementation, with urine from the 10% and 20% DDGS supplemented diets being significantly greater than 0% diet but not significantly different from each other. Concentrations of N_T ranged from 5.5 to 12.6 g N L^{-1} urine from the 0% to 20% DDGS diets, respectively. Similar trends were observed for the forms of urine N as well: N_{Org}, N_{NH4+}, and N_U. Urea N had the highest concentrations, ranging from 1.7 to 7.3 g N L^{-1} urine, followed by N_{Org} which ranged from 3.5 to 5.9 g N L^{-1}, with N_{NH4+} having the lowest concentrations ranging from 0.3 to 0.8 g N L^{-1} urine, from the 0% to 20% DDGS diets, respectively.

Not only did N_U have increasing concentrations with DDGS supplementation, but the proportion of N_U in the urine, expressed as a percentage of N_T, increased from 30% in the 0% DDGS diet to 54% in the 20% DDGS diet. The proportion of N_{Org}, expressed as a percentage of urine N_T, exhibited the opposite trend however, decreasing from 66% to 39% from the 0% to 20% DDGS diets, respectively. The proportion of N_{NH4+} did not change as DDGS supplementation was increased, and accounted for an average of 5.5% of the urine N_T concentration. The interaction effect seen in the proportion of urine N_{NH4+}

indicates that the increased proportion of N_{NH4+} occurred only in the DDGS supplemented diets during the winter sampling period.

The combined N_U and N_{NH4+}, or available N, proportions of feces and urine increased from 19% in the 0% DDGS diet to 40% in the 20% DDGS diet, a 2 fold increase. This is equivalent to 19, 34 and 53% of the intake N excreted in an available N form from the 0, 10, and 20% DDGS diets, respectively.

Table 2.6. Mean† concentrations of total N (N_T), organic N (N_{Org}) and ammonium N (N_{NH4+}) in feces expressed as g kg^{-1} and as a percentage of total N during an 8-hour collection period.

Effect	N_T ‡	N_{Org}	N_{NH4+}	N_{Org} ‡	N_{NH4+} ‡
	g N kg^{-1} DM feces			% of N_T§	
Season					
Fall	16.9 a	15.1 a	0.9 b	90	5 b
Winter	14.6 b	13.1 b	1.2 a	90	8 a
Diet					
0% DDGS	12.2 c	11.0 c	0.8 b	90	7
10% DDGS	15.5 b	13.7 b	1.1 ab	89	7
20% DDGS	19.5 a	17.7 a	1.2 a	91	6
ANOVA					
Season	0.0009	0.0014	0.0030	NS	<0.0001
Diet	<0.0001	<0.0001	0.0094	NS	NS
Season x Diet	NS	NS	NS	NS	NS

NS, not significant.

† Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets.

‡ Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test (n=7 for all winter diets and the 10% DDGS fall diet; n=6 for the 0% and 20% DDGS fall diets).

§ Proportions calculated as feces N form divided by feces N_T x 100.

Table 2.7. Mean[†] concentrations total N (N_T), organic N (N_{Org}), ammonium N (N_{NH4+}), and urea N (N_U) in urine expressed as g L^{-1} and as a percentage of total N during an 8-hour collection period.

Effect	N_T [‡]	N_{Org} [‡]	N_{NH4+} [‡]	N_U [‡]	N_{Org} [‡]	N_{NH4+} [‡]	N_U [‡]
	g N L^{-1} urine				% of N_T[§]		
Fall							
0% DDGS	5.0 (2.1)	3.0 (0.7)	0.3 (0.1)	1.7 (1.6)	66 (16)	5 ab (1)	30 (15)
10% DDGS	10.3 (1.5)	5.9 (0.6)	0.5 (0.2)	3.9 (0.8)	57 (3)	5 b (2)	38 (2)
20% DDGS	12.6 (5.4)	4.1 (1.6)	0.8 (0.5)	7.7 (6.0)	38 (20)	6 ab (1)	55 (18)
Winter							
0% DDGS	6.0 (1.1)	4.1 (1.4)	0.3 (0.09)	1.7 (1.5)	68 (20)	5 ab (1)	27 (19)
10% DDGS	10.8 (1.9)	6.0 (2.0)	0.7 (0.05)	4.2 (0.9)	54 (12)	6 a (2)	40 (11)
20% DDGS	12.6 (3.4)	5.0 (2.9)	0.8 (0.20)	6.8 (3.3)	41 (21)	7 ab (1)	53 (20)
Season							
Fall	9.3	4.3	0.5	4.5	53	5	42
Winter	9.8	5.0	0.6	4.2	54	6	40
Diet							
0% DDGS	5.5 b	3.5 b	0.3 b	1.7 b	66 a	5	30 b
10% DDGS	10.6 a	5.9 a	0.6 a	4.1 a	56 a	6	39 ab
20% DDGS	12.6 a	4.5 ab	0.8 a	7.3 a	39 b	6	54 a
ANOVA							
Season	NS	NS	NS	NS	NS	0.0200	NS
Diet	<0.0001	0.0110	<0.0001	<0.0001	0.0079	NS	0.0021
Season x Diet	NS	NS	NS	NS	NS	0.0141	NS

NS, not significant; Numbers in brackets are standard deviation of the mean.

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets (n=7 for all winter diets and the 10% DDGS fall diet; n=6 for the 0% and 20% DDGS fall diets).

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

[§] Proportions calculated as urine nitrogen form divided by urine N_T x 100.

2.4.4. Excreta phosphorus concentrations

Total P concentrations in feces decreased significantly in the winter sampling period while total P concentrations in urine increased significantly during the winter collection period (Table 2.8). Concentrations of P determined in feces were much higher than that of urine. Mean feces P_T ranged from 2.6 to 6.3 g P kg^{-1} feces DM while urine P_T ranged from 0.02 to 0.52 g P L^{-1}, from the 0% to 20% DDGS diets, respectively. Total P concentration in feces increased significantly as cows received increasing amounts of DDGS supplementation, while urine P_T was found to be significantly lower in the 0% diet compared to the 10% and 20% DDGS diets which had statistically similar P_T concentrations.

The proportions of environmentally and agronomically labile fractions of P as affected by diet and collection period are provided in Table 2.9. Mean concentrations and standard deviations of fractions of P are given in Appendix B. The addition of DDGS supplementation to the diets increased the proportion of H_2O-P and decreased the proportion of Res-P of the total Hedley P concentration, with no effect on the proportion of P measured as $NaHCO_3$-P. This resulted in an increase in proportion of P_L in feces from cows receiving DDGS supplementation. No significant differences were found between the 10% and 20% DDGS diets in terms of proportions of concentrations of fractions of P within the H_2O-P, Res-P, or P_L fractions. The proportion of H_2O-P increased from 36 to 47% and the proportion of Res-P decreased from 35% to 23% from the 0% to 20% DDGS diets, respectively. The proportion of P_L in feces was 65% from the 0% DDGS diet and was 77% from feces in 10 and 20% DDGS diets. The interaction effects in the $NaHCO_3$-P, Res-P, P_L fractions appear to be the result of the changing

effect of DDGS supplementation in the winter sampling period, while the proportions of P in each fraction of the 0% DDGS diet remained relatively constant between both sampling periods. In the $NaHCO_3$-P and Res-P fractions, the winter sampling period resulted in a lower proportion of P extracted from feces from the 10% and 20% DDGS diets, whereas in the P_L fraction the winter sampling period resulted in an increase in the P extracted 10% and 20% DDGS diets. Contrary to this, the $NaHCO_3$-P and Res-P 0% DDGS diet had relatively constant proportions of P extracted in both sampling periods. This same interaction effect did not occur in the H_2O-P fraction, indicating that the H_2O extractable P was not affected by DDGS supplementation, but by changes in animal metabolism and absorption of P during the winter sampling period.

Table 2.8. Mean total P (P_T) concentrations of feces and urine for the 8-hour collection period.

Effect	Feces P_T ‡	Urine P_T ‡
	g P kg^{-1} DM	g P L^{-1} urine
Season		
Fall	4.7 a	0.20 b
Winter	4.2 b	0.31 a
Diet		
0% DDGS	2.6 c	0.02 b
10% DDGS	4.5 b	0.23 a
20% DDGS	6.3 a	0.52 a
ANOVA		
Season	0.0087	0.0127
Diet	<0.0001	<0.0001
Season x Diet	NS	NS

NS, not significant.

† Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets (n=7 for all winter diets and the 10% DDGS fall diet; n=6 for the 0% and 20% DDGS fall diets).

‡ Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

Table 2.9. Mean[†] proportions of concentrations of water (H_2O-P) and sodium-bicarbonate ($NaHCO_3$-P) extractable P, residual P, and labile P[§] (P_L) in fresh feces as determined by modified Hedley sequential fractionation.

Effect	H_2O-P[‡]	$NaHCO_3$-P[‡]	Res-P[‡]	% P_L [‡]
	% of Total P (g kg DM)			
Fall				
0% DDGS	31 (7)	34 a (4)	36 a (4)	64 d (4)
10% DDGS	45 (4)	30 ab (5)	26 b (1)	74 c (1)
20% DDGS	39 (4)	35 a (4)	27 bc (5)	73 bc (5)
Winter				
0% DDGS	36 (4)	30 ab (2)	34 a (3)	66 d (3)
10% DDGS	49 (4)	31 ab (2)	21 cd (2)	79 ab (2)
20% DDGS	55 (6)	26 b (6)	19 d (4)	81 a (4)
Season				
Fall	38 b	33	29	71
Winter	47 a	29	25	75
Diet				
0%	33 b	32	35	65
10%	47 a	30	23	77
20%	47 a	30	23	77
ANOVA				
Season	<0.0001	0.0027	<0.0001	0.0002
Diet	<0.0001	NS	<0.0001	<0.0001
Season x Diet	NS	0.0087	0.0041	0.0482

NS, not significant; Numbers in brackets are standard deviation of the mean.

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets (n=7 for all winter diets and the 10% DDGS fall diet; n=6 for the 0% and 20% DDGS fall diets). Proportions calculated as P fraction concentration divided by the Hedley total P concentration (sum of H_2O-P, $NaHCO_3$-P, and Res-P) x 100.

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

[§] P_L is the sum of H_2O-P and $NaHCO_3$-P.

2.4.5. Ratios of nitrogen and phosphorus excreted

The ratio of total N to P in excreta over the 8 hours was not significantly different between diets or seasons, but diet did affect the ratio of N to P excreted in feces and urine over the 8 hours (Table 2.10). Ratios of N to P in total excreta ranged from 8.3 to 7.3 from cows receiving the 0% to 20% DDGS diets, respectively, averaging 7.9. Ratios of N to P excreted in feces over the 8 hours was greatest for cows receiving no DDGS supplementation, at 4.9, with cows receiving the 20% DDGS diets having the lowest ratios of N to P in excreta at 3.5, while the 10% DDGS diet was intermediate to the two. Cows receiving the 0% DDGS diet also had the highest ratio of N to P in urine, at 491, while cows receiving the 10% and 20% DDGS supplementation had the lowest N to P ratios at 220 and 91.

Table 2.10. Mean[†] ratios of nitrogen to phosphorus excreted per cow during 8 hour collection period in total excreta, feces, and urine.

Diet	Total Excreta[‡] N:P	Feces[‡] N:P	Urine[‡] N:P
	g excreted cow^{-1} $8h^{-1}$		
Season			
Fall	8.5	4.3	290
Winter	7.4	3.7	245
Diet			
0% DDGS	8.3	4.9 a	491 a
10% DDGS	8.2	3.6 ab	220 b
20% DDGS	7.3	3.5 b	91 b
ANOVA			
Season	NS	NS	NS
Diet	NS	0.0264	0.0010
Season x Diet	NS	NS	NS

NS, not significant.

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA considering cows as replicates; 2 seasons, 3 diets (n=7 for all winter diets and the 10% DDGS fall diet; n=6 for the 0% and 20% DDGS fall diets).

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

2.5 Discussion

2.5.1. Relationships between diet and excretion of nitrogen and phosphorus

The addition of DDGS as a protein and energy supplement to the diets significantly increased the mass of N and P excreted in urine and feces per cow during the 8-hour collection period (Table 2.4). This was due to the increased concentrations of N_T and P_T excreted in urine and feces (Tables 2.6, 2.7 and 2.8) combined with the increased volume of urine excreted by cows receiving DDGS supplementation. However, this supplementation also increased both the concentrations and proportions of plant available and environmentally labile forms of N and fractions of P. When the proportions of forms of N or fractions of P in excreta changed as a result of diet, as was seen for both forms of N in urine and fractions of P in feces in this trial, animal metabolism of N and P has been affected. The addition of DDGS to the diets therefore not only caused an increase in the total N and P excreted per animal, but has also increased the plant availability and the risk of loss of this excreted N and P to the environment.

2.5.1.1. Nitrogen

2.5.1.1.1. Nitrogen intake and excretion

It is well documented that increases in dietary N will increase concentrations of total N excreted in feces and urine by cattle (Satter et al. 2002; Powell et al. 2006; Broderick 2003) as was seen in this trial. The increase in N intakes for cows receiving the 10% and 20% DDGS diets was primarily the result of increased dietary protein (Table

2.1). Dried distillers' grains with solubles are considered a high source of protein, as well as an energy source (Gibb 2008), with CP content of the DDGS used in this trial at 38% (dry matter basis). This CP content is comparable to other studies that have used wheat-based DDGS (Hao et al. 2009; Gibb et al. 2008).

The ruminant gastrointestinal tract is highly efficient at breaking down most protein sources, with 80% of intake protein estimated to be truly digestible (Satter et al. 2002). Despite this efficiency, cattle will normally excrete greater than 80% of their intake N (Varel et al. 1999). Overfeeding of N will further increase excretion rates of intake N. The portion of intake protein that remains undigested by the animal is excreted in feces, resulting in most of the fecal N being in organic forms (Powell et al. 2006), as was seen in this data set as well. Fecal N can be divided into two general pools: (1) endogenous N, consisting of microbial products and microorganisms (2) undigested feed N (Powell et al. 2006). The portion of intake protein that is digested and absorbed by the cow is used by the animal for maintenance purposes, to create new tissue, and for milk production, or, it is converted to urea in the liver and excreted by the kidney via urine (Satter et al. 2002).

The protein provided through the DDGS in this study appears to have been mostly digestible protein, as indicated by the significant increase in excretion of urine N with increasing DDGS supplementation. The cattle in this trial received excess N in the 20% DDGS diet and marginally sufficient N in the 10% diet (NRC 2001). Despite this, there were no significant differences in urine N_T concentrations between the 10% and 20% DDGS diets. Therefore, the increase in excretion of urine N in the 20% diet was an effect of both increased N concentration and increased volume of urine produced. This

increased volume of urine excreted with increasing protein supplementation has been noted by others (Valadres et al. 1999; Broderick 2003). It is expected that as dietary CP intakes increase, urine total N concentrations will increase and this increase in N metabolites would require increased volumes of urinary water to dilute the compounds before excretion (Valadres et al. 1999; Broderick 2003).

The proportion of the total N excreted in feces and urine depends highly on diet composition, with ranges of 50-80% of total N excreted reported to be urine N (Varel et al. 1999; Broderick 2003; Powell et al. 2006). The route of N excretion can then greatly affect the proportions of plant available and environmentally labile N excreted by cattle. Ammonium-N and urea-N are generally considered the two forms of N found in livestock excreta that are most readily available to plants as well as available to environmental losses. Ammonium-N from livestock excreta can be lost as ammonia gas through the process of ammonia volatilization (Powell et al. 2008). Ammonium N can also be transformed to nitrate through the process of nitrification by microorganisms, whereby it may be lost in water movement or as nitrous oxide during the nitrification or subsequent denitrification processes (Arriaga et al. 2010). Urea is one of the major nitrogenous compounds of urine and, although it is an organic N form, once applied to soil it is quickly hydrolyzed to ammonium through the process of urea hydrolysis by soil microorganisms (Sherlock and Goh, 1984). As a result, urine N, with high concentrations of urea and ammonium, is often noted as the most plant available and environmentally labile form of N in livestock excreta (Varel et al. 1999; Broderick 2003; Powell et al. 2008). Therefore, practices that increase volumes of urine as well as concentration of N in urine have the potential to increase losses of N to the environment.

As feces contain primarily organic forms of N from undigested feed and of microbial origin, fecal N is less available to crops and less available to be lost to the environment. Organic N of microbial origin can make significant contributions to crop N requirements through the process of soil mineralization (Powell et al. 2006). However, undigested feed N has been found to be relatively recalcitrant in soil (Powell et al. 2006). The relatively high proportion of N_T excreted in feces in this study (Table 2.5) compared to other studies in the literature suggests that low-quality forage based diets will result in higher proportions of undigested feed N excreted in feces. For example, Powell et al. (2006) found that undigested feed N in dairy cattle feces fed diets of alfalfa silage and soybean meal, with CP ranging from 15.1 to 18.3% DM, accounted for only 23 to 25% of feces total N. Although the concentrations of N_T, N_{Org}, and N_{NH4+} in feces increased with increasing DDGS supplementation, the proportions of N_{NH4+} and N_{Org} did not change (Table 2.6). This is likely reflective of the fact that DM intake was not affected by DDGS supplementation (Table 2.1), resulting in increased N concentration in feces proportional to the increases in dietary N. Interestingly, Powell et al. (2006) determined through field trials that increased CP concentration in cattle diets did significantly increase the mineralization of organic forms of N in feces once applied to soil, resulting in increased soil inorganic N concentrations compared to feces from low CP diets.

The addition of DDGS supplementation significantly increased the proportion of urine N excreted and decreased the proportion of feces N excreted, and therefore increased the excretion of environmentally labile N. Urinary N accounted for 42% to 62% of the total N excreted from the 0% and 20% DDGS diets, respectively (Table 2.5). These proportions of N excreted in urine and feces are similar to those reported by

Misselbrook et al. (2005) and Cole et al. (2005) by lactating dairy cows fed varying levels of CP. Misselbrook et al. (2005) reported urine N accounting for 52 to 64% of the total N excreted, from cows fed an alfalfa-corn-soybeal silage with 13.6 to 19.4% CP, respectively. Misselbrook et al. (2005) and Cole et al. (2005) also found the same trend in decreasing total N excreted in feces and increasing total N in urine with increasing CP.

2.5.1.1.2. Protein metabolism and relationship to forms of nitrogen in excreta

Increased protein supplementation in cattle diets has been found to increase concentrations and proportions of urine urea N, as well (Bristow et al. 1992; Broderick 2003). The proportion of urea N of the total urine N concentration for the 0, 10, and 20% DDGS diets was 30, 39, and 54%, respectively (Table 2.7). This increased proportion of urea N accounted for 46 and 86% of the increase in urine total N excreted from the 0 to 10% (6.0 to 8.7% CP) and 10 to 20% (8.7 to 11.5% CP) DDGS diets, respectively. This is similar to that reported by Broderick (2003) where an increase in CP from 15.1 to 18.4% DM, from alfalfa based diets supplemented with soybean meal, resulted in urea N accounting for 82% of the increase in total urine N excreted by lactating dairy cows. This study had a much higher proportion of urine urea N compared to the present study, however, ranging from 85-91% of the total urine N.

Bristow et al. (1992) determined components of urine from dairy cows fed various diets. For dairy cows receiving grass-silage and protein concentrate, total urine N from spot samples of four cows ranged from 6.8 to 9.6 g N L^{-1} and the proportion of urea N, expressed as a percentage of the total urine N, ranged from 59.3 to 71.5%, increasing with increasing total urine N concentration. The proportions of urea N determined by

Bristow et al. (1992) and Broderick (2003) were higher than that determined in the present study despite similar total urinaru N concentrations. The low-quality, forage-based diet appeared to reduce the proportion of urine urea N compared to these studies, resulting in a lower percentage of plant available and environmentally sensitive N excreted compared to cows receiving diets based on silage, even though total N excreted was similar.

Arriaga et al. (2010), however, found no significant differences in proportions of urine urea N or total N of feces and urine from cattle fed high forage (77:23 forage to concentrate ratio) and low forage (45:55 forage to concentrate ratio) diets formulated to meet 500 g N cow^{-1} day^{-1}. These diets consisted of triticale silage fed ad libitum with alfalfa and concentrate added to meet N requirements and concentrate ratios. The concentrate used was a mixture of barley, corn, beet pulp, and soybean meal. The urinary urea N concentrations were within the range of the 10% diet of this study, averaging 3.55 g urea N L^{-1} urine. Proportions of N excreted in urine to feces were similar between diets, and total urinary N did not increase with the increased proportion of concentrate. As the diets were formulated to meet the same high N intake and were sufficient in energy, this may explain why no change in route of N excretion or proportion of urea N was observed.

Hao et al. (2009) also did not find significant differences in terms of concentrations of total N or water soluble ammonium N excreted in feces or manure between a control diet (alfalfa silage and barley) and replacing a portion of the barley with 20% DDGS. As in Arriaga et al. (2010), these two diets were likely similar and sufficient in intake N and energy. When DDGS was used to replace barley at rates of

40% and 60%, however, increases in total N of feces were noted, although manure total N concentration were still not found to be significantly different (Hao et al. 2009). Either the increased N in manure was lost, via ammonia volatilization from potentially increased concentrations of urine N or ammonium N in feces, or the effect of DDGS supplementation did not increase urine N significantly enough between diets to result in differences in manure total N concentrations.

These studies, Bristow et al. (1992), Broderick (2003), Hao et al. (2009), Arriaga et al. (2010), highlight the importance of diet composition on the proportions of forms of N found in excreta. Dietary protein provides metabolizable protein in the forms of rumen degradable protein (RDP), which is used in microbial synthesis by rumen microorganisms, and rumen undegradable protein (RUP), which can be digested directly by the animal without the aid of rumen microorganisms (Broderick 2003). Microbial synthesis is the most efficient, and cheapest, source of dietary N for ruminant livestock production (Satter et al. 2002). Therefore, maximizing microbial synthesis in the rumen will result in the most efficient use of dietary N (Satter et al. 2002). Practices that maximize microbial synthesis include balancing the proportion of RDP and RUP in feed in accordance with animal requirements, increasing DM intakes, and supplying sufficient and efficient energy sources for rumen microorganisms (Satter et al. 2002).

The National Research Council assumes that diets that are formulated to meet CP requirements are also formulated to match RDP and RUP requirements (Satter et al. 2002). However, in practice this can prove to be difficult (Satter et al. 2002). Although animals may be receiving sufficient CP and N to meet nutritional requirement of animals, a diet that is not balanced for RDP and RUP requirements will result in excess N intakes

and therefore excess N excretion as well. A balance of RDP for microbial synthesis and RUP to meet high production requirements is required to make the most efficient use of intake N and minimize excretion of N. In the present study, the cows used were open, non-lactating cows fed only to meet maintenance requirements.

Distillers' grains, along with bone meal and blood meal, are generally considered rich sources of RUP. A range of 47 to 66% RUP is generally used for corn DDGS and 62.7% RUP has been documented for wheat DDGS (Kononoff and Christensen 2007; Klopfenstein et al. 2008). Conversely, alfalfa, a high N containing legume often used in cattle diets, contains 50-60% of its N as non-protein N, making this forage a rich source of RDP (Satter et al. 2002). High production animals, such as dairy cows, will require a higher proportion of RUP in their diet to meet high milk production demands efficiently. Dairy cow rations that use alfalfa as the primary forage source often supply excess N as RDP when protein supplements are added to meet increased N requirements, especially if a protein supplement high in RDP is used as well, such as soybean meal (Satter et al. 2002). The result is inefficient use and increased excretion of N, despite the fact the protein requirements are being met according to feed analysis.

Conversely, diets high in DDGS may increase an animal's requirement for RDP, especially when used in combination with low quality forage (Satter et al 2002; Broderick 2003). This may be more of factor for high production animals such as dairy cows, however. Feedlot animals fed diets containing DDGS that were low in RDP had no significant effect on performance from added urea to the diet, indicating that RUP supplied in the DDGS had been efficiently recycled back to the rumen to be used a source of RDP (Klopfenstein et al. 2008). In general, protein supplements with a high RUP

content such as DDGS are excellent for balancing N intakes in rations using alfalfa silage, while N sources with higher proportions of RDP, such as soybean meal, may be better suited as protein supplements for diets based on corn silage or other lower quality forages.

Highly fermentable starches such as barley or shelled corn will also increase microbial synthesis per unit DM intake, by providing a readily available energy source for rumen microorganisms, stimulating microbial synthesis (Satter et al. 2002; Broderick 2003). Gibb et al. (2008) determined that DDGS could be as effective as barley in providing an energy source for feedlot cattle when included at rates of 20%. Therefore, energy should not have been a limiting factor in 10% and 20% DDGS diets. The addition of energy to cattle diets can also result in increased requirements for RDP, in order to meet the demands of increased microbial activity.

Diets that increase DM intake and thereby rate of passage, will also increase microbial activity and thereby microbial protein synthesis (Satter et al. 2002). In the present trial, however, no significant differences were found in DM intakes due to increased supplementation with DDGS. Therefore, the form and concentration of N supplied through DDGS in this trial is likely more related to changes in nutrient excretion and rate of passage than DM intake. When DM intakes remain unchanged, the balance of RUP and RDP in the diet could have a large impact on the route and forms of N excreted. Even if N requirements are being met, imbalance of RDP and RUP could result in diets that do not maximize microbial protein synthesis, resulting in unnecessary intake and excretion of N. Even though the 10% DDGS diet in this trial was formulated to meet N requirements, it is questionable as to whether the RDP and RUP balance was achieved.

The 20% DDGS diet, which was considered excessive in N, and likely supplied excess RUP, which resulted in increased excretion of absorbed N via urine.

2.5.1.1.3. Minimizing excretion of available nitrogen

For cow-calf producers, protein supplementation to forage based diets is generally used during winter months or during spring calving, to meet nutritional requirements or to meet the increased dietary requirements needed during the later stages of gestation and lactation. This is at a time when plant uptake of N is not occurring. These animals may be housed and fed in dry lots or, as is becoming common practice, they may be fed forage and supplement out on pasture to reduce labour and manure handling costs. If the latter practice results in excreta with high proportions of available N deposited on snow or in wet conditions prior to plant growth, there is a high probability that this available N will be lost in spring snowmelt runoff. If deposited on ground following snowmelt in early spring, as Chapter 3 demonstrates, there is an increased risk of loss of N as nitrous oxide as well.

When considering manure application from a drylot, the increased concentrations and proportions of plant available forms of N in both urine and feces could be a positive implication of DDGS supplementation. Beef cattle manure is generally considered low in plant available N, due to the high proportion of organic N contained in feces (Powell et al. 2006). A higher proportion of plant available N in excreta would mean that a larger area of land could benefit from reduced N-based manure application rates. Carryover of organic N to subsequent crops would be lower for cows receiving a highly supplemented diet, however, due to the decreased proportion of urine N_{Org}. The higher concentrations of

available N in excreta indicate that there is increased risk of loss of N to ammonia volatilization, N_2O emission, and runoff as well. Therefore, depending on management practices, the benefit of increased available N concentrations in manure from DDGS supplementation may be diminished if the available N is lost before application to crops (Powell et al. 2006; Hao et al. 2009).

2.5.1.2. Phosphorus

2.5.1.2.1. Phosphorus Intakes and Metabolism

Similar to the N intakes, the addition of DDGS to the forage diet increased the intake of P (Table 2.1). Distillers grains are known to be a rich source of dietary P, with an average P concentration in the literature reported at 0.83% P (DM basis) and with a standard deviation of 0.12 (Buckley and Penn 2003). The P concentration in the DDGS used in this trial was at the high end of this range, at 0.93% P (DM basis) with a standard deviation of 0.02. As a result, DDGS supplementation significantly increased P intakes and therefore P excretion (Tables 2.4 and 2.8). This is consistent with other studies that found that increased intake of P in cattle diets resulted in increased P excreted with feces. For example, Hao et al. (2009) found that increasing total P concentration in feedlot diets associated with increased distillers' grain supplementation, was highly correlated with increasing concentrations of P in feces and manure. Many studies have documented the relationship between increasing diet total P and increasing fecal total P in dairy cattle as well (Kincaid et al. 2005; Spears et al. 2002; Dou et al. 2002; Rotz et al. 2002; Powell et al. 2001).

Average P requirement for maintenance of a dry beef cow is recommended to be 16 mg P kg^{-1} body weight (BW) (Geisert et al. 2010). For the cows used in this trial, this would be approximately 11 g P cow^{-1} day^{-1}. The 0% DDGS diet was therefore nearly adequate to meet P requirements of the animals while the DDGS supplementation provided excess P in both the 10% and 20% diets (Table 2.1). Many studies have concluded that P recommendations by NRC are too high, however, and that P recommendations could be reduced further, especially for beef cattle (Erickson et al. 2002; Geisert et al. 2010).

The distillers' grains used in this trial were derived from wheat, and therefore, a large proportion of the P fed would have been phytate-P (Maguire et al., 2005). Grains and oil seeds contain high concentrations of organic P, with approximately two-thirds of the P in the form of phytate (Maguire et al. 2006). Unlike monogastric animals which cannot digest phytate-P, the rumen microorganisms of ruminants can synthesise phytase, the enzyme required to break down phytate-P into forms available for absorption. In forages, stems and leaves of plants contain organic forms of P, but unlike grains and seeds, forages contain very little phytate-P (Buckley and Penn 2003; Bravo et al. 2002). The availability of feed P to cattle is considered to be 64% for forages and 70% for grains (Buckley and Penn 2003). Organic P that is available to the animals is converted to inorganic forms of P, mainly orthophosphates, which is absorbed to be used for bodily functions (Buckley and Penn, 2003).

Net absorption of P occurs mainly in the small intestine through two mechanisms, and is directly related to the supply of potentially absorbable P (NRC, 2001). When animals are fed low-P diets absorption occurs mainly through a vitamin-D dependant

active transport mechanism (NRC, 2001). When available P in the diet is adequate or excessive, such as the 10% and 20% DDGS diets, resulting in high availability of P in the lumen and blood plasma, passive absorption dominates (NRC, 2001). Efficiency of absorption of diet P depends on age and body weight of the animal, as well as physiologic state (i.e., non-lactating versus lactating) and amount of dry matter or phosphorus intake (NRC, 2001). All of these factors were constant between periods in the present trial.

Other factors, such as dietary protein content, starch degradability, and grain content of the diet may affect availability of organic feed P (Buckley and Penn, 2003), as well as rumen outflow, rumen pH, and Ca content (Chapuis-Lardy et al., 2004). High concentrate diets generally result in faster rates of passage, which means less time for organic forms of P, such as phytate, to be broken down by rumen microorganisms (Bravo et al. 2002). However, there were no differences found in rate of passage between diets in this trial (Bernier 2010). There were also no significant differences found in rumen pH between diets or season. Calcium to phosphorus ratios in the diets ranged between 1.6 in the 20% DDGS diet and 3.5 in the 0% DDGS diets, and therefore was not low enough to be of concern to P utilization.

2.5.1.2.3. Phosphorus Excretion and Forms of Phosphorus

As with N, the route of P excretion and source of P in the diet can have an effect on excretion of environmentally susceptible fractions of P. Literature suggests that, in cattle, greater than 95% of excreted P is in the feces, with only small amounts of P excreted in urine (NRC, 2001). The 0% and 10% DDGS diets resulted in 99 and 92% of excreted P via feces, which is within range of the literature findings. However, the 20%

diet resulted in only 82% of excreted P via feces during the 8 hours, due to the significant increase in excretion of urinary P. Other studies have also found that cattle fed diets with high concentrations of protein and energy supplement can result in significant increases in urinary P concentrations (Meyer et al. 2006; Geisert et al. 2010). Although this concentration of urinary P was low compared to the feces P concentrations, it is important because it can be considered labile P, as this P is already in dissolved form in urine and therefore highly mobile and easily moved in water (Meyer et al. 2006). Analysis of three randomly selected samples by ion chromatography revealed that greater than 50% of total urinary P was in the phosphate form, confirming the high availability of urine P (Appendix C).

Meyer et al. (2006) determined that excess dietary P was excreted via urine and feces, with urinary P accounting for 29 to 34% of the total P excreted from feedlot cattle fed increasing concentrations of distillers' grains. Urinary P excretion was found to be dependent on apparent digestibility of dietary P. Meyer et al. (2006) hypothesized that when diets were high in concentrate, volume of saliva secreted would be reduced. Ruminants maintain homeostasis of P, and most other essential mineral elements, through salivary recycling and excretion. The higher concentration of P in the saliva as a result would increase the amount of P absorbed in the blood stream but there would be less recycling of P to the digestive tract, thereby less P excreted via feces, due to the lower rates of salivary recycling. This would cause concentrations of P in the blood to increase to the threshold of clearance in the kidney. Meyer et al. (2006) also noted that this increase in urine P excretion could lead to higher P concentrations in runoff from feedlots.

Fecal P is considered to be in three fractions: that of dietary origin unavailable for absorption or not absorbed; that of endogenous origin which is inevitably excreted (inevitable fecal loss); and that of endogenous origin which is excreted to maintain homeostasis (NRC 2001). It is believed that inevitable fecal loss of P, of which about half is associated with microbial debris and nucleic acids, is determined mainly by dry matter intake (DMI) and that this fraction can also vary depending on fermentability of the diet (NRC, 2001). As no significant differences in DMI between diets or seasons were found, DMI should not be a factor in the P fractions of fecal P determined. These results suggest that P intake or diet P was the most important factor contributing to P excreted in this trial.

2.5.1.2.4. Relationship between feces total phosphorus and labile phosphorus

Literature has shown that as total P excreted in feces increases due to increased intake of P, the proportion of P excreted that is susceptible to be lost in water increases as well (Dou et al. 2000; Chapuis-Lardy et al. 2004; Ebeling et al. 2002). As was found in the present study, Dou et al., (2002) indicated that increasing dietary P concentrations through the use of P minerals not only led to higher concentrations of total P in feces, but more importantly increased the amount and proportion of P that was water soluble and thus most susceptible to loss in the environment. They determined that when dietary P was 3.4, 5.1, and 6.7 g P per kg DM, the water soluble fraction of fecal P was 56, 77, and 83% of the total P. This is within the same range of the 65% to 77% labile P found in the current trial. A study by Hao et al. (2009) using wheat distillers' grain supplementation also found that water soluble reactive P increased with increasing supplementation,

however, the labile or water soluble total P fraction was not determined and thus cannot be compared with this study.

The percentage of labile P determined in the feces in this study was higher than that determined by Kumaragamage et al. (2009) from beef cattle manure (containing feces, urine, and bedding material) collected from various farms in Manitoba. Kumaragamage et al. (2009) determined average labile P to be 44%. One notable difference was that the sum of total P concentration determined in the Kumaragamage study were much higher than the sum of total P concentrations in the present study. Despite this, the range of total P extracted in H_2O and $NaHCO_3$ extractants was similar in both studies. For example, the range of P concentrations found by Kumaragamage et al. (2009) in the H_2O extraction was 0.5 to 1.3 g kg^{-1} DM, while in this study H_2O-P total P concentrations ranged from 0.4 to 1.2 g kg^{-1} DM (Appendix B). The lower percentage of labile P determined in the Kumaragamage study is likely due the fact the manure used in that study was a mixture of urine, feces and straw, and not just pure feces. The fact that fresh feces were used in this study instead of dried feces may have also affected the Hedley total P and labile P determinations, due to increased variability in samples as well as the effect of drying on the solubility of fecal P (Ajiboye et al. 2004).

2.5.1.2.5. Effect of Diet on Labile P

As was seen in Table 2.9, the addition of DDGS to diet affected not only the fraction of labile P extracted from the feces but also had an effect on the individual fractions of H_2O-P and Res-P. It has been hypothesised by others that H_2O-P is related directly to P overfeeding and consists of primarily inorganic forms of P (He et al. 2004;

Dou et al. 2002). The addition of DDGS significantly increased the proportion of H_2O-P in the 10% and 20% DDGS diets, whereas it had no effect on the proportion of $NaHCO_3$-P. The increase in labile P with DDGS supplementation, therefore, was directly related to the H_2O-P fraction.

As literature suggests that increasing dietary P increases feces water soluble P, it draws attention to the fact that the percentage of labile P and H_2O-P were not significantly different between feces from the 10% and 20% DDGS diets. Other studies have found that the proportion of water soluble P increases as P intake increases (Dou et al. 2000; Chapuis-Lardy et al. 2004; He et al. 2004). The difference between this trial and others is possibly due to the fact that organic feed stuffs were used in this trial, unlike most of the other studies where increases in total P were due to added mineral P (i.e. inorganic P) supplement. The results of these studies brings up questions as to the bioavailability of organic feedstuff and the effect that bioavailability of feed stuff may have on the water soluble phosphorus content of feces.

In a study by Chapuis-Lardy et al. (2004), using mineral P supplementation, it was determined that feces water soluble P concentrations comprised approximately 50% of fecal total P concentration, and that 83% of the P in these water extracts was inorganic P. He et al. (2004) determined that the inorganic P in water soluble fractions of cattle manure was made up primarily of orthophosphate while the water soluble organic P fraction was primarily composed of phytate like P, which was probably derived from undigested feed residues.

Chapuis-Lardy et al. (2004) hypothesized that the high proportion of inorganic P in the water soluble P fraction of feces in both the control diet and diets with mineral P

supplementation suggested that the form of dietary P did not affect the bioavailability of P. The excess organic P in feed intakes was still hydrolysed, even though it may not have been absorbed by the animal, resulting in the high concentrations of inorganic P in the feces water soluble P fraction for all diets. Conversely, Dou et al. (2002) found that, although it made up a small proportion of the feces water soluble P, organic P concentration in water extractions generally tended to increase as more mineral P was added to the base diets. The animals appeared to be using inorganic sources of P before organic forms, resulting in increased excretion of organic forms of P. Therefore, eliminating or decreasing the amount of mineral P supplementation is likely to provide the benefit of decreasing water soluble organic P in feces as well. Dou et al. (2002) hypothesized that the high concentration of inorganic P in feces from the base diets that consisted solely of organic feed components was an indication that P in the base diets was either largely water soluble to begin with or readily digestible by the animal. He et al. (2004) also found that the water soluble organic P concentration increased as total fecal P increased, suggesting a relationship between mineral supplementation and phytate P digestibility.

As advances in technology have given us the ability to more accurately determine forms of P in manure, phytate-P has now been indentified in some ruminant feces, leading to questions regarding the effect of diet on organic P and phytate-P hydrolysis (Kincaid et al. 2005; He et al. 2004). Considering that phytate makes up the majority of the organic water soluble portion of labile P fractions, phytate digestibility is an important consideration when trying to reduce labile P excretion. In a study by Kincaid et al. (2005), phytase enzyme was added to feedlot cattle diets. This study found that the

addition of phytase enzyme did not reduce total fecal P, but did reduce the fraction of water soluble P in feces. Bravo et al. (2002) found that phytate-P digestibility is actually highly variable, ranging in various studies from 33% to 97%. Phytate-P hydrolysis by ruminant microorganisms can be variable depending on feedstuff, feed processing, and ruminal outflow rates, and diet Ca concentration, which affect the ability of phytases to access and completely hydrolyse phytate (Kincaid et al. 2005; Kebreab et al. 2005; Bravo et al. 2002).

The results of these studies suggest that more research is necessary to determine the digestibility of organic feed P and factors that affect concentrations of phytate-P and water soluble P in feces. These studies are difficult to compare, as they used varying sources and concentrations of feed P, and analysis of feces was not always consistent. As inorganic and organic P fractions were not determined in this study, we cannot make any firm conclusions on the reasons for the similar proportions of labile P between the 10% and 20% diets.

2.5.2. Effect of season on excretion of nitrogen and phosphorus

The colder weather during the winter sampling period resulted in decreased total fecal P concentrations with an increased proportion of labile P and an increase in the concentration of urinary P. As well, total N excreted in feces decreased during the winter sampling period. Bernier (2010) determined that although rate of passage was not different between diets, rate of passage of solids in the hindgut were increased during the winter sampling period. However, DM, CP, N and P intakes were not significantly different between the two sampling periods (Table 2.1). This indicates that animal

metabolic responses to cold weather caused the differences in nutrient excretion during the colder temperatures.

The decrease in total feces P excreted in the winter trial could indicate that animal requirements for P are higher in cold temperatures, as intakes of P were not significantly different between fall and winter sampling periods. The animals did not gain weight between fall and winter periods, however. The increased concentrations of labile P during the winter sampling period can be hypothesized to be the result of the increased movement of solids through the hindgut in the winter sampling period (Bernier 2010). The faster movement of materials through hindgut would result in less absorption of available P, as most absorption of P occurs in the small intestine (NRC 2001). This could be the result of the increased proportions of labile P in feces in the winter sampling period. The higher concentration and excretion of urine P in the winter sampling period indicated that blood P levels were higher in the winter, possibly the result in the decreased fecal P concentrations. Data suggests that the speed of rumen contractions increased during the winter period, which could result in a greater breakdown of organic forms of P and increase available P concentrations in the hindgut (Bernier 2010). Although rate of passage in the hindgut was increased, increased concentrations of P in the hindgut would still result increased absorption of P despite the fact that the efficiency of P absorption would be decreased.

The proportions of $NaHCO_3$-P and Res-P extracted in feces were decreased during the winter sampling period while the proportion of H_2O-P and labile P was increased. However, the interaction effect between diet and season indicate that the effect of season on $NaHCO_3$-P, Res-P, and labile P only occurred in the DDGS supplemented

diets. This interaction effect was not seen the in H_2O-P portion of the feces P however, indicating that the same effect of cold weather was seen the both the DDGS and no DDGS diets.

Hao et al. (2009) found no significant differences in total P or water soluble phosphate concentrations in feces and manure collected in February, in Lethbridge, Alberta, compared to those collected in June from feedlot cattle receiving DDGS supplementation, which is contrary to the results of this study. This could indicate that the increase in labile and H_2O-P in this trial was due to an increase in the organic P fraction of H_2O-P, as the organic P fraction was not measured in the Hao et al. (2009) study. Due to the different diet compositions it is hard to make direct comparisons with the Hao et al. (2009) study, however.

The decreased concentration of N in feces due to cold acclimatization indicates that the animals were making more efficient use of intake N during cold temperatures, when requirements of CP and energy would be greater. The fact that the urine N concentrations were not affected indicates that the organic N portion of the feces was being utilized more efficiently by the cows to compensate for the cold weather. The increased concentration of N_{NH4+} in the winter sampling period (Table 2.6) could also support this hypothesis. The interaction effect found in the proportion of N_{NH4+} indicate that only the DDGS supplemented diets had an increased proportion of N_{NH4+} in the winter. Hao et al. (2009) also noted significantly lower manure total N concentrations in winter months from feedlot cattle fed diets supplemented with DDGS, although no significant differences were found in feces total N concentrations over time indicating

that urine total N concentrations may have been decreased or volatilization losses of ammoniacal N in urine were still occurring during winter months from the manure pack.

2.5.3. Nitrogen to Phosphorus Ratios

As regulations in Manitoba regarding soil test P are now in place, the ratio of N to P applied through manure is of great importance to livestock producers. Plants take up N and P at ratios of 10:1 for legumes, 7:1 for grasses, and 8:1 for annual crops (Penderson et al. 2002). Beef cattle manure N:P ratios range from 2.5:1 to 3.7:1 (Larney et al. 2006). Therefore, if manure is applied to meet crop N requirements, phosphorus accumulation in the soil will occur. The differences in the urine and feces N to P ratios (Table 2.10) again demonstrates that it is the urine which supplies the majority of the N to the soil, while feces are oversupplying P. There were no significant differences in total N to P ratios of excreta from the three diets, however (Table 2.10), which ranged between 7.3 and 8.3. The observed similarity is interesting considering the huge difference in diet composition between the 0% and 20% DDGS diets. The N to P ratios of the diets ingested by the cows were 8, 7, and 6 from the 0, 10, and 20% DDGS diets, respectively, which were all significantly different from one another (Table 2.1). This suggests that the 8-hour excreta collection may have overestimated daily rates of excreta production. However, it has also been found in other studies that N to P ratios will increase following ingestion, due to the double retention rate of P in the animals compared to N, which could also explain the increased N to P ratios of excreta products compared to that found in the diet (Erickson et al. 2000; Jungnitsch 2008).

The total N to P ratios of the excreta were above the plant requirement of grasses, which means that P accumulation in soil should not occur following application on grasslands. However, as organic N, which consists of a large portion of both urine and feces total N, is not immediately available to plants upon application. Available N is generally used to calculate manure application rates and N to P ratios instead of total N. In this paper, available N refers to the sum of N_U and N_{NH4+} portions, or ammoniacal N portion, of excreta. In Manitoba, in accordance with the Tri-Provincial Manure Application and Use Guidelines (MAFRI 2004), available N calculations also include 25% of the organic N portion of the manure, which is considered to be readily mineralized and available for plant uptake within the first year following application. This latter method of determining available N is generally used for manures applied to soil from bedding pack areas, where feces and urine are mixed with straw. If available N is used to calculate N to P ratios, the N to P ratios are significantly lower, and below the plant requirements of both grasses and legumes. Extrapolation of the results from Tables 2.4, 2.6, and 2.7 would result in available N to P ratios of 1.5, 2.2, and 2.9, with 18, 29, and 40% of the total N being in available N (N_U and N_{NH4+}) form, from the 0, 10, and 20% DDGS diets, respectively. If including 25% organic N in the calculations, the N to P ratios increase to 3.0, 3.5, and 3.9, with available N accounting for 38, 47, and 55% of the total N excreted per cow during the 8 hours from the 0, 10, and 20% DDGS diets, respectively.

Jungnitsch (2008) determined that total N to P ratios deposited in-field by grazing animals (including excreta and feed residue) overwinter on forage-based diets were in the range of 9:1. This value is comparable to that in excreta in the present trial. When taking

into consideration losses of N and retention of soil P, Jungnitsch (2008) determined N to P ratios to be 6:1, based on soil test measurements. The same study found that manure application from similar diets fed in a drylot system resulted in manure with N to P ratios of only 3:1. These significantly lower N to P ratios were obtained from soil measurements following application of drylot manure to a forage stand, which was believed to have high losses of available N. Available N in excreta products were also considered to be greater than one third of the total N applied, which are much higher than those determined in the present study.

The available N to P ratios in excreta from cattle in this trial were extremely low, and if this excreta was applied to meet forage crop N requirements P accumulation would occur. As P loss to waterways is becoming an important issue, manure application rates based on crop P requirements instead of N requirements is becoming another increased cost of production for livestock producers, as large land bases and longer hauling distances are generally required to meet these requirements. As such, caution should be taken when using DDGS supplementation, as manure or excreta application could quickly increase soil test P levels above soil test P thresholds. This effect would be increased if the available N portion of excreta is lost through runoff, leaching, or gaseous losses. As the DDGS supplemented diets had significantly greater proportions of available forms N in the urine, the loss of available N following application would quickly result in the 10% and 20% DDGS diets having lower available N to P ratios in soil than the 0% DDGS diet. If forced to apply excreta or manure from these cattle on receiving these diets based on crop P requirements, producers will have a much harder time finding land base to spread drylot manure or may not be able to re-use winter

grazing areas on an annual basis. The environmental implications of accumulating soil test P due to low available N to P ratios is compounded by the fact that P excreted from the DDGS supplemented diets is highly labile and easily lost to in runoff or snowmelt to waterways.

2.6 Conclusions

The addition of DDGS as a protein and energy supplement to low quality forage diets resulted in increased excretion of agronomically and environmentally available forms of N and fractions of P in beef cattle excreta. Urine N was responsible for the majority of the increase in available forms of N, due to increased concentrations and proportions of urea and volume of urine with increased DDGS supplementation. Increases in labile P fractions were largely due increased P concentrations in feces. The addition of DDGS supplementation increased labile P from 65% to 77% in feces. Distillers' grains supplementation also increased urine P concentrations, and as a result urine comprised a significant portion of potentially labile P to the total P excreted from the 20% DDGS diet. Differences in N and P excretion between seasons suggest that cold acclimatization increased proportions of labile P in feces receiving DDGS supplementation, and decreased total N and P concentrations in feces while increasing P concentrations in urine. The ratio of available N to total P in excreta was very low. Application of manure or excreta from cattle receiving these diets could potentially lead increased soil test P if not applied to meet crop P requirements.

Although DDGS may be an inexpensive source of protein and energy for beef cattle, the use of DDGS could have profound environmental implications in terms of nutrient loss of N and P to the environment. If DDGS supplementation is used, timing of application of excreta to soil could be an important factor in reducing losses of N and P to the environment. However, as protein and energy supplements are generally fed to beef cattle when forages are dormant, this may prove difficult, especially if a winter grazing system is being used to reduce cost of production.

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3. SOIL GHG EMISSION FROM EXCRETA COLLECTED FROM OVERWINTERING BEEF CATTLE FED DIETS OF LOW-QUALITY FORAGE SUPPLEMENTED WITH DDGS DEPOSITED ON GRASSLAND FOLLOWING SNOW MELT

3.1 Abstract

Donohoe, Gwendolyn R. M.Sc., University of Manitoba, September, 2010. Soil GHG emission from excreta collected from overwintering beef cattle fed diets of low-quality forage supplemented with DDGS deposited on grassland following snow melt. Major Professor: Dr. Mario Tenuta.

Overwintering of mature beef cattle on pasture, rather than in a drylot, is a practice that Western Canadian beef producers have adopted to lower production costs. However, it has yet to be determined if overwintering on pasture can be considered an environmentally beneficial management practice. The objective of this study was to monitor soil greenhouse gas (GHG) emission of feces and urine deposited on grassland by overwintering beef cows following snow melt and throughout the subsequent growing season, and to determine if differences in GHG emission occur due to supplementation of a low-quality forage diet with dried distillers' grains with solubles (DDGS).

Feces and urine used in the trial were collected from cows in three diet treatment groups fed diets consisting of a low-protein (6% crude protein) forage ration containing increasing concentration of DDGS as a protein and energy supplement: 0%, 10%, and 20% w w^{-1} supplementation. Greenhouse gas emission from feces and urine were also compared to a mixture of feces, urine, and bedding material to simulate drylot manure.

Feces and urine were mixed according to the ratio of feces to urine produced with straw added to meet 23% dry matter. Feces, urine, and simulated bedding pack manure were deposited in April, 2009, and monitored through September. Gas emissions of nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) from patches were determined using static vented chambers and cumulative emissions were estimated. Soil samples were collected weekly to determine soil nitrogen (N) transformations and their relationships to GHG emissions.

Cumulative N_2O emissions over the 147-day study period were significantly greater from urine patches derived from animals receiving DDGS supplementation, being 1 258, 735, and 267 mg N_2O-N m^{-2} produced from urine patches from 20%, 10% and 0% diets, respectively. Feces resulted in consumption of N_2O, with cumulative emissions of -22 mg N_2O-N m^{-2}. Bedding pack emissions were intermediate, at 20 mg N_2O-N m^{-2}. Peak N_2O emissions from urine patches occurred in early July and did not occur immediately following application of excreta, when soil available N concentrations were greatest. Instead, peak N_2O emissions were related to warming temperatures and followed large (>40 mm) precipitation events. Feces had low cumulative emission of CH_4, at 76 mg C m^{-2}, with no significant difference in CH_4 emission between diets or patch type. Nitrogen emission factors for urine, feces, and bedding pack treatments of 0.37, -0.04, 0.06% of applied N, were all lower than current guidelines of 2.0% applied N, set by the Intergovernmental Panel on Climate Change (IPCC 1996). The results of this research will be used in the development of low-cost, environmentally sustainable management practices for Canadian beef producers, as well as, provide emission factors for GHG national accounting and carbon credit models for overwintering beef cattle systems.

3.2 Introduction

As production costs continue to increase for beef producers on the Canadian Prairies, changes in management practices are becoming necessary in order to ensure the survival of the beef industry. Overwintering costs, including feed, labour, and manure management, present significant challenges for Canadian producers, and have stimulated many changes in farm management of cattle. In an attempt to reduce inputs and lower labour costs, production practices involve reducing quality of feedstuff, using cheaper sources of protein and energy supplementation, and reducing time spent in drylots. This has resulted in production practices such as feeding cows on pasture, to reduce manure build-up in pens and the associated costs of corral cleaning and manure management, and low-quality forage based diets, with opportunistic supplementation to meet protein and energy requirements of the animals. Dried distillers' grains with solubles (DDGS), a by-product of the ethanol production industry, have been used as a protein and energy supplement in cattle diets. With ethanol production predicted to increase in the future (Simpson et al. 2008), DDGS may become a more common and readily available, low-cost supplement for beef producers in the future.

These relatively new production practices have much uncertainty associated with them in terms of their environmental implications, however. Beef producers need to be aware of the environmental implications of their management practices as consumers are becoming ever more concerned about the source of their food and the environmental footprint of food production. Government regulations, particularly in the province of

Manitoba, are becoming ever more focused on mitigation of GHG emission and environmental protection, and therefore producers must ensure that both industry viability and environmental protection are of utmost importance. Government regulators and agronomists also need to be aware of the environmental implication of these new low-cost management practices, in order to give sound recommendations to producers and create scientifically sound environmental policy.

There is virtually no peer reviewed information available on soil GHG emission from excreta deposited by overwintering beef cattle on pasture versus the traditional drylot system. Similarly, there is little peer reviewed literature available on soil GHG emission from beef cattle fed a low-quality forage based diet.

Most of the available literature on the subject, and associated emission factors and models, are based on research done in humid and subtropical climates, where cattle, usually dairy, are fed high-quality diets of silage or are grazing grass and grass legume pastures year round (Onema et al. 1997; Bolan et al. 2001: Saggar et al. 2004). Information is available on the effect of diet on GHG emissions from excreta deposited on pasture, but again using dairy cattle that are grazing or receiving high-quality rations, and this information was not collected from a semi-arid continental climate (Arriaga et al. 2010; van Groenigan et al. 2005a).

Previous studies conducted in Manitoba on GHG emissions from grazing beef cattle were conducted during the summer (Tremorin 2008). As climatic conditions in the spring are quite different than those in summer, the effect of temperature and moisture on GHG emission from excreta deposition will be further examined in this study. Nitrous oxide emission from microbial nitrification and denitrification have been found to be very

sensitive to temperature and moisture factors, as well as, nitrate and carbon availability (Oenema et al. 1997; Bol et al. 2004; van Groenigan et al. 2005a; Carter et al. 2007; Luo et al. 2008). As a result of this sensitivity to the environment, other studies have found conflicting reports on the production of N_2O by nitrification and denitrification from excreta deposition, with some studies reporting denitrification as the dominant source, others nitrification, and some studies reporting a combination of the two (Bol et al. 2004; van Groenigan et al. 2005a; Carter et al. 2007; Luo et al. 2008). Therefore, the combination of diet and climatic conditions present during the current study will add new depth to information available on soil processes resulting in N_2O and CH_4 emission from excreta deposition.

The IPCC (1996) uses a factor of 2% of applied N lost as N_2O for all types of livestock excreta. However, N emission factors reported in the literature have high temporal and spatial variability and are dependent on livestock species, type of excreta, and storage (Onema et al. 1997; Bolan et al. 2004; van Groenign et al. 2005a). This suggests that emission factors should be determined for individual regions and production practices (Bolan et al. 2004). Urine deposition on grassland has been found to have values for N emission factors ranging from 0.02 to greater than 6.5%, and while feces deposition were in the range of 0.1 to 0.7 (Onema et al. 1997; Bol et al. 2004; van Groenigen 2005b; Tremorin 2008; Di et al. 2010).

This study examined more closely the effect of a low-quality, forage diet and DDGS supplementation on the emission of N_2O associated with turnout of cattle on pasture in early spring. This is becoming a very common practice on farms in Manitoba, as it reduces manure build-up in drylots, as well as, labour costs. In early spring, no plant

growth occurs and cattle still need to be fed overwintering diets, despite being turned out onto pasture.

The objectives of this study were to answer the following questions:

1. Does supplementation with DDGS to a low-quality, forage diet fed to overwintering beef cows result in increased soil GHG emissions when urine and feces are deposited on grassland following snow melt?
2. How do GHG emissions from a bedding pack compare to fecal and urine patches?
3. What are the seasonal effects of soil nutrient concentrations, soil temperature, and soil moisture on GHG emissions from excreta deposited on grassland?

The results of this study will help provide more insight to the environmental implications of some of the production practices of the Canadian beef industry, and aid in our understanding of the generation of soil GHG emission from excreta deposition on grassland in early spring.

3.3 Materials and Methods

3.3.1. Excreta Collection

Feces and urine used in the field trial were collected during the diet trial described in Chapter 2. In brief, fresh feces and urine were collected individually from 24 mature

beef cows from three diet treatment groups. Diets all contained a baseline low-quality forage (6% crude protein (CP)) and were supplemented with either no DDGS (0% DDGS), 10% DDGS ww^{-1} or 20% DDGS ww^{-1}. The 0% diet was considered to be N deficient to meet animal requirements for N, the 10% diet borderline sufficient N (8.7% CP), and the 20% diet excessive in N (11.5% CP). Excreta was collected frequently and refrigerated over an 8 hour period, and frozen at the end of the collection period until analysis or use in the field study. Composite mixtures of equal proportions of feces or urine from cows within each diet were composed for use in the field study.

In order to make a comparison between a typical drylot feeding system and extended season grazing system, a simulated bedding pack treatment was devised. This simulated bedding pack represented gas emissions that may occur from conventional drylot housing practice in Manitoba or a bedding area on pasture during an extended winter grazing period. The simulated bedding pack was composed of a mixture of urine and feces from a given diet, added according to the ratio of feces to urine produced by the cows, and combined with barley straw to achieve 23% dry matter (DM) (Table 3.3). The barley straw was composed of 41% carbon, 53% acid detergent fibre, 84% neutral detergent fibre (DM basis) with DM determined to be 95%. Average DM content of manure samples in Manitoba is 27.9% (Government of Manitoba 2008). However, this DM content resulted in too much heterogeneity when composing bedding pack mixture. As a result, straw content was reduced and simulated bedding pack DM was reduced to 23%. For each diet treatment, 0.5 kg of the simulated bedding pack mixture was packed into the collars.

3.3.2. Site Description and Study Design

The field study took place at the University of Manitoba's the "Point" Field Research Laboratory. The "Point" consists of 60 acres of agriculture land located on an oxbow of the Red River on the University's Fort Gary Campus in the city of Winnipeg, Manitoba. The area receives an average of 416 mm of precipitation annually. The soil is a lacustrine fine clay, and is part of the Red River association known as St. Norbert Clay. The soil is characterized as a clay loam with 33% clay, 40% sand and 27% silt.

The plot area was grassland that bordered the agricultural fields at the "Point". Although the area was once seeded to tame forages, it consisted mainly of *Poa pratensis*, *Elymus repens*, *Festuca* spp., sedges, and *Trifolium hybrid*; typical species found in naturalized grassland pastures used for cattle production in the region. There was no history of manure or fertilizer applications in the past 10 years and the only maintenance the stand had received was annual mowing. Soil was characterized as follows: bulk density was determined to be 1.2 Mg m^{-3} with 4.8% carbonate, bicarbonate soil test phosphorus at 36 ppm, total C of 4%, and nitrate-N of 8 ppm.

The study design was a randomized complete block with 6 replicate blocks. Each rectangular block was 3 meters wide by 4 meters in length and contained 10 static vented chambers, with 1 meter between chambers in all directions. Blocks were placed to allow 2 meters between blocks in all directions. Each block contained 9- treatment by diet combinations plus one control (no excreta addition) to monitor background gas emissions. Treatments consisted of patches of feces, urine, and a simulated bedding pack mixture, with each patch type containing excreta from the three diets (0% DDGS, 10% DDGS, or 20% DDGS). To simulate cows defecating or urinating on pasture, 1 kg of

feces or 1 L of urine composite were placed into static vented chamber collars (Tremorin 2008), while 0.5 kg of simulated bedding pack was placed into collars. Nitrogen added in urine and fecal patches are given in Tables 3.1 and 3.2.

Excreta treatments were placed on the grassland on April 21, 2009 (DOY 111), following snow melt. Soil temperature measurements taken at 15 cm depth revealed that the soil at 15 cm was still frozen.

Table 3.1. Forms and quantity of N in urine added to soil.

Diet	N_{Total}	N_{NH4+}	N_U	N_{Org} †
	g m^{-2}			
0% DDGS	102.3	4.1	28.6	69.6
10% DDGS	209.0	10.5	81.5	117.0
20% DDGS	259.6	15.6	140.2	103.8

N_{Total} = Total N; N_{NH4+} = Ammonium N; N_{Org} = Organic N; N_U = Urine Urea N.
†Organic N in urine does not include urea-N.

Table 3.2. Moisture and forms and quantity of N in feces added to soil.

Diet	Moisture	N_{Total}	N_{NH4+}	N_{Org} †
	%		g m^{-2}	
0% DDGS	83	46.2	3.0	41.7
10% DDGS	85	54.2	3.8	50.0
20% DDGS	80	73.8	4.6	66.9

N_{Total} = Total N; N_{NH4+} = Ammonium N; N_{Org} = Organic N; N_{Urea} = Urine Urea N.
†Organic N in feces includes urea-N

Table 3.3. Composition of simulated bedding pack, composed from a mixture of feces and urine, added according to the ratio of kg feces to L urine produced by beef cows, and straw[†].

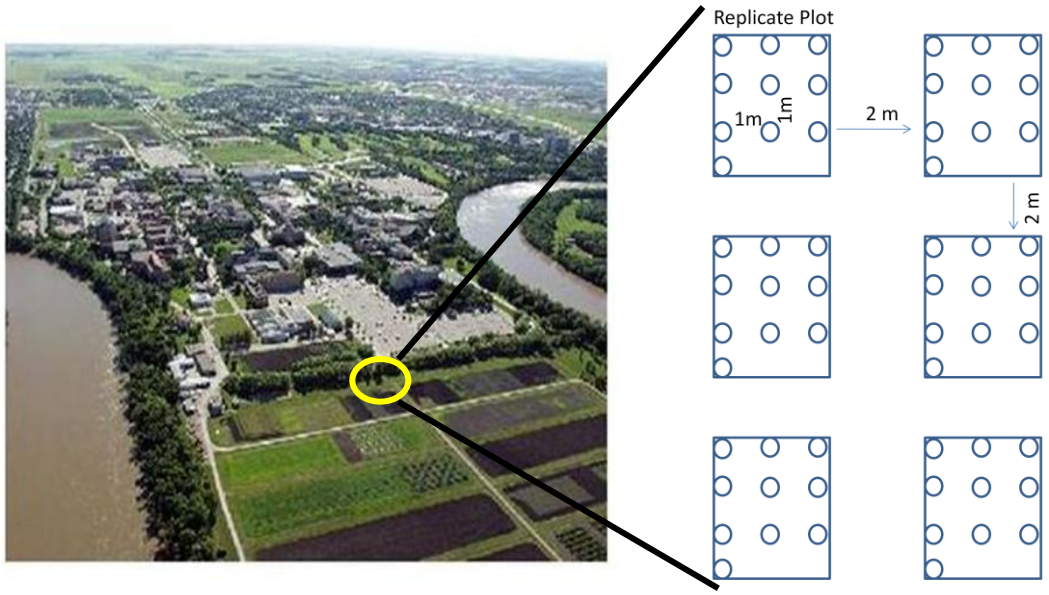
Diet	Bedding Pack Component					
	Straw[†]	Feces and Urine				
		Ratio	N_{Total}	N_{NH4+}	N_{Org}[‡]	N_{Urea}
	g m^{-2}	g feces: L urine	g m^{-2}			
0% DDGS						
Total	1902	4	31.3	1.7	22.0	7.6
Feces			20.2	1.3	18.9	ND
Urine			11.1	0.4	3.1	7.6
10% DDGS						
Total	2079	4	44.7	2.5	30.1	12.1
Feces			23.2	1.6	21.6	ND
Urine			21.5	0.9	8.5	12.1
20% DDGS						
Total	1953	3	76.2	3.7	54.5	18.0
Feces			31.1	1.9	30.2	ND
Urine			45.1	1.8	24.3	18.0

ND = not determined; N_{Total} = Total N; N_{NH4+} = Ammonium N; N_{Org} = Organic N; N_{Urea} = Urine Urea N.

[†] Straw added to achieve 23% dry matter.

[‡] Organic N in feces includes urea-N; Organic N in urine does not include urea-N.

Fig. 3.1. Location of the study site and randomize complete block design at the "Point" Field Research Laboratory on the University of Manitoba campus.



3.3.4. Measurements

3.3.4.1. Gas Sampling

Static vented chambers were used to measure GHG emissions from beef cattle excreta applied to the grassland. The chambers were made of two pieces, a collar and a lid, made from PVC pipe (Hutchingson and Livington 2006). Both the collar and lid had an inner diameter (i.d.) of 23 cm and were 10 cm in height. The collars had one bevelled edge which was pounded into the ground 2 cm on April 20, 2009. Chamber lids were placed on collars only during gas sampling. For more detailed methodology on construction of lids see Tremorin (2008).

Gas sampling occurred on the day the treatments were deposited (April 21, DOY 111) and then at intervals of 3 sample per week for the first 6 weeks of the study, weather permitting. Sampling was then reduced to 2 times per week, weather permitting, for the remainder of the study (DOY 258). Gases were sampled between the hours of 0900 and

1200. Gas samples were collected from a static vented chamber following capping and then at intervals of 15 minutes until 4 samples had been collected from the chamber, and average flux over 45 minutes was determined. Gas samples were obtained using a 20 mL syringe (Becton-Dickinson) with a 23-gauge luer-lock needle through the rubber septum in the lids. Exetainer vials (Labco, UK) were used to store gas samples. Prior to their use, Exetainer vials were sealed with silicone and then flushed with helium three times and evacuated to 500 mTorr. As a check of the vial handling and gas storage procedures, six vials of 20 mL samples of two standard gas mixtures (N_2O, CH_4 and CO_2) were put into evacuated Exetainers and handled in the same way as the other gas samples on every day of gas sampling. Soil temperature (0-5 cm), using hand held thermometers, and volumetric soil moisture, using a theta probe, were measured at each gas sampling period as well.

3.3.4.2 Soil Sampling

In order to monitor soil N transformations throughout the study period, additional patches were also deposited in each plot for the purpose of soil sampling. Due to a shortage of fresh urine collected over the feces and urine sampling period, full replicates of urine patches for soil sampling could not be conducted. Instead, 0.5 L of urine, or 0.5 kg of feces or simulated bedding pack, was placed around the outside of their respective gas sampling patches, creating enlarged patches. Soil samples to monitor for N transformations were then taken weekly, weather permitting, from the area directly around the outside of the gas sampling patches so as not to disturb gas sampling treatments. A push probe was used to sample the top 5 cm of soil from the area directly

around each patch. Feces and simulated bedding pack material collected from the surface of the soil in these samples were separated from soil samples, and all materials collected were frozen (-20^{o}C) until analysis.

3.3.4.3. Meteorological Data

The "Point" Weather Station was located <100 m from the plot area. Average daily temperature and total daily precipitation was monitored using a 21x data logger (Campbell Scientific, Canada) from dual, stand alone air temperature sensors (Campbell Scientific, Canada) and tipping bucket rain gauges with 0.25 mm tips (Campbell Scientific, Canada).

3.3.5 Analysis

3.3.5.1. Nutrient Analysis

To determine soil N concentrations of NH_4^+, NO_3^- and NO_2^- the method of Keeney and Nelson (1982) was followed. Moist soil samples were mixed and 5 g sub-samples were shaken in 50 mL conical polypropylene tubes (Fisherbrand) for 30 minutes at 120 oscillations per minute on a reciprocating shaker with 25 mL of 0.5M K_2SO_4 and the suspensions were centrifuged for 1.5 min at 1350 x *g*. Fifteen mL of clear supernatant were removed and placed into scintillation vials and refrigerated. All samples were analyzed within 24 hours of extraction on a Technicon II Auto-analyser (Pulse Instruments, Saskatoon, SK) for. Gravimetric moisture content (GMC) was determined by drying 10-g sub-samples at 105°C for 24 hours.

Composite fecal, urine, and bedding pack samples were analysed for nitrogen concentration as described in Chapter 2, prior to application in plots.

3.3.5.2. Flux Determination

Gas samples contained in Exetainer vials were analyzed for the greenhouse gases N_2O, CH_4, and CO_2 on a Varian gas chromatograph (GP3800; Varian Canada, Mississauga, ON) fitted with an electron capture detector, flame ionization detector, and an automatic sampler (Combi-PAL; CTC Analytics, Zwingen, Switzerland). Gas standards, containing mixtures of N_2O, CH_4 and CO_2 (Welders Supplies, Winnipeg, MB) were included with each set of samples analyzed to calibrate fluxes. Fluxes were calculated using the following formula (Hutchinson and Livingston, 1993):

$$F = \frac{dC}{dT} x \frac{V}{A} x \frac{M}{V_{mol}} \quad (1)$$

where F is the rate of gas emission (μg N or C m^{-2} min^{-1}), $\frac{dC}{dT}$ is the linear rate of change of chamber gas concentration (mol mol^{-1} min^{-1}), V is the chamber headspace volume (m^3), *A* is the surface area of the chamber (m^2), M is the molecular weight of the gas and V_{mol} is the volume of a mole of the gas (m^3 mol^{-1}) at the air temperature during the time of sampling. Chamber head space was corrected for the volume of feces and simulated bedding pack.

Cumulative emissions were calculated using linear interpolation (Pennock et al. 2006). Background (control) emissions were subtracted from those of treatment patches so that emissions presented may be attributed to the treatment imposed.

3.3.6. Statistics

The Statistical Analysis Software (SAS) version 9.2 (SAS Institute Inc. 2000) was used to perform all statistical analyses. Proc Mixed was used to perform a 2-way analysis of variance on cumulative emissions of N_2O and CH_4, between patch type and diet, and considering patch type*diet interactions. Tukey's test for multiple comparison of means was used to determine significant differences at $P \leq 0.05$.

Spearman rank correlation analysis was used to determine associations between gas emission and measured soil parameters. Only days when both soil and gas samples were taken were used. Fluxes did not have background emission subtracted; however, background gas and soil samples were used in the analysis of all the treatments. A factor of soil temperature by soil moisture was also used in the correlation analysis, called the temperature moisture factor (TMF) (Akinremi et al. 1999).

3.4 Results

3.4.1. Meteorological conditions

Monthly temperature and precipitation over the sampling period and the long-term (29-year) area averages are given in Table 3.4. With the exception of the months of April and September, all 2009 mean monthly temperatures during the sampling period were below the long-term average. May, in particular, was 3^{o}C cooler, lower than the long-term average. Total precipitation in the spring months of April and May was also below average, at 20 and 64 mm compared to the long-term averages of 59 and 90 mm, respectively. However, precipitation from June through September was above average,

with the month of July and August being particularly wet, receiving 111 and 92 mm compared to the long-term averages of 75 and 52 mm, respectively.

Table 3.4. Mean monthly air temperature and total precipitation at the study site in 2009 during the months of April through September compared to the climate normal data for the area (1971-2000).

Month	2009		Climate Normal†	
	Air Temp	Total Precip	Air Temp	Total Precip
	^{o}C	mm	^{o}C	mm
April	4	20	4	59
May	9	64	12	90
June	16	88	17	71
July	18	111	20	75
August	18	92	19	52
September	18	44	12	31
Mean	12	70	14	63

† Climate normal (1971-2000) data was obtained from Environment Canada (2010) for the Winnipeg International Airport, Winnipeg, Manitoba, Canada.

3.4.2 Nitrous Oxide Emissions

Figure 3.2 reveals the timing and magnitude of N_2O emission from the different patch types throughout the sampling period and complements the ANOVA analysis. Peak N_2O emission from the urine patches did not occur immediately following application, but occurred instead in mid-summer, during the growing season. Beginning on day of year (DOY) 160 (June 8), all three urine diet treatments showed an increased flux of N_2O. The 20% DDGS urine patches had N_2O fluxes that increased to 1145, 1646, and 1640 µg N m^{-2} h^{-1} on DOY 175, 181, and 191, respectively, while the 10% DDGS urine patches reached average peaks fluxes of 811 and 805 µg N m^{-2} h^{-1} on DOY 175 and 191, respectively, dropping down to 495 µg N m^{-2} h^{-1} on day 181. Mean N_2O fluxes from the

10% and 20% DDGS urine patches returned to near control values by DOY 225 (August 12). The 0% urine patches also had a mid-summer peak which occurred on DOY 166, reaching an average flux of 339 µg N m^{-2} h^{-1}, but fluxes quickly returned to control values by DOY 175. The 0% urine patches reached similar N_2O emission levels following application early in growing season as well, with fluxes of 351 and 298 µg N m^{-2} h^{-1} occurring on DOY 124 and 126 (May 3 and May 5). Maximum mean fluxes from 10% and 20% DDGS urine patches following application in spring occurred on day 124 for 10% DDGS, at 275 µg N m^{-2} h^{-1}, and on DOY 111 for 20% DDGS, at 325 µg N m^{-2} h^{-1}.

Fecal and bedding pack patches did not have a mid-summer peak of N_2O similar to that from urine patches, with the exception of the 0% bedding pack patches which had a similar flux pattern to the 0% urine patches. Fecal and bedding patches produced peak N_2O flux occur by DOY 126, or within the first 2 weeks of application of treatments to the grassland. Mean peak N_2O flux from background (control) patches occurred on DOY 126, reaching 154 µg N m^{-2} h^{-1}.

Cumulative N_2O emission from urine patches was significantly greater than that from feces or bedding pack treatments and these urine patches also had significantly greater N_2O emission from urine from cattle receiving DDGS supplementation (Table 3.5). There were no differences in cumulative N_2O emission between the fecal or bedding pack patches or from the diet treatments within the feces and bedding pack patches (Table 3.5). Analysis of variance revealed that there was a significant patch type by diet interaction controlling the emission of N_2O from the treatments. This appears to be the result of increasing N_2O emission with DDGS supplementation in the urine patches

combined with the decreasing emission of N_2O with DDGS supplementation in the bedding pack patches and no effect of diet in the feces patches. Due to the high flux of N_2O from urine patches, however, the 20% DDGS diet was still considered to be a significantly greater emitter of N_2O despite the fact that it was not the highest emitter in the bedding pack or feces patches.

To determine the amount of N applied with each treatment that evolved as N_2O, N emission factors were also determined. Again, analysis of variance revealed that a significant patch type by diet interaction was controlling the percentage of applied N lost as N_2O. Unlike the cumulative N_2O emission results, however, N emission factors from the diets within the urine patches were not significantly different. Again, the same patch type by diet interaction occurred, with urine patches having increased N emission factors with the addition of DDGS supplementation and bedding pack patches having decreasing emission factors with DDGS supplementation, and no effect of supplementation on emissions from fecal patches.

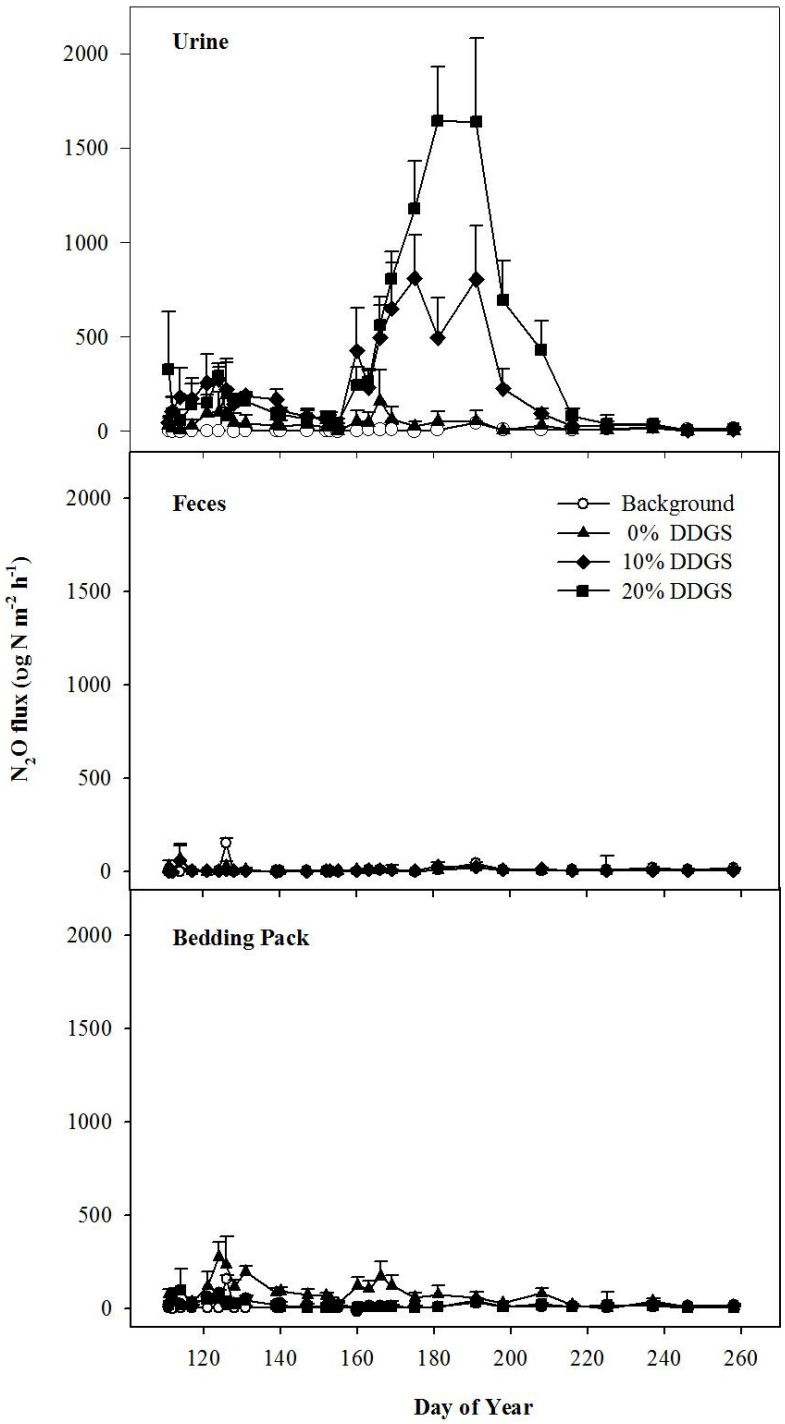


Figure 3.2. N_2O flux from patches of urine, feces, and simulated bedding pack, from three diet treatment groups, and grassland background emission, deposited on grassland in April 2009 and measured until September 2009. Average values (n=6) +1 standard error of the average is shown as bars.

Table 3.5. Means[†] of cumulative N_2O emission above background levels and associated N_2O emission factors for patches of urine, feces, and simulated bedding pack from three diet treatments deposited on grassland in April 2009 and gas emissions measured until September 2009.

Effect		Cumulative N_2O[‡]	Emission Factor[‡]
		mg N_2O-N m^{-2}	% N added evolved as N_2O[§]
Patch x Diet			
Urine	0%	267 c (123)	0.26 ab (0.12)
	10%	730 b (310)	0.35 ab (0.15)
	20%	1 258 a (470)	0.49 a (0.18)
Feces	0%	-25 c (13)	-0.03 c (0.03)
	10%	-16 c (18)	-0.03 c (0.03)
	20%	-24 c (15)	-0.05 c (0.02)
BeddgP	0%	46 c (74)	0.15 bc (0.24)
	10%	11 c (25)	0.02 c (0.06)
	20%	2 c (21)	0.003 c (0.03)
Patch			
Urine		752	0.37
Feces		-22	-0.04
Bedding Pack		20	0.06
Diet			
0%		96	0.12
10%		241	0.12
20%		412	0.15
ANOVA			
Patch		<0.0001	<0.0001
Diet		0.0244	NS
Patch x Diet		<0.0001	0.0247

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA with 6 replicates; 3 patch types, 3 diets, and 1 control randomly placed in 6 replicate blocks. Mean control cumulative emissions have been subtracted from each patch type and diet combination. Multiple comparison of means were performed on log transformed data, actual means are shown.

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P < 0.05$ by Tukey's test.

[§] Emission factor calculated as g of N added per treatment divided by the g N lost as N_2O x 100.

3.4.3 Methane Emissions

No significant differences were found in the CH_4 emissions from the different patch types or due to diet (Table 3.6). Appendix D also shows that CH_4 flux was quite variable throughout the sampling period, with patches acting as both sinks and sources at times. Overall, the three patch types were sources of methane.

Table 3.6. Means[†] of cumulative CH_4 emission above background levels for patches of urine, feces, and simulated bedding pack from three diet treatments deposited on grassland in April 2009 and gas emissions measured until September 2009.

Effect	Cumulative CH_4[‡]
	mg CH_4-C m^{-2}
Patch	
Urine	92
Feces	76
Bedding Pack	62
Diet	
0% DDGS	57
10% DDGS	101
20% DDGS	71
ANOVA	
Patch	NS
Diet	NS
Patch x Diet	NS

[†] Means computed using Proc Mixed (SAS version 9.2) for two-way ANOVA with 6 replicates; 3 treatments, 3 diets, and 1 control randomly placed in 6 replicate plots. Mean control cumulative emissions have been subtracted from each treatment x diet combination.

[‡] Means within the same column followed by the same lower case letter are not significantly different at $P \leq 0.05$ by Bonferroni test.

3.4.5. Spearman Rank Correlation Coefficients

Spearman rank correlations, given in Table 3.7, were low but significant, and determined that soil NO_3^--N concentration was the most significant parameter related to N_2O flux from all types of patches (including background patches; N=615) over the sampling period, at 0.23 (P<0.0001; Table 3.7). Carbon dioxide flux was also highly correlated to N_2O fluxes, following closely at 0.20 (P <0.0001). Other significantly correlated parameters included soil NH_4^+-N, soil NO_2^--N, soil temperature, and TMF. Scatter plots of the correlation analysis are shown in Appendix F.

Spearman rank correlation coefficients for individual types of patches over the sampling period (N=184) revealed that none of the parameters were significantly correlated with urine patch N_2O emission. However, N_2O emissions from fecal patches were found to be correlated with CO_2 flux, soil NO_2^--N, soil temperature, and TMF. Emission of N_2O from bedding pack patches were correlated with soil temperature and TMF, along with soil NO_3^--N.

In an attempt to further investigate parameters correlated with urine patch N_2O emission, the sampling period was further divided into sampling periods that occurred early in the season (following application of treatments but prior to grass growth; days 111 through days 152) and sampling that occurred through the remainder of the growing season (days 152 to 258). These periods are termed, respectively, Early Season and Growing Season throughout the remainder of the chapter.

Early Season correlations revealed that neither soil NH_4^+-N, soil temperature, GMC or TMF were related to N_2O emissions from any of the individual types of patches or from all types of patches combined, unlike that observed in the full season correlation.

Combining all the patch types (N=199) resulted in correlations between N_2O emissions and CO_2 flux, NO_3^--N and NO_2^--N. Analysis of individual patch types (N=60) revealed significant correlations between urine patch N_2O flux and soil NO_3^--N and CO_2 flux, at 0.26 (P=0.0444) and 0.27 (P=0.0355), respectively. N_2O emissions from fecal patches early in the season were related to CH_4 flux, CO_2 flux, soil NO_3^--N and soil NO_2^--N, while bedding pack patches were related to CO_2 flux and soil NO_3^--N.

Unlike the Early Season correlations, Growing Season N_2O emission from all patch types combined (N=416) was found to be significantly correlated to CO_2 flux, soil NH_4^+-N, soil temperature (-0.17, P<0.0001) and TMF (-0.13, P = 0.001). However, during the growing season when peak N_2O flux from urine patches occurred, there were again no correlations between N_2O emission from urine patches and measured soil parameters (N=124). Emission of N_2O from fecal patches were found to be correlated with CH_4 and CO_2 flux while bedding pack patches were correlated only with CO_2 flux.

Table 3.7. Spearman rank correlation coefficients for the associations between N_2O emission from urine, feces, bedding pack patches and measured soil variables over the growing season.

Trt[†]	Variable							
	CH_4	CO_2	NH_4^+ N	NO_3^- N	NO_2^- N	GMC[††]	Soil Temp	TMF[‡]
Early Season								
Urine	NS	0.27*	NS	0.26*	NS	NS	NS	NS
Feces	0.26*	0.36**	NS	0.31*	0.44***	NS	NS	NS
BP	NS	0.36**	NS	0.28*	NS	NS	NS	NS
All[§]	NS	0.51***	NS	0.55***	0.15*	NS	NS	NS
Growing Season								
Urine	NS	NS	NS	NS	NS	NS	NS	NS
Feces	0.17*	0.34***	NS	NS	NS	NS	NS	NS
BP	NS	0.20*	NS	NS	NS	NS	NS	NS
All[§]	NS	0.18***	0.19***	NS	NS	NS	-0.17***	-0.13**
Total								
Urine	NS	NS	NS	NS	NS	NS	NS	NS
Feces	NS	0.40***	NS	NS	0.27***	0.20**	NS	0.22**
BP	NS	NS	NS	0.15*	NS	-0.21**	NS	-0.17*
All[§]	NS	0.20***	0.18***	0.23***	0.09*	NS	-0.11**	-0.08*

*, **, *** Indicate if the correlation is significant at $P<0.05$, $P<0.01$ or $P<0.001$, respectively; NS represents pairs of variables that are not significantly correlated ($P>0.05$).

[†] Treatment emission of N_2O over the total sampling period (total) and divided into emissions produced during periods of no grass growth (Early Season) and Growing Season. Only sampling dates where soil conditions and gas fluxes were measured on the same day were used in the analysis.

[††] Gravimetric soil moisture content.

[‡] Factor of soil temperature multiplied by GMC.

[§] Correlation determined using all N_2O emission from all treatments including background N_2O emissions.

3.4.6. Soil Conditions

Application of urine to soil immediately increased average soil NH_4^+-N and NO_2^--N concentrations (Fig. 3.3). Peak soil NH_4^+-N concentrations occurred on DOY 112, at 91, 761, 273 mg kg^{-1} dry soil for 0%, 10% and 20% DDGS diets, respectively. Peak soil NO_2^--N concentrations occurred on DOY 112 for 0% and 20% DDGS diets at 6 and 12 mg kg^{-1} dry soil, and DOY 114 for 10% DDGS at 13 mg kg^{-1} dry soil. Urine patch soil NO_3^--N peaked on DOY 133, approximately 3 weeks following application of urine, reaching concentrations of 50, 79, 60 mg kg^{-1} dry soil for 0%, 10% and 20% DDGS diet , respectively. Soil NO_3^--N and NO_2^--N for 10% and 20% DDGS urine patches also spiked relative to the control midseason. Beginning on DOY 175, soil NO_3^--N and NO_2^--N increased, reaching concentrations of 30 and 4 mg kg^{-1} dry soil by DOY 180, respectively, for the 20% diet, and returned to control levels by DOY 210. The 10% diet reached similar concentrations of 25 and 3.8 mg kg^{-1} dry soil for NO_3^--N and NO_2^--N on DOY 180.

Soil moisture for urine patches was highest following application of urine, with a decreasing trend over the study period. During days 181 to 196, soil moisture increased notably but did not reach Early Season peak levels. Soil temperature (0-5cm) had an increasing trend over the growing season, peaking at 23^{o}C on DOY 225. Patch type did not affect soil temperature or moisture over the study period.

Mean and minimum air temperatures were cool and variable over the first month of the study, with minimum temperatures ranging between -5 and 9^{o}C, and mean daily air temperatures ranging between 0 and 13^{o}C (Fig. 3.4). Average daily air temperature began increasing on DOY 160, and average daily temperature ranges were between 10 and 25^{o}C

for the remainder of the sampling period. Several very large precipitation events occurred throughout the study period of greater than 40 mm, on days 135, 179, 192.

Lush plant growth in and around all treatments, extending laterally much further than the application area, was noted on DOY 150. Grass growth protruded from underneath the fecal and bedding pack patches as well. The 10 and 20% DDGS urine treatments, however, resulted in burning of the grass inside the collars, and little to no plant growth in the center of the collars occurred for the entire study period.

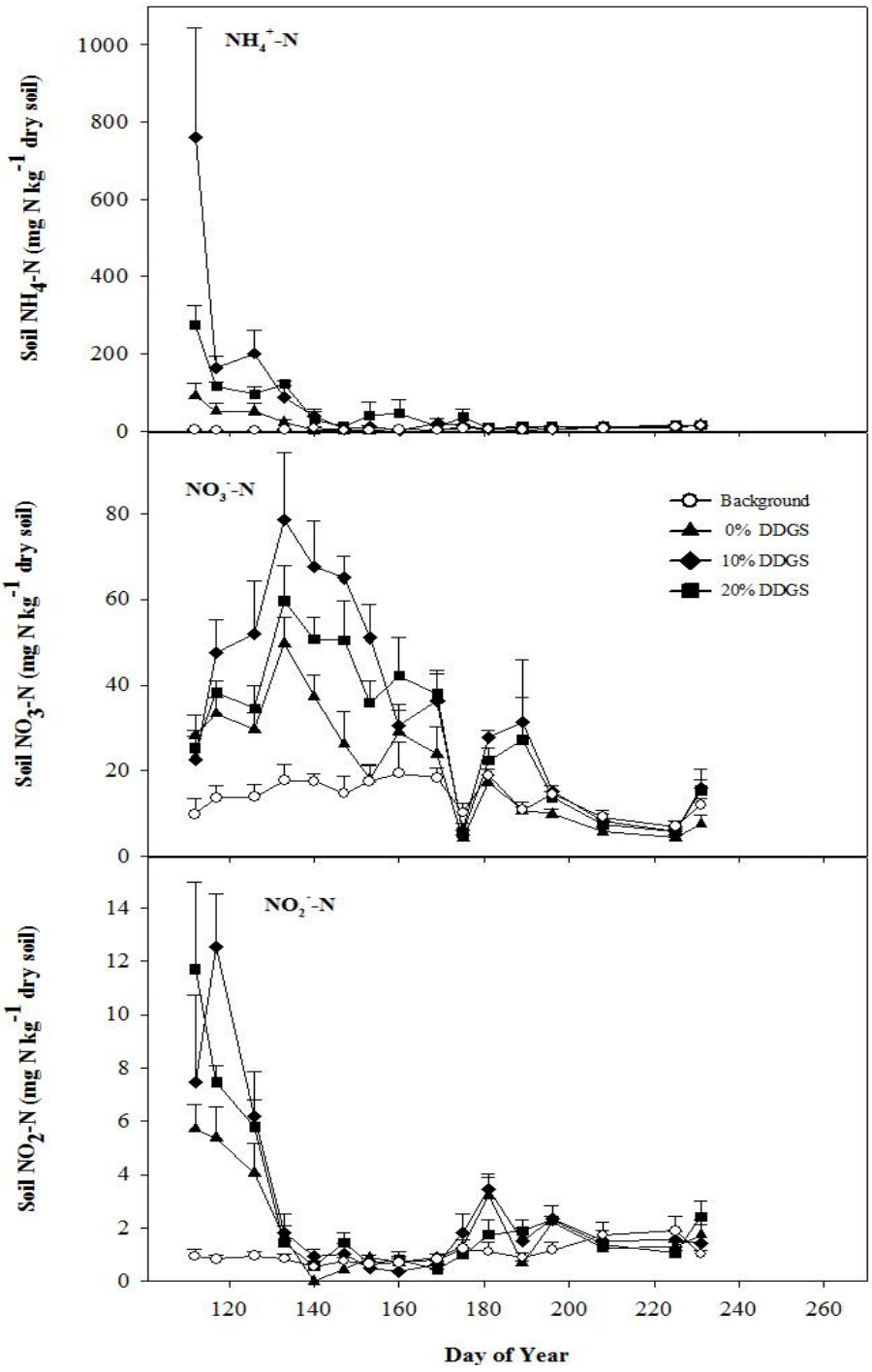


Figure 3.3. Urine patch soil NH_4^+-N, NO_3^--N, and NO_2^--N, mean temperature and precipitation events for urine, feces, bedding pack and background patches during 2009 sampling period. Average values (n=6) +1 standard error of the average is shown as bars.

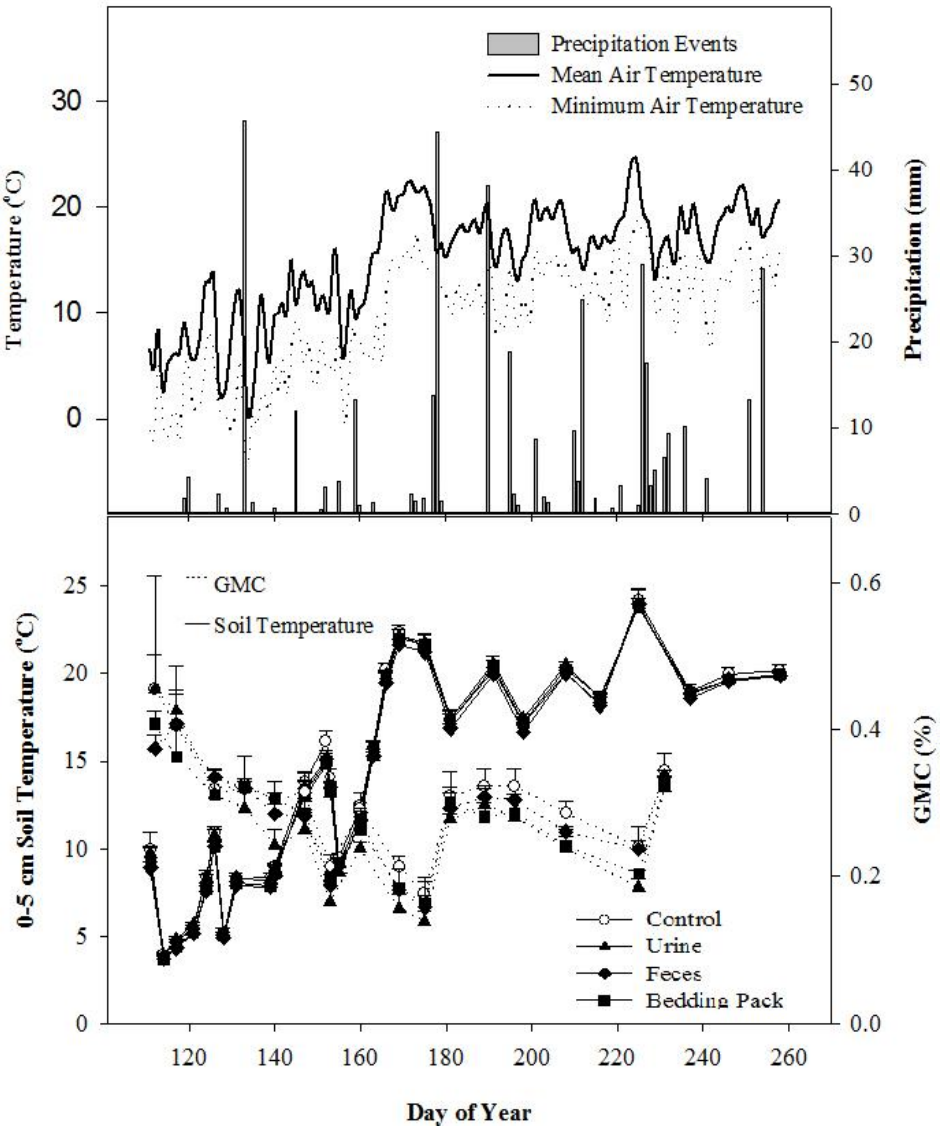


Figure 3.4. Mean temperature and total daily precipitation and mean 0-5 cm soil temperature and gravimetric moisture content (GMC) for urine, feces, bedding pack and background patches during 2009 sampling period. Average values (n=6) +1 standard error of the average is shown as bars.

3.4.7. Carbon Dioxide Emission

For urine, feces, and bedding pack patches, CO_2 emission increased immediately following application. For the urine and bedding pack patches, this increase was comparable to the peak fluxes that occurred in late-summer, ranging between 300,000 and 500,000 µg C m^{-2} h^{-1}. Fecal patches had a small increase in CO_2 emission following application, with 10% and 20% diets reaching near 220,000 µg C m^{-2} h^{-1}, but did not reach peak emissions as great as for the other two treatments. Following this burst in emission, CO_2 fluxes quickly stabilized and remained relatively constant, similar to background levels throughout the growing season, with no significant difference due to diet (Fig. 3.5). Appendix E helps to further demonstrate this point, and shows that once background emissions were taken into account, CO_2 emission from urine, feces and bedding pack remained relatively low and constant throughout the sampling period. Carbon dioxide emission increased steadily throughout the growing season, with the peak for all treatments and the background patches on DOY 225.

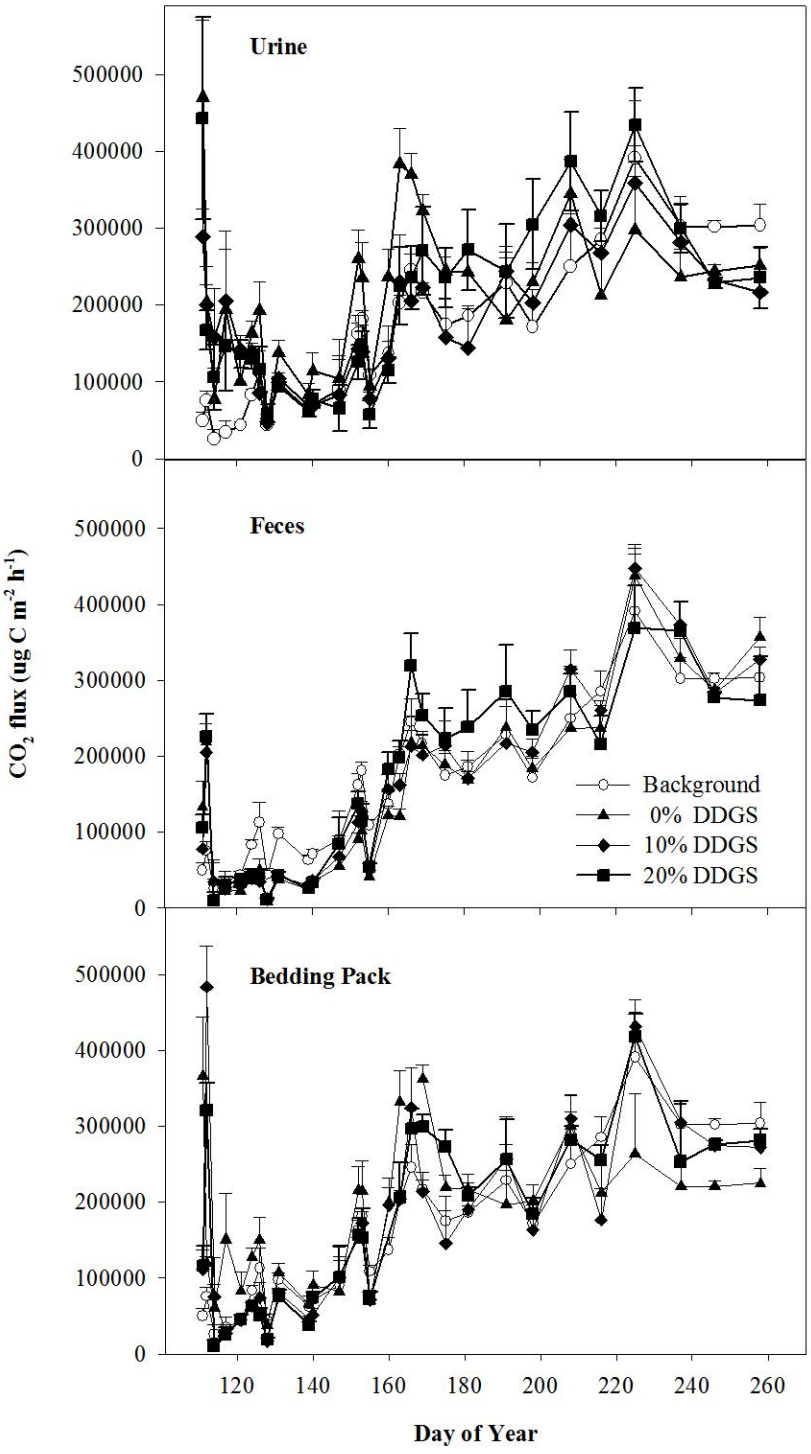


Figure 3.5. CO_2 flux from patches of urine, feces, and simulated bedding pack, from three diet treatment groups, and grassland background emission, deposited on grassland in April 2009 and measured until September 2009. Average values (n=6) +1 standard error of the average is shown as bars.

3.5 Discussion

3.5.1. Limitations

It is important to note that due to the methodology, the soil data presented can only be used to estimate the soil N transformations throughout the sampling period, as the soil samples used in the analyses were not taken from inside the collars where the gas measurements were taken. Due to the lack of available fresh urine, the application of treatments around the outside edge of the collar, to form an enlarged patch, was the only option available to make the best use of limited resources. The soil analyses for these treatments may not be a good representation of what was happening inside the collars as a result. Incidentally, there were no significant gas fluxes from the latter two treatments, so the relationships between soil N transformations and N_2O flux from the fecal and bedding pack patches will not be discussed in detail. It is also important to note that soil sampling occurred weekly and did not always occur on days of gas sampling. Therefore, some daily variations in soil moisture due to precipitation events may have been missed from the dataset. Some days of significant N_2O or CH_4 emission may not have been included in the spearman rank correlation analysis as well because of the lack of accompanying soil data for that particular day.

3.5.2. N_2O Emission

The main source of N_2O release from soil is through the linked microbial processes of nitrification and denitrification. Both processes are responsible for N_2O emission from excreta deposited on grassland, with no agreement on which process is the

most important (Bol et al. 2004; van Groenigen et al. 2005a; Carter et al. 2007; Luo et al. 2008). This is likely due to the fact that a combination of many factors contribute to the emission of N_2O from each of these processes including N forms and concentrations in excreta, soil texture, soil moisture, soil temperature, dissolved organic carbon (DOC), plant growth and uptake of N, soil compaction, and microbial community health and composition (Oenema et al. 1997; Bol et al. 2004; van Groenigen et al. 2005a; Carter et al. 2007; Luo et al. 2008; Orwin et al. 2010). Therefore, the proportion of N_2O generated from nitrification relative to denitrification is highly variable. The main source of Early Season N_2O flux from urine patches appears to have been nitrification, while denitrification is more likely to have been the dominant process governing N_2O emission during the Growing Season mid-summer peak.

3.5.2.1. Early Season N_2O Emission from Urine Patches

3.5.2.1.1. Soil Available-N and Soil Texture

The addition of urine to the grassland immediately increased the concentration of soil available-N above background concentrations, and this seems to have been the limiting factor controlling N_2O emission. The peak N_2O fluxes from urine during the Early Season occurred as soil NH_4^+-N and NO_2^--N were decreasing and NO_3^--N was increasing, through the process of nitrification. The small, yet significant, positive Early Season spearman rank correlation found between NO_3^--N and N_2O flux reveals that nitrification and its product NO_3^--N were important factors contributing to N_2O flux following urine application (Table 3.7). Soil NO_3^--N concentration has been found by

others to be an important factor controlling N_2O emission from urine patches as well. It serves both as a product of nitrification and a substrate necessary for denitrification (Luo et al. 2008; Di et al. 2010; Arriaga et al. 2010).

The majority of the N found in the urine was in the form of urea (Table 3.1) and the high NH_4^+-N concentrations found in the urine patches during first two weeks of the sampling period are likely the result of urea hydrolysis. This is also indicated by the high flux of CO_2 in urine patches during the first two weeks of the experiment (Fig. 3.5). These increased CO_2 fluxes are also seen, although to a lesser extent, in the bedding pack patches, as urine was included in the mixture of feces and straw (Table 3.3). Urea hydrolysis is rapid and has been found to be able to completely hydrolyse in 24 hours, depending on soil temperature and moisture, leading to increased CO_2 emission as microorganisms respire and as CO_2 is released directly during hydrolysis (Bolan et al. 2004). The presence of hippuric acid, a nitrogenous compound of cattle urine, is also known to speed up hydrolysis (Whitehead et al. 1989). Carbon dioxide emission (Fig. 3.4) and high NH_4^+-N concentrations in the first two weeks following application indicated that urea hydrolysis occurred rapidly, despite the low mean air and soil temperatures. Sherlock and Goh (1984) determined the half life of urea hydrolysis was 4.7 hours at mean air temperatures of 8.3^{o}C. These low temperatures during the first few weeks of the experiment would have slowed ammonia volatilization losses from the newly formed NH_4^+-N. At average daily temperatures of 8^{o}C it is predicted that ammonia volatilization losses will only be in the range of 10% of applied urine N (Bolan et al. 2004). This would result in a greater opportunity for N available in the soil to be lost as N_2O, leaching or runoff, or for plant uptake.

The rate of N applied within urine patches ranged from 327-920 kg available N ha^{-1} from the 0% to 20% DDGS diets, much higher than crop requirements for grassland. A maximum of 168 kg N ha^{-1} is recommended by the Manitoba Soil Fertility Guide (MAFRI 2007). Even after considering volatilization losses, this leaves much N available for loss to the environment, particularly following application in April. Significant plant growth around the collars was not noted until day 150, or May 30th, so until this point all of the N applied would be at risk for loss to the environment. The below average temperatures and precipitation in the spring would have decreased the amount of N lost through leaching and runoff in this particular year, however, and the medium textured soil would also reduce leaching losses following application. If this trial had been performed on coarse textured soil, it is likely that we would have seen lower emissions from the urine patches due to greater leaching losses and lower soil moisture holding capacity (Wachendorf et al. 2008), although a coarse textured soil would have warmed faster in the spring.

3.5.2.1.2. Soil Moisture

Soil moisture is another factor that has been found to be important in controlling rates of N_2O emission from nitrification and denitrification following the addition of N to soil. Interestingly, soil moisture was highest following application of treatments and decreased as N_2O flux increased until DOY 124 (Fig. 3.3). Water filled pore space (WFPS) was calculated (Luo et al. 2008) and found to be in the range of 75% on DOY 111, dropping down to 55% by DOY 124. Despite this high soil moisture content

following urine application, nitrification did not seem to be inhibited, as was demonstrated by the increasing soil NO_3^--N and decreasing NH_4^+-N concentrations. Nitrification has been found to be inhibited by high soil moisture (>80% WFPS; Zaman et al. 2007), as oxygen becomes limited to nitrifying microorganisms, while denitrification is promoted by increasing anaerobic conditions. At WFPS between 60% and 80%, denitrification is the dominant soil process contributing to N_2O emission, while nitrification is responsible for the majority of N_2O emission at WFPS between 30% and 60% (Arriaga et al. 2010). The WFPS estimated in this study would then suggest that denitrification is responsible for N_2O emission following application. However, as the increasing soil NO_3^--N and decreasing NH_4^+-N concentrations indicate, nitrification was still occurring at this point as well, supplying the substrate for denitrification, and could also be responsible for the Early Season N_2O emission.

3.5.2.1.3. Nitrification

Despite Early Season rainfall events, including one > 40 mm event on day 136 (Fig. 3.3), the added soil moisture did not seem to trigger increased N_2O emission, as would be suspected if denitrification was producing N_2O. Instead, following this precipitation event N_2O flux began decreasing. Soil NH_4^+-N and NO_3^--N concentrations were also decreasing by this point. Significant plant growth was not noted until day 150, but it is possible that plant uptake was starting to become a factor along with the loss of N through gaseous losses. The fact that moisture from precipitation events did not seem to increase Early Season N_2O flux, and that soil moisture content was decreasing as N_2O

flux increased, indicates that denitrification was at least not the dominate process producing N_2O during this time.

Nitrification has been found by others to be the dominant process resulting in N_2O emission in the first 2-6 weeks following application of urine to soil (Bol et al. 2004; Carter et al. 2007; Luo et al. 2008; Kool et al. 2006; Koops et al. 1997). Koops et al. (1997) found that initial N_2O flux from urine patches was the result of nitrification following deposition on dry top soil. Bol et al. (2004) also determined that WFPS was too low to favour denitrification following application of urine on pasture, and concluded that soil conditions at the time of application play an important role in initial N_2O flux. Luo et al. (2008) noted that application of urine to soil that does not increase WFPS >60% will likely result in N_2O production from nitrification as the ratio of N_2O:N_2 produced during nitrification will be high if oxygen concentrations are non-limiting.

However, with the exception of Luo et al. (2008), the amount of urine used in these previously mentioned studies was in the range of 40 to 55 g N m^{-2}, with concentrations in the range of 4 to 11 g N L^{-1} urine. In the present experiment, urine N applied was in the range of 150 to 250 g N m^{-2} with a similar range in N concentration. This means that a much higher volume of urine per unit area was added in the present study compared to the previously mentioned experiments, causing WFPS to increase following urine application more than the previous experiments where nitrification was determined to be the dominant N_2O producing process. The application rate used by Luo et al. (2008) was comparable, however, at 1000 kg N ha^{-1}, but no conclusions could be made as to which process was contributing more to N_2O flux following application, indicating that it may be dependent on the season of application.

3.5.2.1.4. Denitrification

Nitrification and denitrification can take place simultaneously (Arriaga et al. 2010), so it is impossible to rule out the role of denitrification in the Early Season N_2O peak. At intermediate WFPS, both nitrification and denitrification have been found to contribute to N_2O flux from urine patches. Carter et al. (2007) found that nitrification and denitrification contributed equally to N_2O emission from urine applied to soil at an intermediate WFPS of 45%, due to the presence of both aerobic and anaerobic micro-sites. In the present study, the 75% WFPS that resulted following application of urine indicated that denitrification could be taking place, however, nitrification was still active as is indicated by the increasing NO_3^--N concentration. As a large precipitation event seemed to inhibit rather than simulate N_2O flux, it is likely that, if denitrification was occurring during this time, complete reduction of NO_3^--N to N_2 was occurring or that the ratio of N_2O: N_2 produced was low (Carter et al. 2007).

Several factors can affect the ratio of N_2O:N_2 produced during denitrification. Application of urine to soil will markedly increase soil pH in the top 5 cm, and this is known to increase rates of denitrification (van Groenigen et al. 2005b). However, high pH has been found to cause a reduction in the ratio of N_2O:N_2 produced during denitrification, especially if high concentrations of NO_3^--N are present (van Groenigen et al 2005), as they were in this study. Conversely, high soil pH has been found to increase N_2O emissions from nitrification (van Groenigen et al 2005b) despite the fact the nitrification rates may be retarded (Tenuta and Beauchamp 2000).

Another factor affecting N_2O:N_2 ratio of end products of denitrification is the availability of oxidant to denitrifying bacteria. As the availability of oxidant increases, the reduction of NO continues incompletely, increasing the ratio N_2O:N_2 (Carter et al. 2007). These same authors concluded that addition of urine to soil resulted in a high ratio of NO_3^--N: DOC compared to a water treatment, resulting in higher N_2O:N_2 ratios. Although the addition of DOC may decrease N_2O:N_2 ratios, the increased rate of denitrification due to the addition of an easily available C source to denitrifying bacteria may still increase the amount of N_2O formed overall however (van Groenigen et al. 2005a). The addition of feces to urine patches have been noted to cause such effects (van Groenigen et al. 2005a).

In field experiments that have determined denitrification as the dominant soil process causing N_2O flux following application of urine to soil, it is often partially a result of the application of urine increasing soil moisture in the patch area to values of greater than 60% WFPS (Saggar et al. 2004). As WFPS in our experiment was greater than 60% following application of urine, it seems that other factors were limiting N_2O production from denitrification, such as the low soil and average air temperatures or low concentrations of available C, similar to that observed by Luo et al. (2008).

3.5.2.2. Growing Season N_2O Emission from Urine Patches

Growing Season N_2O emission, which included the period of peak N_2O flux from urine patches, appeared to be related more to denitrification than to nitrification soil processes. Very large N_2O fluxes from urine patches have often been attributed to

denitrification in the literature, usually as a response to increased WFPS (Saggar et al. 2004; Luo et al. 2008). Spearman rank correlations found N_2O emission during this period were positively correlated to NH_4^+-N, CO_2 flux, soil temperature, and TMF, with the latter 3 parameters indicating relationships to microbial activity. Peak N_2O flux for 10% and 20% DDGS urine patches occurred on sampling days immediately following the large precipitation events (Fig. 2). The 10% urine patch N_2O flux appeared to be on a declining trend after peaking on DOY 175, but the large precipitation events on DOY 178 and 190 appeared to result in a second N_2O flux peak on DOY 191. The 10% and 20% DDGS diets soil NH_4^+-N concentrations increased just prior to peak N_2O flux, and soil NO_3^--N and NO_2^--N concentrations increased during peak N_2O flux, indicating that organic forms of N were being mineralized. This also indicates that again both nitrification and denitrification processes were taking place, with denitrification being the dominant soil process at this point.

3.5.2.2.1. Temperature

Temperature also appeared to be an important factor influencing N_2O emissions, as Fig. 3.3 demonstrates that the Growing Season N_2O peak seemed to occur as mean air temperature began to increase on DOY158. Up until this point, Early Season mean air temperatures ranged between 0 and 13^oC, with minimum air temperatures reaching below zero several nights. Starting on DOY 158, however, mean air temperatures began to increase and did not drop below 10^oC for the remainder of the sampling period, with mean 0-5 cm soil temperature following the same trend. Coincidently, DOY 158 also marks the start of the large Growing Season mid-summer peak of N_2O.

Dobbie and Smith (2001) also found that increases in temperature were highly correlated to N_2O emission. They determined Q_{10} rates of 8.9 and 2.3 for an increase in temperature of 12-18^oC for arable and grassland soils, respectively. These emissions were determined to be mainly from denitrification at WFPS > 60%. A similar temporal trend in N_2O flux over the experiment, as compared to the present experiment, was also observed. Abdalla et al. (2009) also found a strong correlation with temperature and N_2O emission in an incubation experiment, where Q_{10} values ranging from 4.4 to 6.2 were determined for temperatures between 10 and 25^oC. Fig. 3.4 shows increasing CO_2 emission as temperatures increased over the sampling period, supporting the theory that microbial activity, and therefore rates of denitrification, were increasing over time as the soil warmed.

3.5.2.2.2. Microbial Health

High urine-N application rates have been known to cause microbial stresses that limit nitrification activity and caused death of plant roots (van Groenigen et al. 2005a). The high soil NH_4^+-N concentrations and increased soil pH due to urine application would also result in conditions favourable to ammonia accumulation. The high concentration of NH_4^+-N alone would have resulted in extremely high osmotic pressure. Polonenko et al. (1986) determined that osmotic pressures of -0.5 MPa could inhibit microbial activity, and application of urine at a rate of 40 g N m^{-2} in an experiment by Bol et al. 2004 resulted in osmotic pressures of -0.33 M Pa. The fact that there was no difference in CO_2 flux between treatments throughout most of the sampling period indicates that additional N and increased pH from urine treatments did not inhibit or

stimulate microbial activity compared to background patches, however. Orwin et al. (2010) found that the addition of urine to wet soils resulting in a reduction of microbial community by nearly half, with less negative effects seen when urine was applied to a dry soil. The low temperatures at the time of urine application in the present study may have reduced the negative impact on microbial community compared to that observed in the study by Orwin et al. (2010).

3.5.2.2.3. Mid Season N_2O Peak

The presence of a second and larger flux of N_2O following application of urine to grassland has been seen in other experiments as well, although not always as a result of the same soil properties and seasonal effects. Van Groenigen et al. (2005b), in an experiment using several concentrations of urine N applied in the same volume of urine, found the highest urine-N concentration had a delayed peak N_2O flux, although there was no sound explanation of the occurrence. Compaction and addition of dung to urine patches also resulted in delayed fluxes during a laboratory experiment (van Groenigen et al. 2005a). The addition of dung to the urine patches added a large portion of readily available C which was attributed to the overall higher emissions. Allen et al. (1996) reported a similar temporal trend in N_2O emission from both feces and urine compared to that of the urine patches for this experiment, with excreta applied in the spring and monitored through the summer. At temperatures in the range of 1-10^{o}C, a quick burst of N_2O was seen following the application of treatments. A second larger and longer flux then occurred on days 30 through 60 following warming of soils and several rainfall events.

The timing of application of excreta was an important factor in the temporal trends observed for N_2O emission as well. Earlier application of excreta would have resulted in excreta placed on snow pack or in melt waters and in those conditions it is likely that all of the urine treatments, and a large portion of the feces and bedding pack treatments, would have been lost with snowmelt. In contrast, when feces and urine were deposited on grassland in July in a similar study in Manitoba by Tremorin (2008), a very different temporal pattern in GHG emission was observed. Nitrous oxide emission from treatments in that study peaked within 10-30 days following application, with peak fluxes ranging between 200-400 and 600-1600 µg N_2O-N m^{-2} day^{-1} from fecal and urine patches, respectively. This appears to be the result of application during warmer temperatures later in the growing season.

Other studies have looked at the effect of application date on N_2O emission from fecal and urine patches, although none of the studies took place in a humid continental climate (Anger et al. 2003; van Groenigen et al. 2005b; Luo et al. 2008). Instead, these studies were conducted in areas where average winter temperatures did not drop below freezing and year round grazing could be performed. These studies have found N_2O emission to be greater in seasons receiving high amounts of precipitation, due to the promotion of denitrification (Anger et al. 2003; van Groenigen et al. 2005b; Luo et al. 2008). This is not always during seasons of peak temperature, however, and in fact often includes fall and winter months as cooler temperatures are often associated with higher water filled pore space (WFPS).

The measured soil conditions did not seem to explain entirely the high midseason peak of N_2O from urine patches. Despite the small but significant correlations with

measured soil conditions and fecal and bedding pack patch N_2O flux, no correlations were found with urine patch N_2O flux during the growing season or when the entire sampling period was used in the spearman rank analysis. Soil conditions inside the patches could have been different from outside the patches, where soil measurements were taken, due to the methodology used.

It is interesting to note that despite the fact that warming temperatures and large precipitation events were considered to be two important factors of N_2O emission through the experiment, the TMF used in the Spearman rank correlation analysis did not result in greater or more significant correlations. With the exception of the Total Feces correlation, TMF was less significant and had a lower value compared to the corresponding soil temperature correlation. Also, there were no instances of a significant TMF correlation occurring when both soil temperature and GMC were not found to be significant. Akrinremi et al. (1999) had found that this factor useful for predicting rates of soil respiration in annual cropland.

One soil parameter that was not measured was dissolved organic carbon (DOC), and this may have been a significant factor in the Growing Season mid-summer N_2O peak. Roots from dead and damaged plants can release high amounts of easily available organic C throughout the growing season (Bolan et al. 2004; Shand et al. 2002). This organic C can then be used as a carbon source for denitrifying bacteria, promoting denitrification losses (Carter et al. 2007). In fact, it has been hypothesized that the addition of DOC to urine patches, not the just addition of N, may be the primary stimulus resulting in release of N_2O from urine applied to grasslands (van Groenigen et al. 2005a). The severe death of grass observed in the collars receiving 10% and 20% DDGS urine

treatments was not observed in any of the other treatments, and this could have resulted in a large amount of DOC becoming available over the sampling period. Grass under the feces and bedding pack treatments was not killed, as grass began to emerge from underneath the fecal and bedding pack patches during the summer.

3.5.2.3. N_2O Emission from Feces

The negative N_2O fluxes observed from the feces treatment have been noted by others. Allen et al. (1996) also found negative, or consumptive, N_2O fluxes on some sampling days from feces and urine deposited on a grassland soil with a high WFPS, although these did not result in an overall net negative emission factor as did the present study. These days of N_2O consumption were determined to be caused by either reduced rates of diffusion due to the high WFPS or enhanced rates of reduction of N_2O to N. There were no differences in WFPS between treatments in this study, however, so the deposition of feces must have resulted in another soil condition that contributed to the soil becoming a sink for N_2O.

Agricultural soils have been documented as capable of being sinks for N_2O, due to the consumption instead of the release N_2O (Chapuis-Lardy et al. 2007). Denitrification has been determined as the main process resulting in N_2O consumption by soils, although nitrifier-denitrification has also been found to be possible by some nitrifying bacteria, causing consumption of N_2O as well (Chapuis-Lardy et al. 2007). High WFPS and low NO_3^--N concentrations generally associated with denitrification can cause N_2O consumption, because microorganisms will use N_2O as electron acceptor

when NO_3^--N is not available. This has also been reported by Tenuta et al. (2010) who found negative N_2O fluxes during a year of above normal precipitation and associated high water tables from unfertilized grassland. In the present study, increased microbial activity due to added organic matter from the deposition of feces may have led to consumption of N_2O during the wet summer as microorganisms needed electron acceptors, however, CO_2 flux data did not show increased microbial respiration in fecal patches to support this theory.

3.5.2.4. Diet effect on N_2O emission

An important aspect related to the timing of excreta application is the forage-based set of diets used in this trial. These diets are typical of feeds that would be fed to beef cattle during April in Manitoba, including those animals turned out on pasture in early spring. Cattle are often turned out on to grasslands in early spring to reduce manure build-up in dry-lots and for spring calving on pasture and the cattle would still require feeding until grass growth is sufficient for grazing in mid- to late May. These forage based diets are therefore an important factor to consider when determining management practices that reduce GHG emission from livestock excreta.

As is demonstrated in Tables 3.1, 3.2, and 3.3, the addition of DDGS to the cattle diet increased the concentration of N in urine most significantly, with comparably small increases in fecal total N. This resulted in a treatment by diet interaction, with the DDGS supplemented diets resulting in significantly greater cumulative N_2O emission from urine patches but with no difference noted from the feces or bedding pack treatments. Few

studies have looked at the effect of diet on N_2O emission from feces and urine deposited on grassland, and they have generally resulted in no significant difference in emission. Van Groenigen et al. (2005a) used synthetic urine of different N concentrations, in the same volume, in attempt to see if diets with higher urine N would result in higher N_2O emission. No significant differences were found, however. Similarly, Arriaga et al. (2010), found no significant differences in N_2O emission from cattle slurry applied to grassland that was collected from cattle receiving diets composed of different ratios of roughage to DDGS concentrate.

It is interesting to note that increases in dietary crude protein, causing increased urinary N excretion, have been found to increase emission of ammonia from cattle urine mixed with soil (Cole et al. 2005). These authors found that increasing in dietary CP from 11.5 to 13% resulted in an increase in ammonia emission from 60 to 200% from urine and feces when mixed with soil, which was attributed primarily to increased urinary N excretion. Ammonia emission can lead to indirect loss of N_2O. Currently, IPCC uses a factor of 1% applied N in livestock excreta to estimate indirect emission of N_2O from ammonia and nitrogen oxide deposition that occurs as a result of livestock excreta and manure (IPCC 1996). A factor 0.2 kg NH_3-N/NO_x-N per kg N excreted is used to estimate ammonia and nitrogen oxide that occurs directly from livestock excreta (IPPC 1996). Ammonia volatilization can account for 10% to 70% of N applied in feces, urine, or manure (Bussink and Oenema 1998). In the case of the 20% DDGS supplemented diet, where a large increase in available urine-N occurred, there could potentially be a large loss of N as ammonia and increased indirect losses of N as N_2O as well.

Saggar et al. (2004) hypothesized that a diet low in crude protein and therefore higher in available C and lower in available N, should decrease N_2O emission. Although increased available C:N ratio increases rates of denitrification, it should also favour a lower ratio of N_2O:N_2 produced (Saggar et al. 2004). However, work with swine manure has found no significant differences in N_2O emission from lower vs. higher crude protein diets (Misselbrook et al. 1998).

Not only did the addition of DDGS to the diets increase the total N concentration of the urine, but also increased the proportion of available-N (ammonium-N and urea-N) in urine, decreasing the proportion of organic N in the total urine N applied. Available-N is more readily available for plant growth, losses due to runoff and leaching, and gaseous losses. It appears that the higher the proportion of available-N applied per unit area, the greater the potential for loss as N_2O-N.

Hippuric acid is another nitrogenous compound found in cattle urine and literature suggests that its concentration increases with increased protein supplementation (Kool et al. 2006; van Groenigen et al. 2006). The presence of hippuric acid in urine has been noted to have an inhibitory effect on denitrification and, therefore, N_2O production (van Groenigen et al. 2006). Hippuric acid breaks down in the soil to form benzoic acid, which inhibits denitrifying microorganisms. This effect could offer an explanation as to why there was no increase in the N emission factor for urine patches with increased DDGS supplementation, despite the significantly higher cumulative N_2O emission. The increase in hippuric acid that occurs with increased diet supplementation may prevent a compounding effect from occurring, decreasing rates of N lost as N_2O from diets that result in high urine-N concentrations.

Hippuric acid concentration in urine could also offer an explanation as to why the 0% bedding pack diet had a higher emission factor than the 10% and 20% bedding pack patches, despite the fact that no significant differences were found in cumulative N_2O emission from the bedding pack treatments and that more available N was added to the soil in the latter two treatments. A larger proportion of urine was added in the 10% and 20% bedding treatments, as increased supplementation with DDGS also resulted in increased urine production (Bernier 2010). This would have resulted in increased volume and concentration of hippuric acid in the 10% and 20% DDGS treatments, causing an inhibitory effect on rate of N_2O production.

3.5.2.5. N Emission Factors

The N emission factors determined for feces, urine and the bedding pack were lower than current IPCC guidelines, which are currently estimated to be 2.0% for livestock excreta from drylot areas and deposition of excreta (both urine and feces) on grassland (IPCC 1996). There is a wide range of emission factors reported in the literature and they seem to depend on climatic conditions at the time of the study, study location, and methodology. Allen et al. (1996) reported a range of 0.8 to 2.3% from excreta N from grazing cows (urine and feces) applied in and measured over the summer and winter, respectively. Di et al. (2010) reported N emission factors of 0.3 to 2.1% for cattle urine deposited on pasture in New Zealand, depending on timing of application and soil type. In an experiment examining the effects of compaction on urine patches, van Groenigen et al. (2005b) found an increase in N emission factors for urine patches of 1.30 to 2.92 as a result of compaction, and but these patches were only monitored for 1 month

after deposition. Van Groenigen et al. (2005b) also found no significant differences in N emission factors from different synthetic urine N concentrations applied in the same volume, with urine N concentration ranging between 18.6 and 76.6 g N m^{-2}, which are much lower N concentrations than this study. Tremorin (2008), reported N emission factors in the range of 0.03 to 0.35% for feces and 3.7 to 6.5% for urine, from grazing beef cows over the summer, also measured for a one month period after deposition, with these ranges being much higher than the present study. Contrary to this, Bol et al. (2004) in a 14 day study reported N emission factors of 0.02% of applied cattle urine urea-N. Therefore, nitrogen emission factors from feces and urine need to be looked at with caution as soil type, methodology, season of application, and diet could result in large variations in N emission factors, even from studies performed in the same regions.

Currently, IPCC Guidelines do not recognize consumptive N_2O fluxes in national GHG inventories, such as those determined in the feces patches, despite several findings of N_2O consumptive behaviour of soils (IPCC 1996). The N_2O consumption reported by others has been inconsistent and has varied with climatic and soil conditions (IPCC 1996). The negatives fluxes associated with the feces patches will help provide more evidence as to the conditions necessary for the consumptive behaviour of soils for N_2O, potentially providing a basis to reduce a livestock producers GHG footprint.

The bedding pack patches appear to be limiting direct emissions of N_2O compared to the urine patches, as can be seen by significantly lower emission factors. This is noted particularly from the 20% DDGS diet bedding pack patches where the majority of the N applied was in the form of urine-N. The higher ratios of C:N and presence of physical organic materials preventing application of urine directly to the soil surface significantly

reduce the emission of N_2O compared to urine patches, although loss of N through ammonia volatilization, and subsequent indirect losses of N as N_2O, could still be occurring. Cole et al. (2005) found that as dietary crude protein increased from 11.5 to 13%, ammonia volatilization from excreta applied to soil increased 60 to 200%, which was determined to be primarily the result of increased urinary N concentration.

3.5.3 Methane emission

Typically, fecal patches deposited on grassland will generate a significant amount of methane following application (Saggar et al. 2004). Tremorin (2008) found cumulative methane emission in the range of 510-598 mg C m^{-2} from fecal patches over a 30 day period, peaking following application to grassland at greater than 10,000 mg C m^{-2} day^{-1}. The present study, on the contrary, found cumulative methane emissions from fecal patches to be much lower, at 76 mg C m^{-2} over a 147 day period, with no peak following application and maximum fluxes in the range of 30-40 mg C m^{-2} day^{-1} over the sampling period.

Low CH_4 emission from feces has been found to occur when climatic conditions at the time of deposition favour drying out of the fecal patches (i.e., warm temperatures), creating aerobic conditions which inhibit CH_4 production (Saggar et al. 2004). As anaerobic conditions are necessary for the production of CH_4, peak fluxes are also most often observed during cool, wet seasons, despite the fact that most CH_4 is derived mainly from within feces and not from soil (Saggar et al. 2004). Methane generally to peak immediately following application, and depending on climatic conditions, will begin to

drop within a few days up to three weeks following application (Saggar et al. 2004). The feces and bedding pack treatments dried out with the first three weeks in this study, despite the cool temperatures and wet soil conditions (Fig. 3.3), and CH_4 emission did not peak following application. An interesting observation was that little microbial activity was observed in the feces throughout the study period. This could be the result of the below average spring precipitation and temperatures, or an effect of the composition of the feces due to diet (Saggar et al. 2004).

The low-quality, forage-based diet may have favoured more aerobic conditions to be present in the feces compared to feces voided by cattle on grass based diets. Parts of undigested feed could still be seen in the feces creating, a very non-uniform composition with little fluid, much different than the feces voided by grazing cattle. Other studies have noted that diet composition can affect CH_4 emission from feces as well (Saggar et al. 2004), something that we did not find in this study with the added DDGS supplementation. Jarvis et al. (1995) found a strong correlation between increasing N content of feces and increased CH_4 emission. The increased DDGS supplementation resulted in a higher partitioning of N to urine, with only a small increase in total fecal N content (Table 3.2), possibly explaining why no diet effect was seen for CH_4 emission.

Generally, urine patches are not considered a significant source of methane due to unfavourable soil conditions created following application of urine. Urine patches in the study by Tremorin (2008) inhibited CH_4 emission, due to inhibition of methanogenesis, likely due to high concentrations of soil NH_4^+-N, or increased methanotrophy in soil. It is interesting to note that although no significant differences were found between treatments, cumulative CH_4 emission from urine patches was greater than from fecal and

bedding pack patches, indicating that the soil processes responsible for urine's inhibition of methanogensis or increased methanotrophy in the Tremorin (2008) study did not occur in the present study. There were no trends observed between measured soil parameters and methane flux over the sampling period (Appendix D).

3.5.4. Extrapolation of Effect of Diet on Excretion Rates and Relationship to GHG Emission

As was demonstrated with the bedding pack patches, the addition of DDGS to the diets increased not only the concentration of N in urine, but the volume of urine excreted as well (Bernier 2010). Therefore, not only did the DDGS supplemented diets result in significantly greater cumulative N_2O emission, but would also have generated a greater number of urine patches or a higher volume of urine excreted per patch per cow (van Groenigen et al. 2005a).

On average, daily excretion rates of these cows during the diet trial were 26 kg of feces and 7 L of urine from the 0% diet and 24 kg of feces and 11 L of urine from the 20% diet (Bernier 2010). To put these results into perspective, one cow from the 0% diet turned out onto this grassland on day 111 would have resulted in 7 urine patches and 26 fecal patches. Assuming each patch to be 0.04 m^{-2} in size, this would have generated 73 and -23 mg N_2O-N (Chapuis-Lardy et al. 2007) from urine and fecal patches, respectively, for a total of 50 mg N_2O-N evolved up until day 258. A cow receiving the 20% DDGS supplementation would have produced 543 mg N_2O-N from urine patches and -21 mg N_2O-N from fecal patches, for a total of 522 mg N_2O-N evolved over the

sampling period, assuming volume per urine excretion remained the same. Cumulative methane emissions for urine and feces extrapolated in the same manner would have resulted in 25 and 78 mg CH_4-C from urine and feces for a total of 103 mg CH_4-C produced from a 0% DDGS diet, and 40 and 74 mg CH_4-C from urine and feces, for a total of 114 mg CH_4-C produced from the 20% DDGS diet.

The assumption that volume of urine per urination would remain the same had to be made, as this could not be determined from data obtained by Bernier (2010) because catheters were used to collect urine. Bussink and Oenema (1998) concluded that if diet changes resulted in increased urine N excretion along with an increased N concentration, (i) urine volume per urination may increase with the same number of urinations, or (ii) the number of urinations may increase with the volume of urine per urination remaining unchanged. Results from this dataset suggest that it is likely that scenario (ii) would cause a greater N_2O emission due to the larger surface area covered. Van Groenigen et al. (2005a) found that increased volume of urine per patch resulted in increased N_2O emission as well. It is also important to note that these estimations do not take into account bedding areas, watering areas, or mixing of feces and urine of which, as discussed previously, are suspected to cause increased N_2O emission. Bedding pack patches in this study only saw a small stimulation of N2O emissions from mixing of feces and urine however. This is possibly due to immobilization of N from the addition of the large amount of organic C through the straw bedding, or possibly because no compaction was simulated.

Using IPCC (1996) global warming potential (GWP) factors of 21 for CH_4 and 310 for N_2O from the IPCC Second Assessment Report (IPCC 2007), we can calculate

CO_2 equivalent (CO_2-eq) emissions as well. When the cumulative CH_4 and N_2O emissions are used, mean CO_2-eqs for the 0% DDGS diet are 132 g CO_2 m^{-2} for urine, -10 g CO_2 m^{-2} for feces. for a total of 122 g CO_2 m^{-2}. The 20% DDGS diet resulted in a total of 605 g CO_2 m^{-2}, with 616 and -11 g CO_2 m^{-2} from urine and feces, respectively. The bedding pack treatment would have resulted in 24 and 8 g CO_2 m^{-2} over the study period from the 0% and 20% DDGS diets, respectively.

Using the extrapolated N_2O emission results from above, CO_2-eq from feces and urine deposited by one cow on DOY 111 and measured through September are 27 and 257 g CO_2 m^{-2} from the 0% and 20% diets, respectively. Therefore, there is a large increase in direct soil GHG emission from feeding a 20% DDGS supplemented diet compared to forage based diet from grazing animals. These measurements do not take into account indirect losses of N_2O from feces, urine, and manure, however. Currently, IPCC uses emissions factors of 1.5% and 3% to determine indirect N_2O emission from N lost as runoff/leachate and ammonia volatilization from manure and livestock excreta applied to soil (IPCC). This includes estimates that N losses from runoff and leachate average of 30% of N applied in excreta or manure and losses of ammonia average 50% of the available-N applied in excreta or manure (IPCC 1996). As the 20% DDGS supplemented diet increased available-N application to soil, mainly via urine, indirect losses of N_2O have also been increased as a consequence.

3.6 Conclusions

Urine from cattle fed forage-based diets deposited in April on grassland had significantly greater N_2O emission than that derived from feces or from a simulated

bedding pack. The addition of DDGS increased the cumulative emission of N_2O from urine patches and it also increased the volume of urine produced per cow per day, causing a compounding effect of supplementation on soil GHG emission. Cumulative N_2O emission over the 147 day study period was 1 258, 735, and 267 mg N_2O-N m^{-2} from urine patches deposited from cattle fed diets with 20%, 10% and 0% DDGS, respectively. Nitrogen emission factors from urine patches were not significantly different between the diets, however, averaging 0.37% applied N lost as N_2O. Peak N_2O emissions from urine patches occurred in early July and did not occur immediately following application of excreta, when soil available N concentrations were highest. Instead, peak N_2O emissions were related to warming temperatures and followed large (>40 mm) precipitation events.

Contrary to the urine patches, feces patches resulted in N_2O consumption. Cumulative N_2O emission from fecal patches was -22 mg N_2O-N m^{-2}, with N emission factors determined to be -0.04%.with no significant differences between diets. The simulated bedding pack treatment appeared to reduce the effect of the DDGS supplementation on direct N_2O emission from excreta. Bedding pack patches were intermediate between feces and urine patches, with cumulative N_2O emission at 20 mg N_2O-N m^{-2} and an N emission factor of 0.06%. Methane emission from excreta were unexpectedly low, likely the result of cool temperatures, lower than normal spring precipitation, and the forage based diets. Feces cumulative emission of CH_4, was 76 mg C m^{-2}, with no significant difference in CH_4 emission between diet or the other patch types.

It seems that urine application early in the season, when temperatures are not favourable to denitrification, will not result in significant emission of N_2O unless diets result in high concentration of N in urine. The nitrification process appeared to be the

dominant process controlling N_2O emission when temperatures were low, despite ample soil moisture conditions and it can be speculated that denitrification processes were either not occurring at this time due to low temperatures or that low N_2O:N_2 ratios were occurring due to soil conditions. Denitrification, associated with warming soil temperatures and large precipitation events, resulted in the peak flux of N_2O from urine patches, which occurred nearly 10 weeks following application of treatments.

More trials are needed to determine if the temporal trends observed in N_2O and CH_4 emissions are typical for excreta from beef cows fed forage-based diets, deposited in April in Manitoba, on various soil types and varying climatic conditions. The results from the study by Tremorin (2008), which is the only other study of this kind in Manitoba, were markedly different from this study, which was to be expected as the soil type, diets, animal type, and timing of application were different from the present study. As well, the heavy textured soil used in the study is not representative of all soil types used for overwintering and pasturing beef cattle, and emissions may be very different from those in observed from other soil textures and locations.

Feeding forage-based diets to cattle on pasture early in spring, immediately following snow melt could potentially result in low soil GHG emission and be considered a best management practice. The fact that the feces patches from these forage based diets had a negative N_2O emission factor and very low CH_4 emission when deposited at this time of year is an important finding for livestock producers. As well, the 0% DDGS diet resulted in low N_2O emission from the urine patches. However, the addition of DDGS to the diet resulted in a significant increase in soil N_2O emission and caution should be taken when using DDGS supplementation while grazing animals. These diets and

management practices would need to be modelled more completely, including the contribution of enteric methane to overall GHG emission before any firm conclusions on environmentally beneficial management practices can be made.

3.7 References

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4.0 SYNTHESIS

The results of the studies in Chapters 2 and 3 suggest that feeding overwintering beef-cows forage based diets supplemented with dried distillers' grains with solubles (DDGS) increased the supply of plant available nitrogen (N) and phosphorus (P), and the potential for both soil greenhouse gas (GHG) emission and losses of N and P to the environment when excreta was applied to soil. From this data it would appear that using DDGS as a protein and energy supplement in overwintering beef-cow diets may not be a uniformly beneficial management practice to recommend to cow-calf producers. However, it is important to keep in mind that other components of the management practice need to be taken into consideration before arriving at any firm conclusion. In particular, the potential for reduction in enteric methane emission due to DDGS supplementation of the low-quality forage diets needs to be considered. Enteric methane emission accounts for 31% of GHG emission from agricultural operations in Manitoba (MAFRI 2010) and therefore a reduction in enteric methane would be an important environmental implication of DDGS supplementation for cow-calf producers.

The increase in soil GHG emission was only measured from the urine patches, and in fact decreased N_2O emission occurred from the bedding pack treatment due to DDGS supplementation. This would suggest that DDGS supplementation may be more appropriate for use in a drylot system than in an extensive overwintering system. This is also suggested by the increased potential of available N and labile P to be lost in runoff, which can have severe implications if urine and feces are deposited directly on snow pack or frozen soil in a winter grazing system. If the excreta are deposited on a straw pack, the

direct contact of excreta with snowmelt will be reduced. The fact that drylot manure can be spread at a time and location more appropriate for plant uptake of N and P must be considered as well. Loss of N from fresh or stockpiled drylot manure can be significant, however, with estimates at 22% for stockpiled manure in Western Canada, while P is generally conserved (Larney et al. 2006). Other studies have found that mixing of urine and feces, combined with compaction from animal trampling, has resulted in increased N_2O emission compared to feces and urine patches (van Groengian et al. 2005). However, as the bedding pack patches used in this study were simulated, much caution needs to be taken when interpreting this data. Future studies on actual bedding pack material are necessary to make comparisons with urine and feces patches deposited overwinter.

The emission of ammonia and subsequent indirect N_2O emission is an important factor to consider and, as it was not measured in this study, assumptions that the drylot system may be more appropriate if supplementation is used is impossible to confirm. Although little N_2O emission was seen from the feces and bedding pack patches compared to the urine patches, ammonia emission could have been significant from these patches (IPCC 1996). Ammonia emissions from urine patches have been well documented (Cole et al. 2006). Urine was a significant source of environmental concern in both studies. Reducing the concentration of N in urine patches, or increasing volume of urine excreted per urination event and effectively diluting N concentration of urine, may be an important consideration when determining overwintering management practices in terms of decreasing loss of N in runoff and leachate and mitigating GHG and ammonia emission (van Groenigan et al. 2005).

The significant increase in P excreted by cows receiving DDGS supplementation may be one of the most important factors for producers and agricultural professionals in Manitoba to consider. As nutrient management regulations regarding soil test P thresholds are coming into effect for the majority of agricultural producers in the year 2013, the development of management practices that reduce P import onto livestock operations will be essential to the long-term viability of livestock operations. Eutrophication of surface water bodies is a serious environmental threat to all Canadians and practices that potentially increase the loss of P to surface water bodies must be avoided, especially if alternative supplementation sources are available. In terms of P loss to the environment, the use of DDGS in an extensive winter grazing system compared to a drylot would be the least appropriate management practice due to the high proportion of labile P excreted in feces and increase in P excreted in urine.

As the nutrient excretion data was determined using an 8-hour collection period, caution should be taken before further extrapolation of this data. Literature suggests that short-term collection periods cannot be used for determining daily rates of excretion from cattle due to diurnal differences in excretion volumes and masses, and as such only 8-hour collection data was presented in Chapter 2. Animals have been found to urinate and excrete feces less at night as they are not as active (Powell et al. 2009; Nsahlai et al. 2000). However, nutrient concentrations, in particular N, have been found to be statistically constant between diurnal periods as well as between collection days (Powell et al. 2009; Misselbrook et al. 2005; Leal et al. 2009). Short term collections (i.e., less than 24 hours) and fecal grab or urine spot samples have been found to be accurate predictors on the effect of diet on N concentration in feces and urine, without the need of

expensive and laborious 24-hour, 3 to 5 day collection periods (Cole et al. 2006; Chizzotti et al. 2008; Janicek et al. 2008; Powell et al. 2009). The effect of short-term sampling on P concentrations has not been studied as extensively, however, and warrants future work.

For example, Powell et al. (2009) determined that urine and feces collected over 11-hour periods from cows fed *ad libitum* did not differ in total N concentrations if sampled in the morning or evening, and that the concentrations determined in these collections did not differ significantly from the total daily collection nutrient concentrations. Leal et al. (2009) determined that urine urea N and total N concentrations were not significantly different between days during a 6-day diet trial. Similarly, Misselbrook et al. (2005) also found no differences in nutrient concentrations in urine or feces over a 40-hour collection period from cows in Metabolism stalls during a diet trial. Although Knowleton et al. (2010) did find significant differences in urine urea N and total N concentrations between days during a 5-day sampling period, he concluded that these differences were suspected to be a result of an inadequate period of adaptation to diet, inadequate adaptation period to confinement and collection procedures, or changes in animal health. However, if these conditions are met, it should be possible to obtain accurate nutrient concentrations from a single day of collection (Knowleton et al. 2010).

The methodology used by Bernier (2010) in this trial allowed for more than adequate adaptation periods to diet, collection procedures, and no animal health issues were noted during the trial on replicates used in the analysis. As both the N and P concentration in this trial were within range of the previous 5-day collection by Bernier (2010), these nutrient concentrations should be reasonable estimates of the effect of diet

on N and P excretion of environmentally labile nutrients due to diet. The 8-hour masses and volumes of urine collected should not be used, however, and these nutrient concentrations should be used with average 24-hour collection data.

The use of mature, non-pregnant, non-lactating cows in the study may also present a limitation to this study. The nutrient requirements of pregnant and lactating cows are greater than those of the cows used in this study, and therefore nutrient excretion and subsequent GHG emission from excreta may be different and warrants further investigation.

Although the 0% DDGS diet appeared to be best diet in terms of reducing soil GHG emission and reducing losses of N and P to the environment, it should be noted that this diet was not meeting animal requirements for N and therefore is not practical for producers to use. Future work is needed to look at the effects of forage based diets, either a grass or grass-alfalfa mix that meet animal requirements for N, to determine the partitioning and proportions of environmentally labile N and P in comparison to a diet similar to the 10% DDGS diet, which was also meeting animal requirements for N. This study and literature findings suggest that the forms of N and P ingested could have a large impact on the forms of N and P excreted. These types of forage-based diets for beef cows, which are typically fed to cow-calf herds in Manitoba, have not been well documented in peer reviewed literature, and the impact of these diets on nitrous oxide and enteric methane emissions would provide valuable information for Manitoba producers in terms of national inventory data.

The low N_2O-N emission factors determined in this study emphasize the importance of the generation and use of a Tier II equation by the IPCC for estimating

direct emission of N_2O from livestock excreta. Dry matter intake and crude protein content, in combination with animal type and production stage, should be used to determine excretion rates of N in feces and urine. Nitrogen emission factors can then be applied to these excretion rates of N. Urine and feces should be considered separately in grazing systems, with different N emission factors, as well as, separate from drylot manure. Season and timing of excreta or manure application, soil type, and cropping system should also be considered in the generation and use of N emission factors.

The following recommendations can be made from the results of this thesis for use in development of environmentally beneficial management practices:

- The inclusion of DDGS to meet protein requirements of beef cattle may result in P intakes above animal requirements and should be used with extreme caution, particularly in Manitoba where soil test P regulations will be enforced. If used as a supplement, farms should have on farm P balance plan, a manure management plan, and be soil testing for P annually in fields where manure or excreta is applied. Use of DDGS in a winter grazing system should not be recommended at the 10% or 20% level where soil test P is already sufficient for forage growth.
- Producers should use NRC recommendations to formulate DDGS rations to meet animal requirements, and ensure that management strategies that deal with any concerns regarding the environment are applied.
- If DDGS is used in beef cattle diets, it is important that the diet is balanced to meet animal N requirements, in a ration where RDP and RUP protein requirements are balanced to minimize unnecessary excretion of N via urine.

- A forage diet supplement with 10% and 20% DDGS will result in excretion of feces with the majority of P at 77% of the total P excreted. Furthermore, the proportion of labile P in excreta from the 20% DDGS diet will be higher due to the increased excretion of urine P. Cold weather also needs to be taken into consideration, as it significantly increases the proportions of labile P excreted in feces.
- DDGS supplementation increases the proportion of plant available N in excreta, but this available N may be highly susceptible to be lost in runoff, leaching, or as nitrous oxide. Management practices should reflect the need to apply excreta with high available N concentrations to land at appropriate site location, rates, and timing for plant uptake. The following estimations can be used for determining N content of manure and excreta: 10% and 20% DDGS supplementation results in available N to P ratios ranging from 2.2 to 2.9, with 29 and 40% of the total N being in available N form. If including 25% organic N, the N to P ratios range from 3.5 to 3.9, with available N accounting for 47, and 55% of the total N excreted.
- Nitrogen emissions factors for urine, feces and bedding packs, from beef cows receiving low quality forages, deposited on grassland in April on fine textured soils can be estimated at 0.37, -0.04, and 0.06, respectively.
- Before generating overall conclusions about the role of DDGS supplementation in a beef overwintering system, potential reductions in enteric methane emission need to be taken into consideration in the net GHG emissions of the production system.

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APPENDIX A

Modified method of Hedley et al. (1982) for determination of labile P in manure

Materials:

1. Screw cap centrifuge tubes, 50mL
2. Reciprocating shaker
3. Centrifuge machine
4. 0.45-um Cellulose membrane filter
5. Suction pump
6. Digestion block
7. Kjeldahl digestion tube
8. Vortex
9. ICP-OES
10. Techinicon autoanalyzer or spectrophotometer (882ug)

Reagents:

1. Deionized water
2. 0.5 M $NaHCO_3$ at pH 8.5
3. Digestion Mixture

Preparation of digestion mixture:

Materials:

1. Water bath
2. Fume hood
3. 2-L Flat bottom beaker
4. 1L Plastic container

Reagents:

1. Se Powder
2. Lithium sulphate ($Li_2SO_4.H_2O$)
3. H_2O_2: 30% hydrogen perioxide (P free)
4. Concentrated (18 M) H_2SO_4

Preparation

1. Transfer 350mL H_2O_2 (30% hydrogen peroxide free, P free) into a glass beaker
2. Place it in a cold water bath under fume hood

3. Add 0.42 g Se powder and 14 g $LiSO_4.H_2O$
4. Add 420 mL conc. H_2SO_4 (18 M) carefully and slowly to the mixture with swirling and let it cool.
5. After cooling, transfer the digestion mixture from the beaker into a plastic container.
6. Store at 2^oC

P fractionation steps in detail:

Day 1:

Weigh 0.3g (dry weight) feces in 50-mL screw cap centrifuge tube and add 30 mL deionized water. Shake for 16 h on end to end shaker at 80epm.

Day 2:

Centrifuge manure suspension at 10,000 rpm for 10 minutes. Decant water extract through a 0.45-µm Cellulose membrane filter into a clean tube using a suction pump. Determine inorganic P (technicon) and total P (ICP) on water extract. Wash any particles off filter back into the tube using 10 mL (split into two) $NaHCO_3$ solution and 20 mL more $NaHCO_3$ solution to bring solution volume to 30mL and shake suspension for 16h. Make sure all manure is free from bottom of tube before putting into shaker.

Day 3:

Centrifuge manure suspension at 10,000 rpm for 10 minutes. Decant $NaHCO_3$ extract through a 0.45-µm Cellulose membrane filter into a clean tube using a suction pump. Determine inorganic P (technicon) and total P (ICP) on $NaHCO_3$ extract. Wash any particles off filter back into Kjeldahl digestion tube using a little amount of deionized water and transfer all manure residue washing with deionized water several times to make sure that all the manure has been transferred into the digestion tube (use minimum amount of water possible). Then place digestion tubes into the digestion block and reduce the volume near dryness by evaporation at low temperature. Thereafter, add 4.4 mL of digestion mixture. Raise the temperature 6^oC per minute, digest for 1 hour at 100^oC and then raise temperature to 350^oC (6^oC per minute) and digest for 3 h at 350^oC. Clear colour is the indication of complete digestion. Remove the tubes from heat and let cool to hand warm. Transfer the content into 50mL volumetric flux by washing the digestion tube several times (use vortex) and make to volume with deionized water and transfer into vials (either filter or allow residue to settle overnight). Determine total P in solution using ICP.

Determination of total P in H_2O and $NaHCO_3$ extracts:

Transfer 5 to 15 mL of extract into a Kjeldahl digestion tube. Then place digestion tubes into the digestion block and reduce the volume below 5mL by evaporation at low temperature (110^oC). Thereafter, add 1.1 mL of digestion mixture. Raise the temperature 6^oC per minute, digest for 1 hour at 350^oC. Clear colour is the indication of complete digestion. Remove the tubes from heat and let cool to hand warm. Transfer the content into 50mL volumetric flux by washing the digestion tube several times (use vortex) and make to volume with deionized water. Determine total P with ICP.

APPENDIX B

Table B.1. Fecal P fraction concentrations and standard deviation determined by modified Hedley sequential fractionation (dry matter basis).

Diet	H_2O-P	$NaHCO_3$-P	Residual P
%DDGS		µg P g^{-1} feces DM	
Fall			
0	345	387	404
	(77)	(74)	(64)
10	760	685	579
	(187)	(439)	(375)
20	994	902	651
	(334)	(395)	(77)
Winter			
0	1298	1109	1259
	(653)	(631)	(670)
10	916	754	530
	(272)	(517)	(429)
20	1194	595	395
	(453)	(323)	(104)

Numbers in brackets are standard deviation of the mean.
† Removed cows J47 and H54 (pregnant) and F55 (deceased) from both seasons, J84 and G79 from fall trial (low intakes)

APPENDIX C

Table C.1. Ion analysis of urine using Ion Chromatography.

Diet	Na^{+} (mg/L)	NH_4^{+} (mg/L)	K^{+} (mg/L)	Mg^{+2} (mg/L)	Ca^{+2} (mg/L)	Cl^{-} (mg/L)	SO_4^{-2} (mg/L)	PO_4^{-3} (mg/L)
0% DDGS	1510	57.1	13500	360	15.4	3780	229	57.9
10% DDGS	1740	11.9	12600	122	8.7	3510	620	182
20% DDGS	1830	12.5	8840	52.4	7.9	2240	827	1110

APPENDIX D

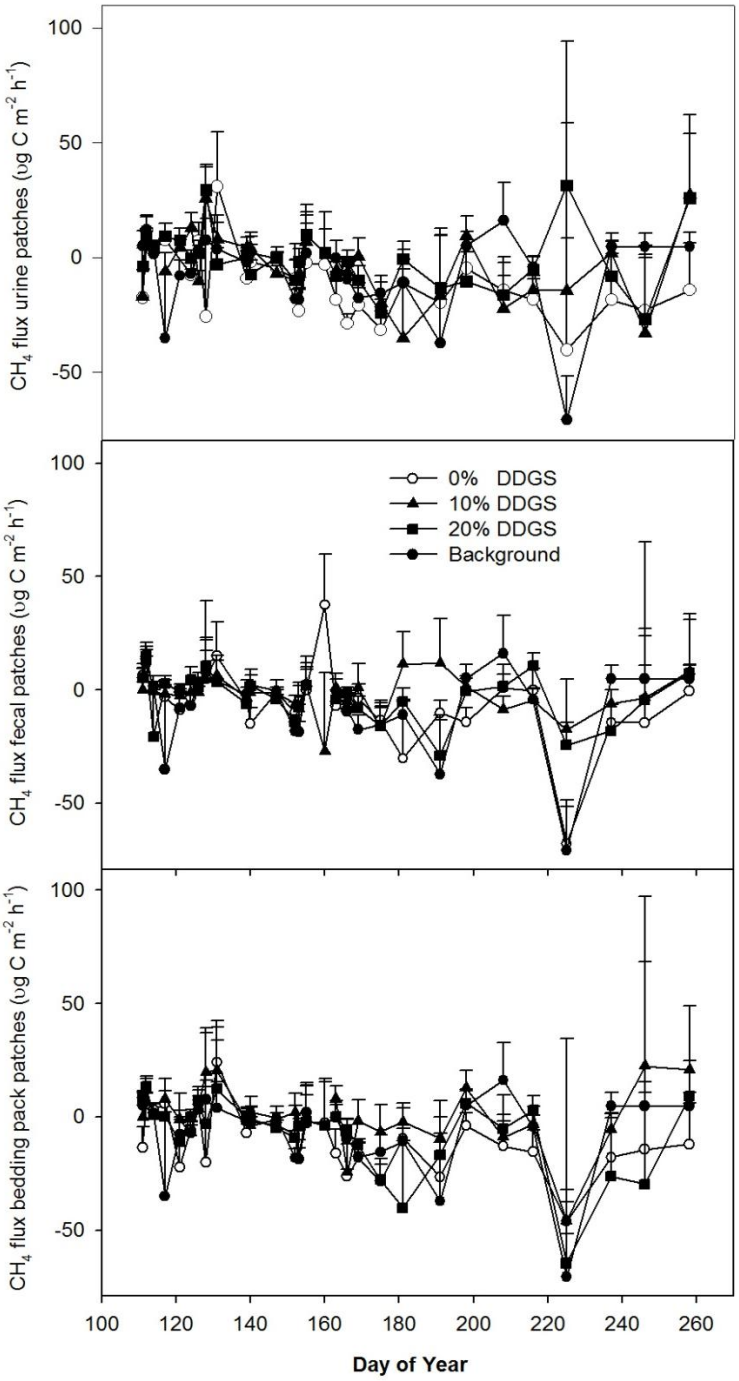


Figure D.1. Methane emissions from urine, fecal, simulated bedding pack, and background patches over 2009 growing season. Average values (n=6) +1 standard error of the average is shown as bars.

APPENDIX E

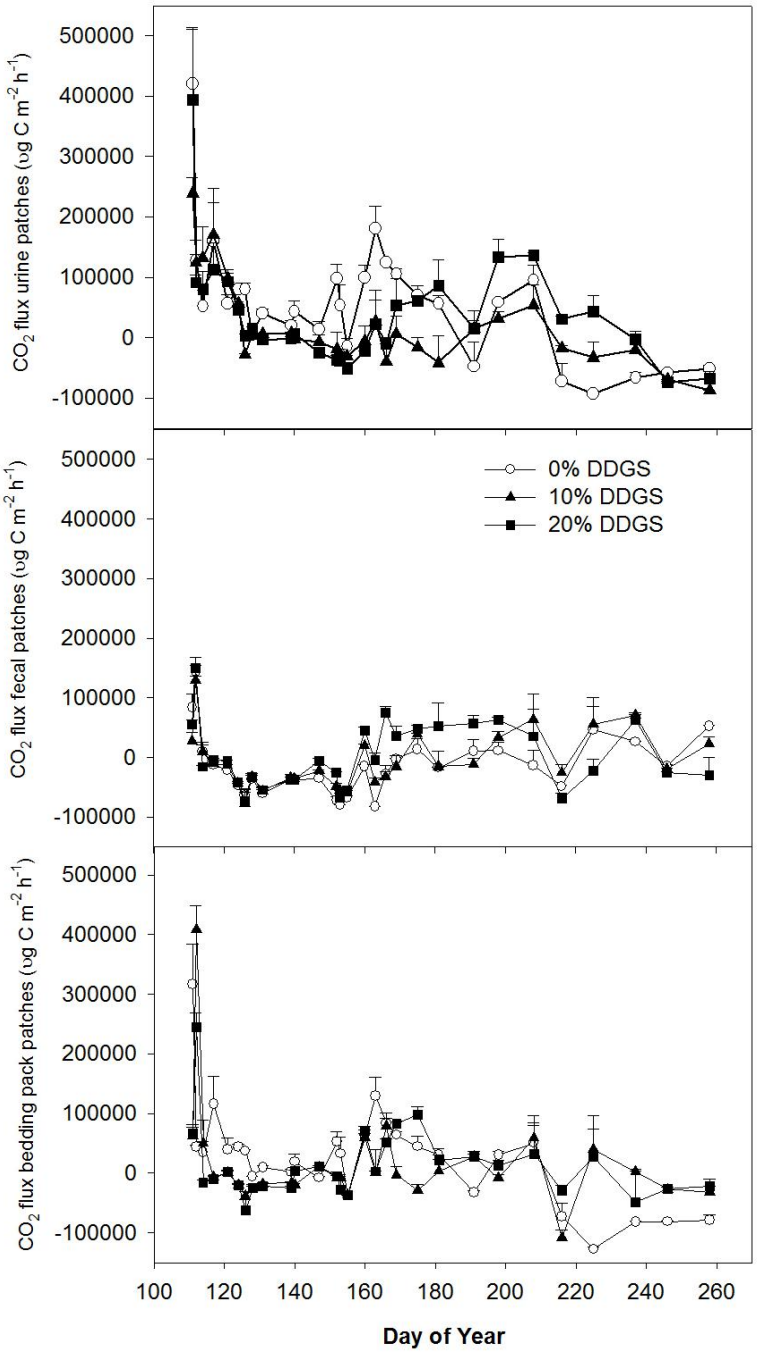
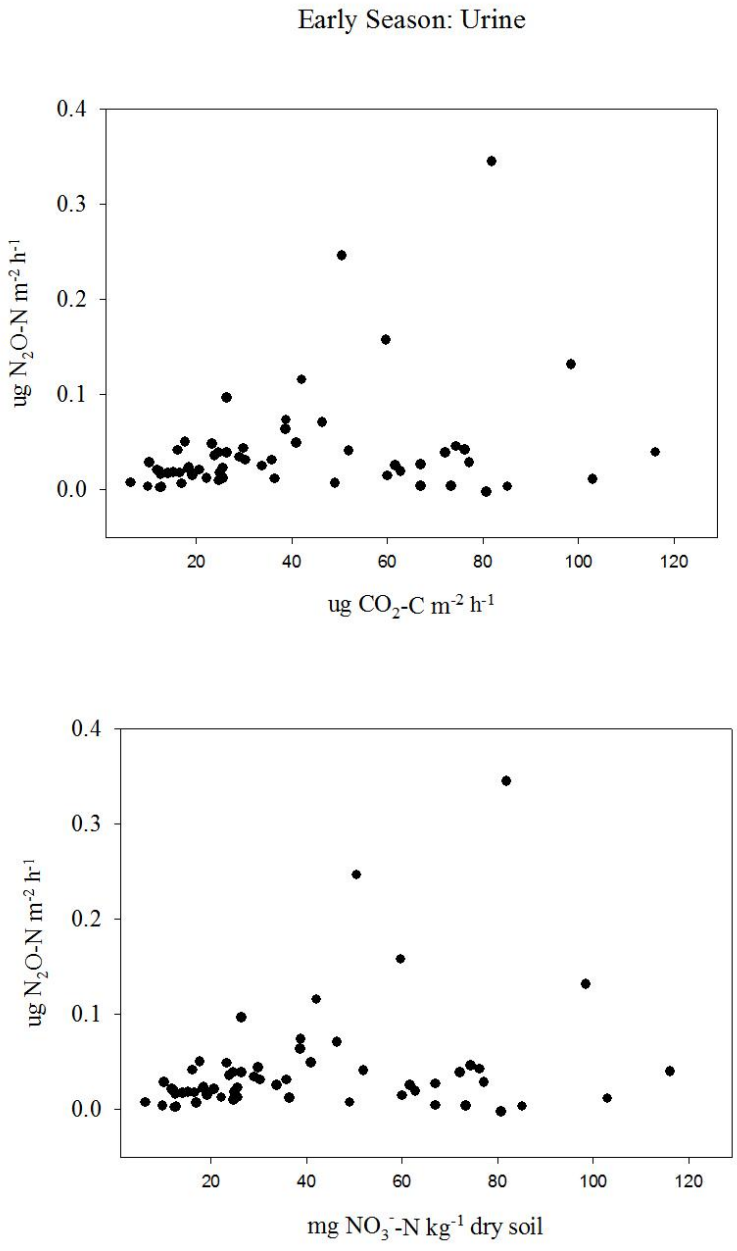


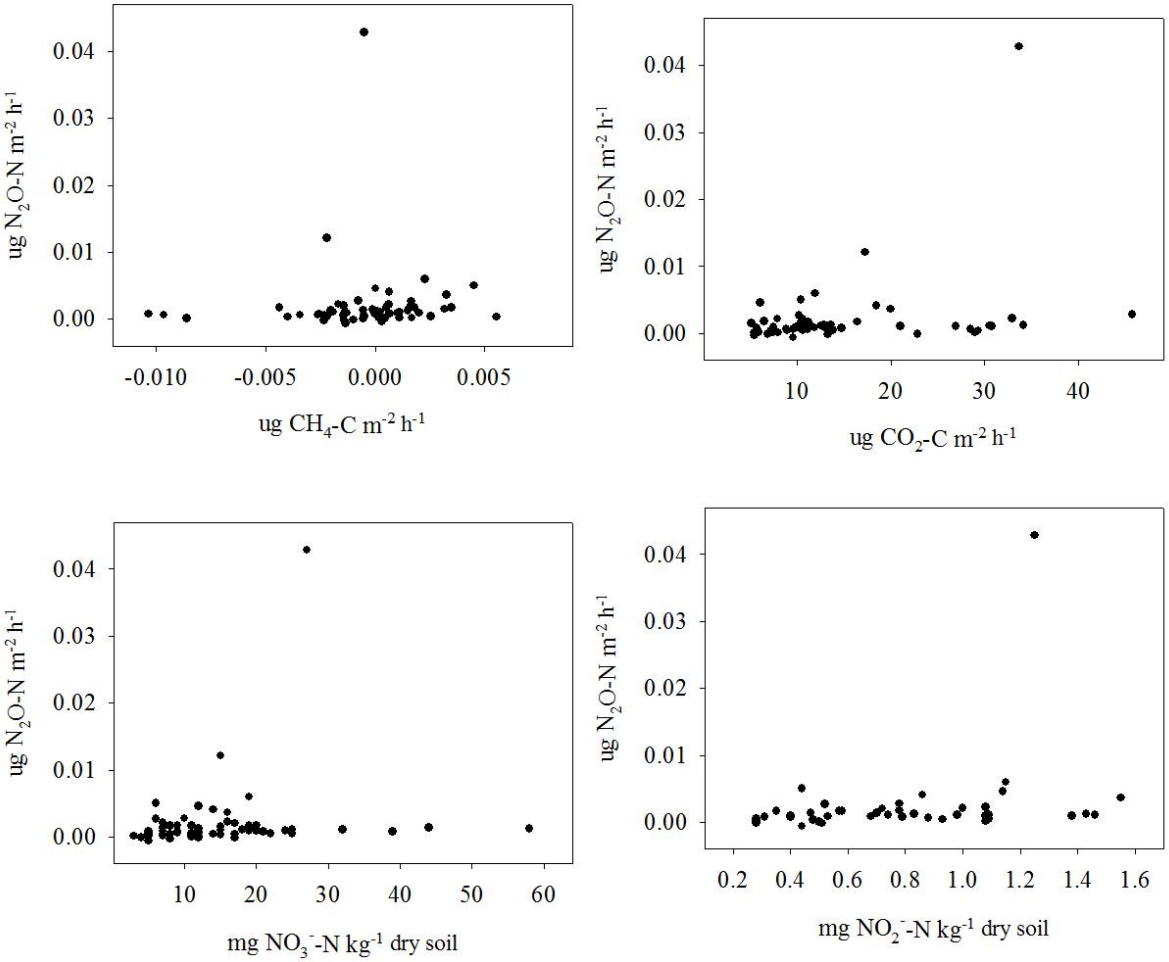
Figure E.1. Carbon dioxide emissions from urine, feces, and simulated bedding pack patches with background emissions subtracted over 2009 growing season. Average values (n=6) +1 standard error of the average is shown as bars.

APPENDIX F

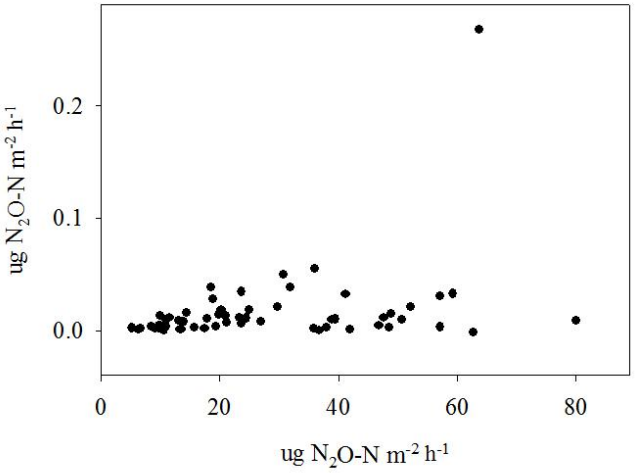
Figure F.1. Spearman Rank Correlation Analysis Scatter Plots

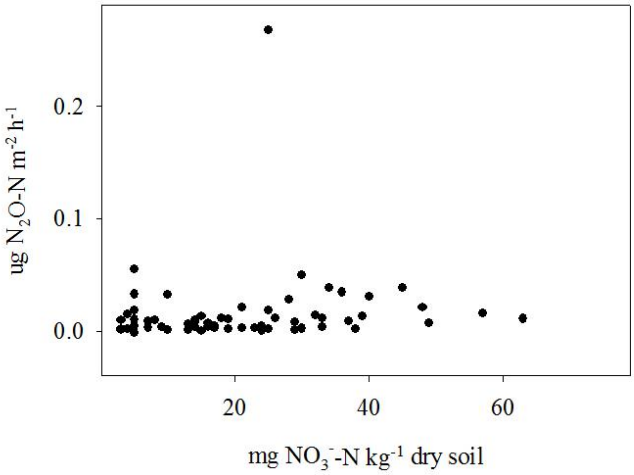


Early Season: Feces

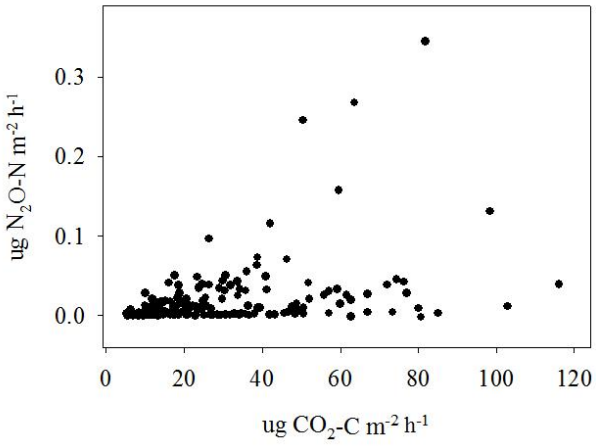


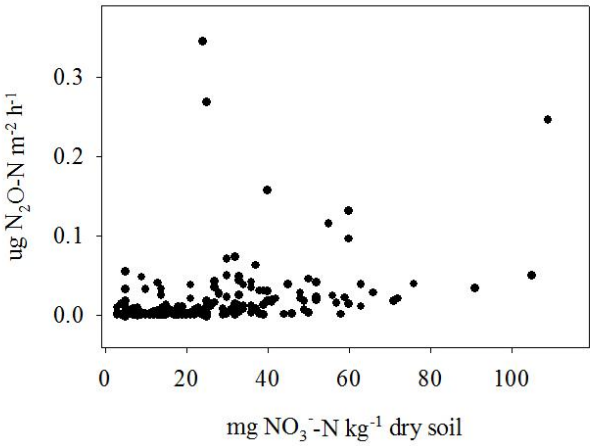
Early Season: Bedding Pack

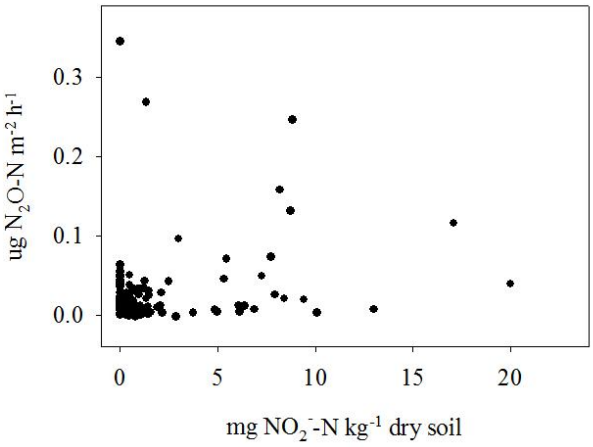




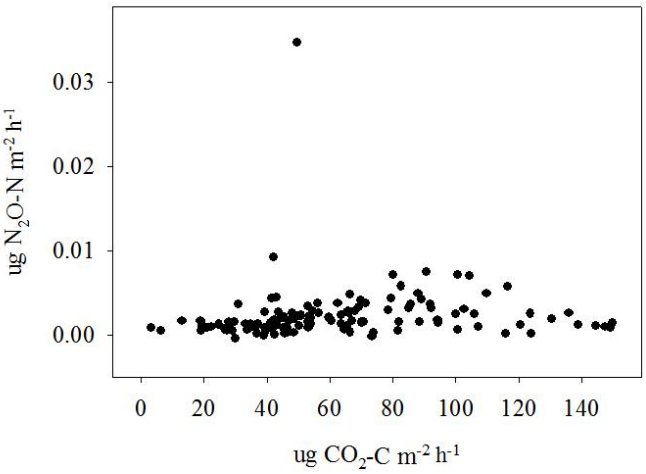
Early Season: All

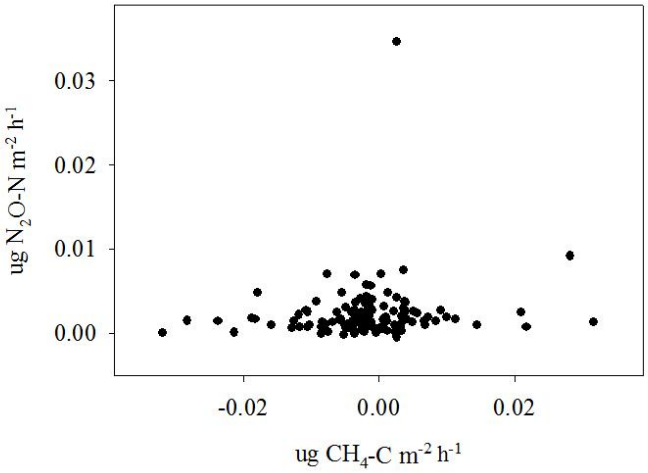




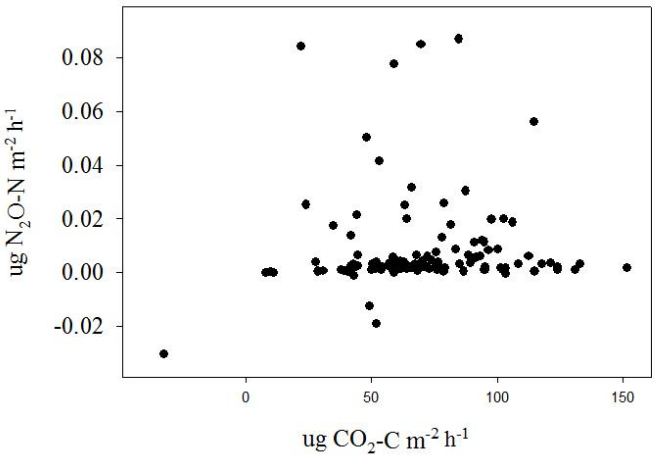


Growing Season: Feces

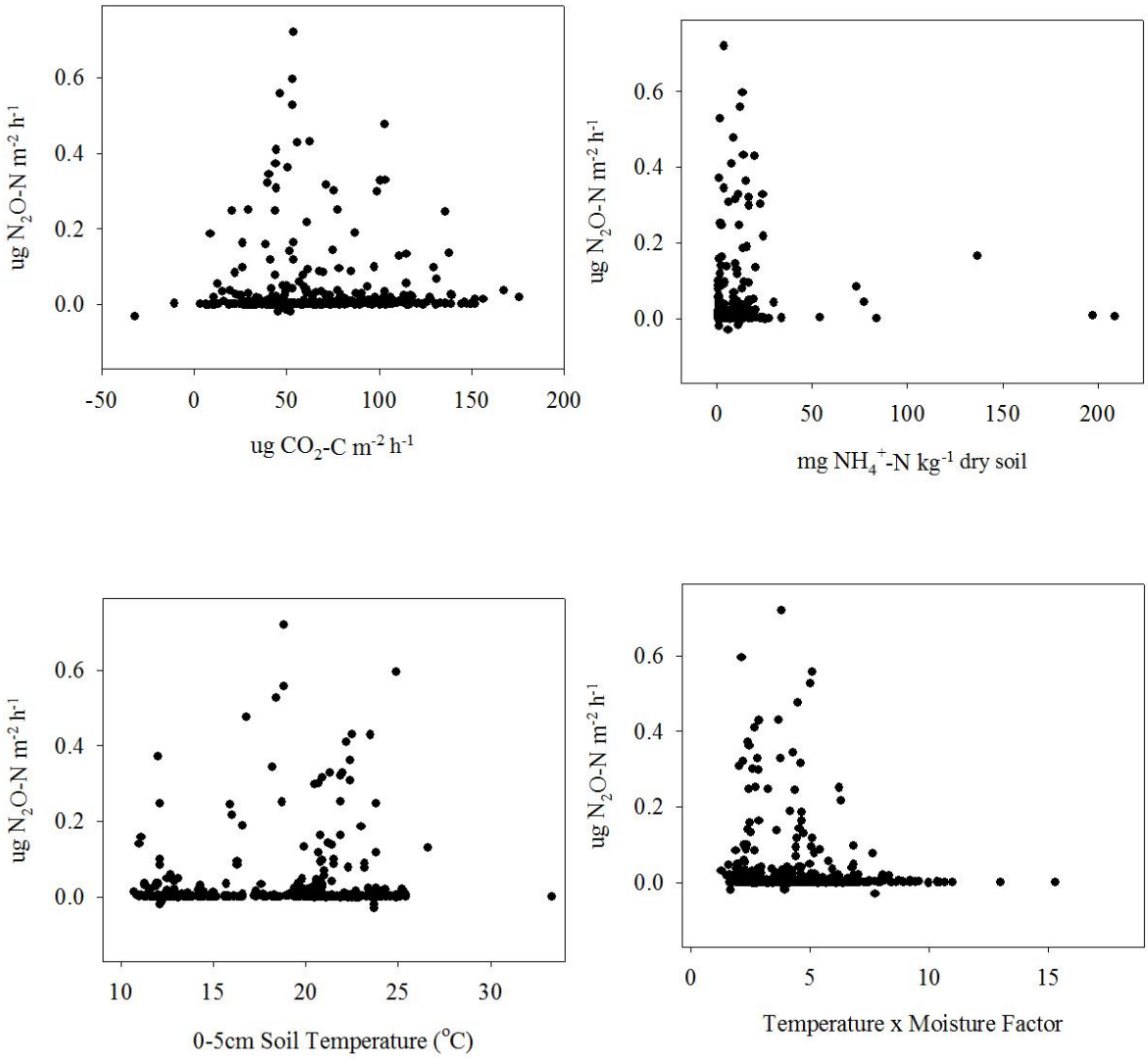




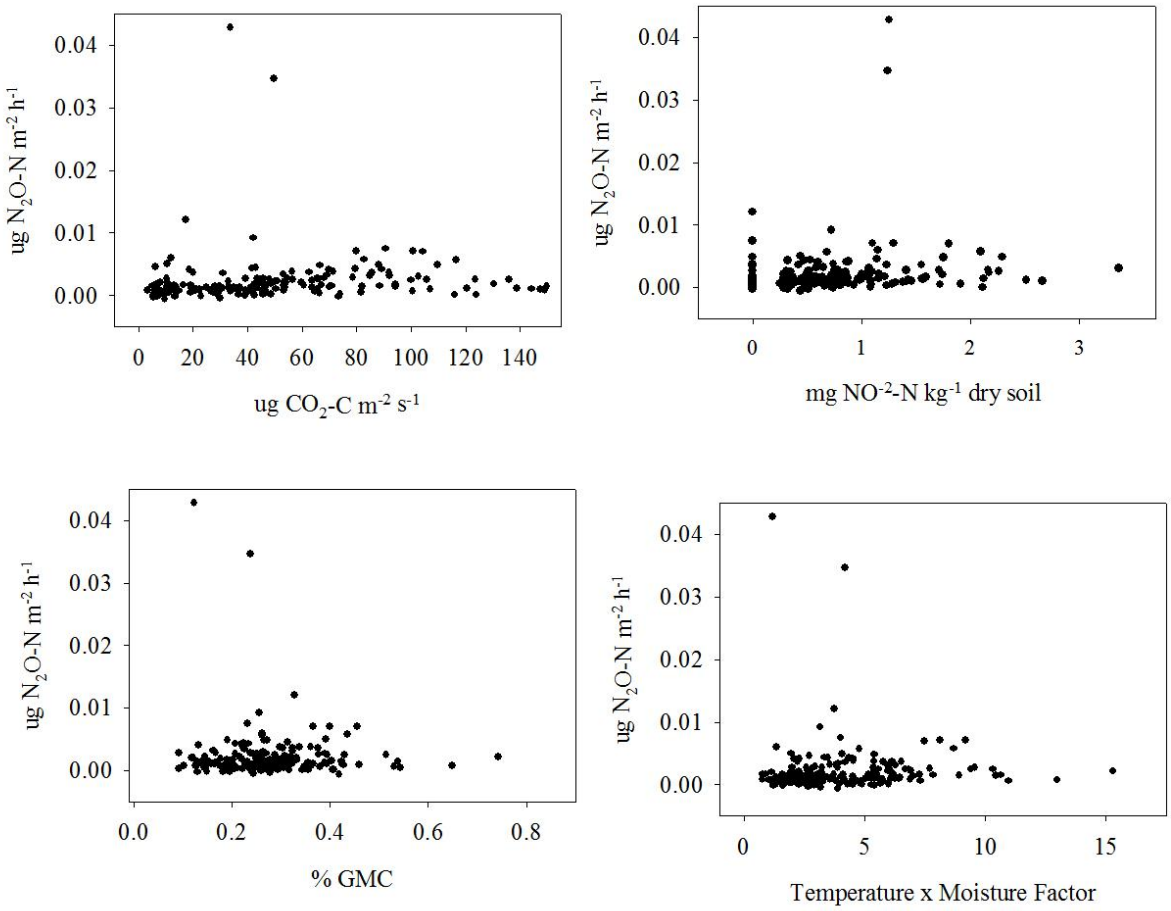
Growing Season: Bedding Pack

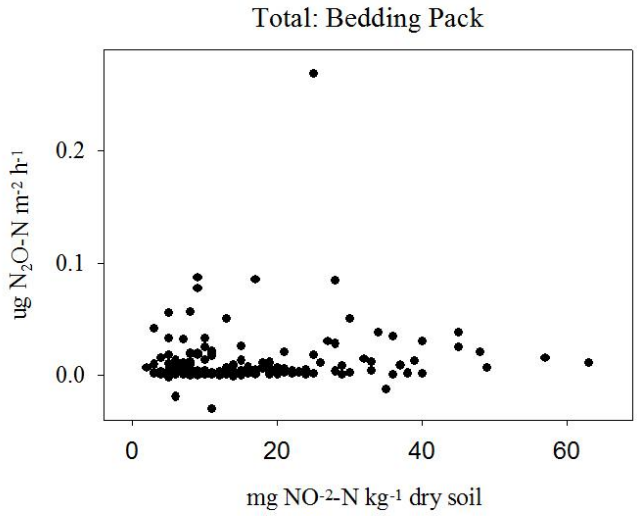


Growing Season: All



Total: Feces


ug N_2O-N m^{-2} h^{-1}
0.04
0.03
0.02
0.01
0.00
0 20 40 60 80 100 120 140
ug CO_2-C m^{-2} s^{-1}
ug N_2O-N m^{-2} h^{-1}
0.04
0.03
0.02
0.01
0.00
0 1 2 3
mg NO^{-2}-N kg^{-1} dry soil
ug N_2O-N m^{-2} h^{-1}
0.04
0.03
0.02
0.01
0.00
0.0 0.2 0.4 0.6 0.8
% GMC
ug N_2O-N m^{-2} h^{-1}
0.04
0.03
0.02
0.01
0.00
0 5 10 15
Temperature x Moisture Factor


Total: Bedding Pack
ug N2O-N m-2 h-1
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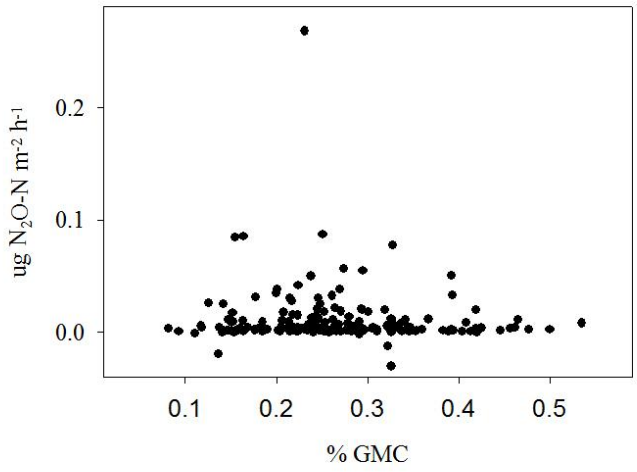
0.1
0.0
0
20
40
60
mg NO-2-N kg-1 dry soil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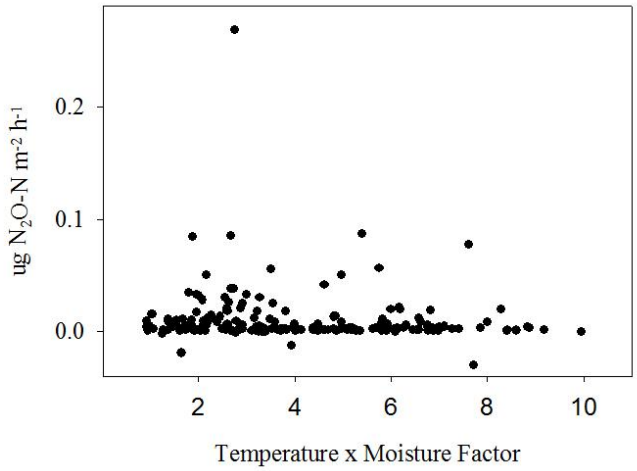
0.0
0.1
0.2
0.3
0.4
0.5
% GMC


ug N2O-N m-2 h-1
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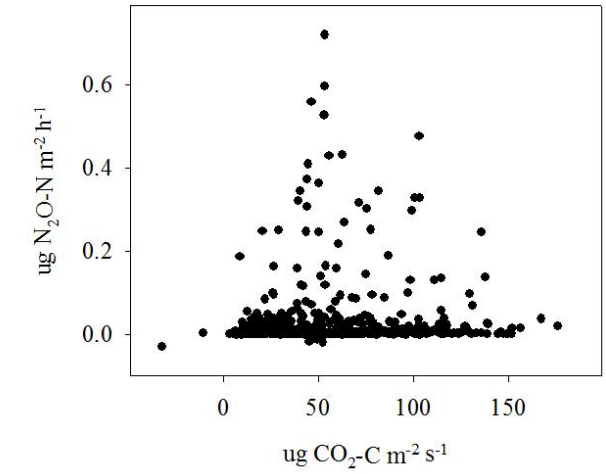
0.0
2
4
6
8
10
Temperature x Moisture Factor

Total: All


ug N_2O-N m^{-2} h^{-1}
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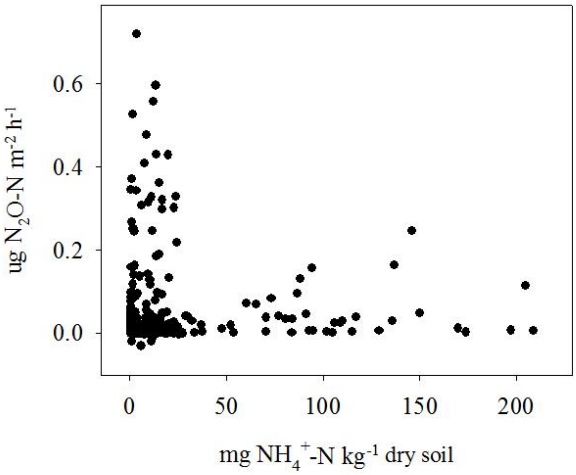
0.4
0.6
0
50
100
150
ug CO_2-C m^{-2} s^{-1}


ug N_2O-N m^{-2} h^{-1}
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0.4
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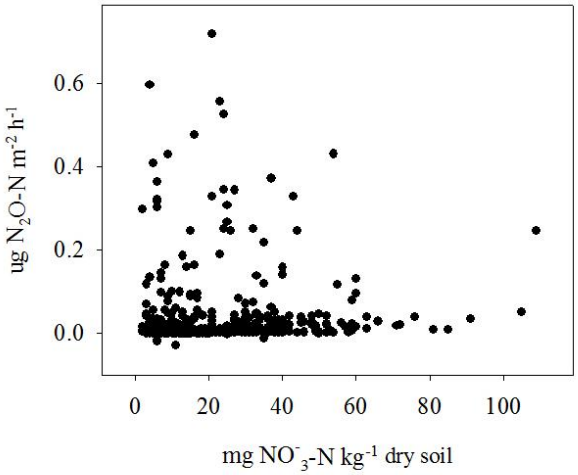
0
50
100
150
200
mg NH_4^+-N kg^{-1} dry soil


ug N_2O-N m^{-2} h^{-1}
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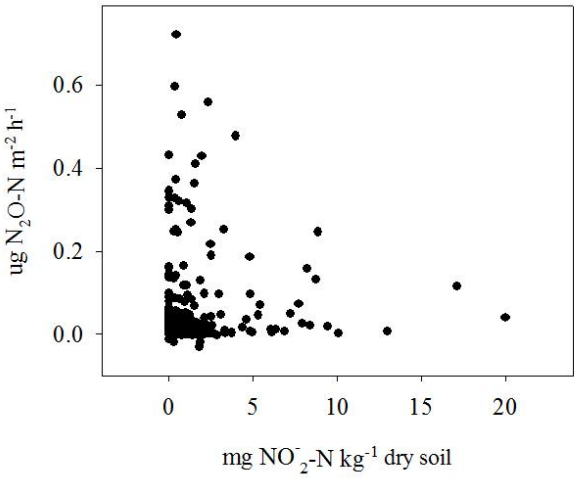
0
20
40
60
80
100
mg NO^-_3-N kg^{-1} dry soil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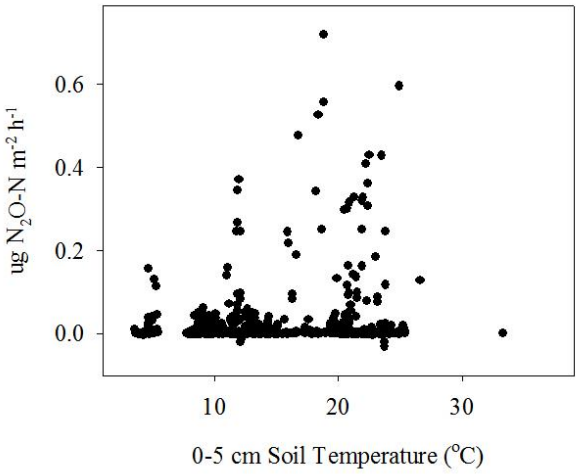
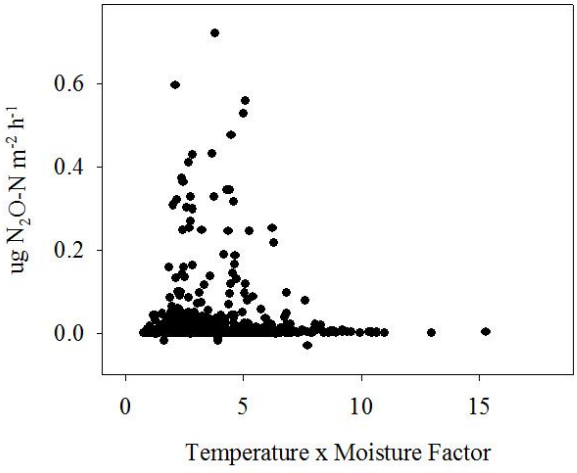
0.2
0.4
0.6
0
5
10
15
20
mg NO^-_2-N kg^{-1} dry soil


ug N_2O-N m^{-2} h^{-1}
0.0
0.2
0.4
0.6
10
20
30
0-5 cm Soil Temperature (°C)


ug N_2O-N m^{-2} h^{-1}
0.0
0.2
0.4
0.6
0
5
10
15
Temperature x Moisture Factor