Measured and modelled enteric methane emissions from beef cattle as affected by dietary crude protein of forage diets

By

Kristine E. Blair

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Department of Animal Science

University of Manitoba

Winnipeg, Manitoba, Canada

ABSTRACT

Methane emissions of 60 steers (321 ± 14 kg) fed isocaloric forage diets differing in crude protein (CP) content were measured at ambient daily temperatures averaging -17.5°C to determine if increased nitrogen status, measured by blood urea nitrogen (BUN), decrease CH_4 as a percent of gross energy intake (% GEI) from backgrounding cattle. Average BUN concentrations (mmol L^{-1}) were 0.81, 1.82, 3.05 and 3.51 (SE ± 0.108) for diets with 6.9% (low), 10.3% (adequate for rumen microbes), 11.1% (adequate for muscle growth) and 13.6% (excessive) CP respectively. Methane (% GEI) emissions decreased with increasing CP over time (P=0.04). Increasing CP content increased BUN levels and decreased methane emissions (% GEI). Although models were developed to predict CH_4 emissions (% GEI) from steers and cows using a backward-elimination process, BUN accounted for only 0.7 to 5.7% of the partial R^2 and therefore has limited value when modelling methane emission predictions.

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ABREVIATIONS

% GEI	=	Percent gross energy intake
°C	=	Degrees Celsius
µm	=	Micrometer
AA	=	Amino acid
ADF	=	Acid detergent fibre
ADG	=	Average daily gain
AMG	=	Adequate for microbial growth diet
ARM	=	Adequate for rumen microbes diet
BG	=	Bale grazing
BUN	=	Blood serum urea nitrogen
BUNX	=	Blood serum urea nitrogen effect
BW	=	Body weight
CH_4	=	Methane
CO_2	=	Carbon dioxide
CP	=	Crude protein
CPI	=	Crude protein intake
d	=	Day
Df	=	Degrees of freedom
DM	=	Dry matter
DMI	=	Dry matter intake
DxPd	=	Diet by period interaction
eq	**=**	Equivalents
E	=	Excessive diet
GE	=	Gross energy
GEI	=	Gross energy intake

GHG	=	Greenhouse gas
H_2	=	Hydrogen gas
h	=	Hour
IPCC	=	Intergovernmental panel on climate change
kJ	=	Kilojoule
km	=	Kilometer
kPa	=	Kilopascal
L	=	Litre
MBW	=	Metabolic body weight
Mg	=	Magnesium
min	=	Minute
MJ	=	Mega joule
mL	=	Millilitre
mm	=	Millimeter
mm Hg	=	Millimeter of mercury (Torr)
mmol	=	Millimole
N	=	Nitrogen
NADH	=	Nicotinamide adenine dinucleotide
NDF	=	Neutral detergent fibre
NE_g	=	Net energy for gain
NE_m	=	Net energy for maintenance
ng	=	Nanogram
NH_3	=	Ammonia
Pd	=	Period
ppt	=	Parts per trillion
psf	=	Pounds per square foot
SE	=	Standard error

SF_6	=	Sulfur hexafluoride
TDN	=	Total digestible nutrients
TMR	=	Total mixed ration
VFA	=	Volatile fatty acid

FORWARD

This thesis is written in manuscript style, with each manuscript having its own abstract, introduction, materials and methods, results, discussion and conclusions. There is also a literature review, discussion and direction of future research, followed by the literature cited. None of the manuscripts have been submitted for publication at the time of thesis completion.

1. LITERATURE REVIEW

1.1 Forage-fed beef cattle production cycle in Canada

Many places in the world offer forage diets to cattle for the entire production cycle. It is common for growing cattle in Canada to be offered forage diets for some period of time after weaning, referred to as a backgrounding phase, where the aim is to grow lean tissue and increase the animal's frame size before moving to the finishing phase where they are fed a grain-based concentrate diet for fat deposition. Cattle enter this phase at approximately 245 kg and leave at 370 kg yielding gains of approximately 0.95kg d^{-1} (Sheppard et al., 2014).

In the last two decades the cattle industry has endured tight profit margins due to several events impacting cattle and beef markets including export restrictions caused by bovine spongiform encephalopathy and mandatory country of origin labelling (Carlberg et al., 2009). The impact on the industry can be seen in cattle inventories which have dropped by 15% between 2003 and 2014 (Statistics Canada, 2015). The industry also experienced a 33% drop in Canadian farm cash receipts from cattle and calves between 2002 and 2003 (Mitura and Do Piétro, 2003). The producers that remain in the industry have been forced to adopt low-cost production systems to stay profitable. A study by Kelln et al. (2011) considered the economic implications of bale grazing, swath grazing, straw-chaff grazing and drylot winter feeding systems for dry pregnant cows. A three year cost analysis revealed that cost ($ cow^{-1} day^{-1}) was 0.98, 0.76, 1.27 and 1.07 respectively. Similarly, a study by McCartney et al. (2004) reported costs ($ cow^{-1} d^{-1}) of traditional feeding, swath grazing and alternate day feeding of 1.54, 0.84 and 1.40 respectively.

In Canada, the range in quality of forage-based feeds can vary dramatically due to differences in input prices, labour availability, plant species, precipitation, temperature, preservation practices, etc. Often forage is preserved later in the season for a higher yield but with more mature plants resulting in lower quality feed. Forage quality can also be compromised due to weather conditions delaying harvest, lack of labour to preserve forage quickly, common use of poor quality native hay and limited fertilization (Sheppard et al., 2014). A forage survey conducted by the Government of Saskatchewan, Ministry of Agriculture (2013) collected over 200 forage samples (classified as alfalfa, grass, alfalfa-grass mix or cereal green-feed hay), with a range in crude protein (CP) from 6.0 to 19.5% and total digestible nutrient (TDN) ranging from 45.2 to 64.5%. Sheppard et al. (2014) observed that most beef cattle operations in Canada harvested forage after full head or full bloom. If fed alone, it may not contain sufficient nutrients to meet the requirements of cows in the last trimester of pregnancy (545 kg), which require 9 to 10% CP and 57% TDN (NRC, 1996). Nutrient demands for backgrounding steers (334 -590 kg) growing at 1.7 kg d^{-1} are even greater at approximately 60% TDN and 10.2% CP (NRC, 1996). For both classes of animals, requirements increase when animals are exposed to cold temperatures. In many cases, the forage produced is not able to meet the requirements of backgrounding animals and supplementation should be used as part of the feeding strategy. A more appropriate energy to protein ratio may be achieved with greater use of legumes, earlier harvesting or nitrogen (N) fertilizer application (Sheppard et al. 2014). Inadequate dietary N concentration is not only detrimental to rumen microbial efficiency; animal growth and productivity (ARC, 1980); it can also cause negative environmental implications as a consequence of unutilized N and P excretion.

There are limited data regarding the quality of diets backgrounding animals receive because of the lack of feed analysis on both pasture and preserved forage. However, it is known that poor-quality forage diets can result in increased enteric methane (CH_4) emissions (Bernier et al., 2012), though making recommendations regarding feeding strategy is of limited value if the quality of feed is unknown.

1.2 **Enteric methane production in beef cattle**

Enteric CH_4 is a by-product of microbial fermentation and is primarily produced in the rumen but it can also be produced in the hindgut of ruminants and monogastrics (Immig, 1996). Of the CH_4 produced in the lower digestive tract, 89 ± 2.3% is absorbed into the blood stream and exhaled and the remaining 11% is excreted through the anus (Murray et al. 1976).

Within the microbial community are a group of anaerobic organisms that produce CH_4 (Wolin and Miller, 1988) called methanogens (Kumar et al., 2009). Methanogens use hydrogen gas (H_2) and carbon dioxide (CO_2), produced during carbohydrate fermentation, which aids in the complete oxidization of those substrates (Hook et al. 2010). Without methanogens in the rumen, H_2 would build up and carbohydrate fermentation would be compromised (McAllister et al. 1996). The production of CH_4 beyond that required for optimal carbohydrate utilization can have negative environmental and production efficiency implications.

1.2.1 Environmental impacts of enteric methane emissions

The loss of feed energy as CH_4 has broad implications as it contributes to the greenhouse gas (GHG) inventory both in Canada and globally. The National Inventory Report: Greenhouse Gas

Sources and Sinks in Canada states that agricultural enteric CH_4 emissions contribute 18 Mt of CO_2 equivalents (CO_2-eq) per year (Environment Canada, 2013). This is 2.56% of total GHG emissions in Canada and makes up one third of the emissions attributed to the Canadian agricultural sector.

Several studies have compared the GHG emitted during the production of food proteins. In a Canadian study by Dyer et al. (2010), it is suggested that beef production contributes 119.0 kg CO_2-eq kg^{-1} of protein and that the production of milk, pork and eggs produces significantly less GHG at 31.7, 24.9 21.9 kg CO_2-eq kg^{-1} protein respectively. An assessment of meat production in 27 countries of the EU (Lesschen et al. 2011), using IPCC conversion factors for nitrous oxide (N_2O) and CH_4, showed that beef had the highest production of net GHG (22.6 kg CO_2-eq kg^{-1} of product) compared to other proteins including; pork, poultry and milk with 3.5, 1.6 and 1.3 (kg CO_2-eq kg^{-1} product). Further, a life cycle analysis study from the UK (Garnett, 2009) found beef emissions (16 kg CO_2-eq kg^{-1} of beef) to be significantly higher than wheat (0.8 kg CO_2-eq kg^{-1} of wheat). Therefore, improved rumen fermentation efficiency and a reduction in CH_4 emissions are worthy goals for Canada's cattle industry.

It is important to critically consider the unit of CH_4 expression used to describe a CH_4 mitigation strategy. For the purposes of this thesis, efficiency was classified as CH_4 produced as a percentage of gross energy intake or % GEI (Johnson and Johnson, 1995) and net CH_4 emissions (L) per feeding period. Improved efficiency was measured by a decrease in CH_4 (% GEI) or by decreased total litres produced during the feeding period.

1.2.2 Production loss associated with increased enteric methane

Although the environmental impacts of CH_4 production are important, the animal production and economic consequences are also considerable. Enteric CH_4 has no nutritional value for the ruminant and therefore it is considered a loss of dietary energy (Hungate, 1975) ranging from 3% from grain diets (Beauchemin and McGinn, 2005) up to 11% GEI from forage diets (Ominski et al., 2006). An overwintering study conducted by Ominski et al. (2006) measured CH_4 emissions from growing cattle offered all-forage diets of varying quality as defined by neutral detergent fibre content (NDF, %). Average daily gains (ADG) were recorded to be 0.83, 1.06, 1.04 and 1.00 (SE ± 0.03) for diets with 60.8, 53.2, 51.2 and 46.4 (%) NDF respectively. The slower rate of weight gain lead to a greater number of days in the backgrounding phase and thus higher emissions expressed as CH_4 L kg^{-1} of ADG with 231.1 ± 18.2, 218.7 ± 17.4, 208.1 ± 16.3 and 211.9 ± 18.1, for diets with 60.8, 53.2, 51.2 and 46.4 (%) NDF respectively. Adequate protein intake is also an important consideration for optimizing animal performance. In situations where a nutritional deficiency can be corrected and animal production can be maximized, wasted feed energy will be reduced by decreasing CH_4 emissions thereby reducing cost of production.

1.3 **Protein utilization in ruminants**

The CP requirement of a 320 kg backgrounding steer expected to finish at 500 kg and maintaining a 0.79 kg d^{-1} ADG, is approximately 10.75% CP, dry matter basis (DM), in a thermal neutral environment (NRC, 1996). Animal protein requirements can be sourced via the ruminant's diet or the microbial community (Janssen, 2010) with the degradation of rumen microbes in the gastrointestinal tract. Dietary protein can be ruminally degradable or undegradable. Rumen degradable protein is the portion of protein that is degraded in the rumen

and acts as the primary source of N in the form of amino acids (AA) and peptides for rumen microorganisms (Waterman et al. 2014).

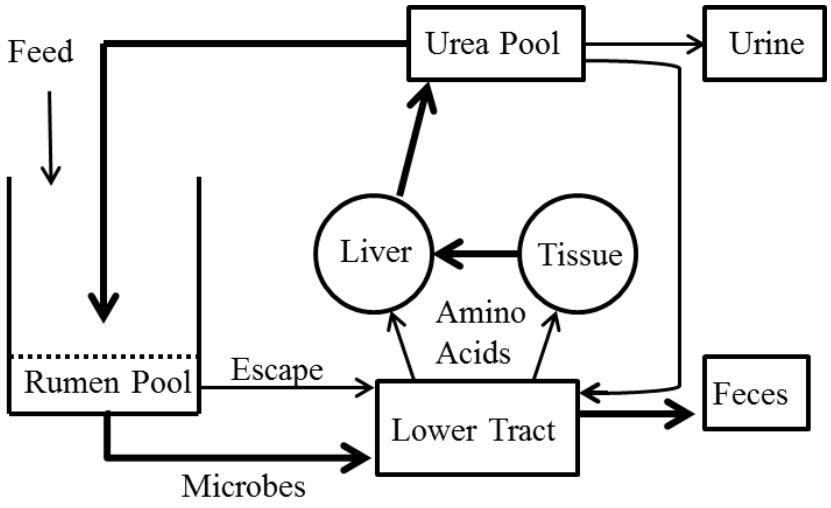
Undegradable protein escapes from the rumen to the small intestine for absorption into the blood stream or may even be excreted from the body unused (NRC, 2000), as indicated in Figure 1. Non-protein nitrogen compounds can also be used by rumen microorganisms as an amino acid source for cell growth when broken down to ammonia and combined with carbohydrate metabolites (Waterman et al., 2014). In this way, adequate energy is required for microbes to incorporate NPN into microbial protein which can be digested and also used for animal protein (Waterman et al., 2014). Total AA-N is about 80% of microbial N and the rest is considered non AA-N, 15.4% of which is attributed to nucleic acids (Ørskov, 1982). Whereas microbial protein is fairly consistent in composition and digestibility, dietary protein entering the small intestine can be variable in digestibility (Ørskov, 1982). Most ruminant feeds will yield approximately 200 g of microbial protein from every kilogram of organic matter ingested (McDonald et al., 2002). However, the amount of microbial protein produced is greatly dependant on the type and quality of feed ingested and can reach 260 g of microbial protein for every kilogram of organic matter ingested of immature forage containing a high proportion of soluble carbohydrates (McDonald et al., 2002).

Many environmental factors can increase rumen degradability of forage protein including; increased soil fertility, presence of vegetative plant growth and sufficient biomass to support selective grazing of high quality forage. The undegradable fraction of forage can increase from inadequate preservation practices such as baling of damp forage for hay, poor packing of silage leading to non-anaerobic conditions or low soluble sugar levels at the time of ensiling, resulting

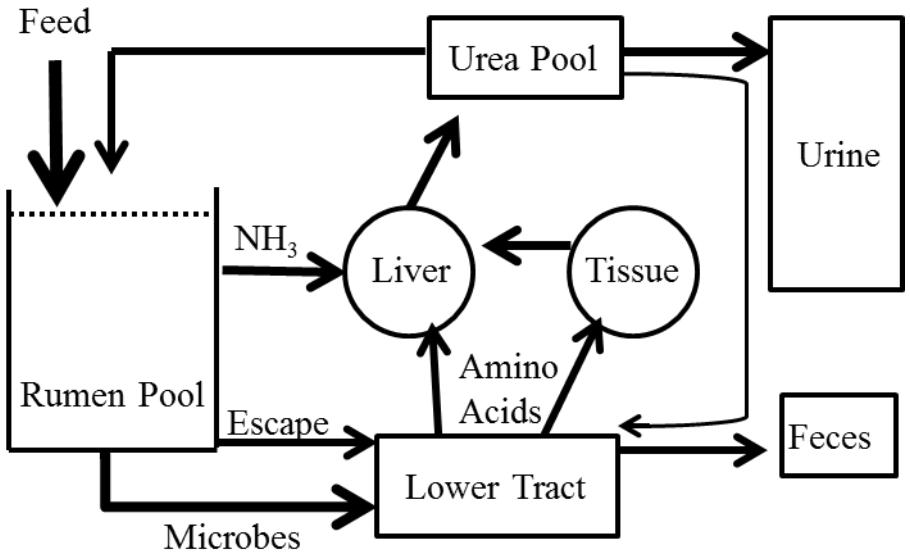
in spoilage. The subsequent heat can be enough to damage protein quality and potentially make proteins resistant to rumen microbial

Figure 1. Representing nitrogen flow through the ruminant body as modified from Van Soest (1982).

A. Low Intake



B. High Intake



fermentation or digestive enzymes (Baah and Shelford, 1999). Preserved grass in the form of hay often contains more soluble protein than fresh grass pasture since the drying process converts some insoluble protein to soluble (Baah and Shelford, 1999). However, the slower rate of protein degradation makes the hay protein less available overall (Baah and Shelford, 1999) and may result in inefficient bacterial CP synthesis (NRC, 1996). Further, slower passage rates require more energy for microbial maintenance also lowering efficiency (NRC, 1996). Ritzman and Benedict (1938) found that ruminant CH_4 emissions were lower when animals were offered protein rich diets and higher when offered diets with higher crude fibre. Dry matter intake (DMI) and nutrient digestibility are typically higher in the early stages of plant maturity and decrease with increasing plant physiological stage (DeRamus et al. 2003).

Rumen degradable protein is required for rumen microbial growth and therefore microbial fermentation, DMI and nutrient flow to the small intestine for use by the animal. It is estimated that CP required for microbial communities in the rumen is 6-8% of DM fed (Van Soest, 1982). In most cases this does not satisfy the animal's protein requirements for production which can differ depending on many factors including age, breed, production stage of animal, environmental conditions etc. The amount and type of protein impacts animal's intake and rate of feed passage, especially with high fibre diets, where decreased protein availability may decrease the rate and amount of bacterial CP synthesised (NRC, 1996), slowing digestion and decreasing the dietary and microbial protein available to the animal.

1.3.1 Feed intake and rate of passage

The volume of the rumen dictates the meal size that cattle are able to consume. Forage diets often bulky and/or have high fibre contents and therefore rumen fill may limit intake before a sufficient quantity of nutrients has been consumed (Baile and Forbes, 1974). Feed in the rumen has to be fermented and passed into the lower gastrointestinal tract before more feed can be consumed. Typically, forage-based diets have a slower rate of passage because of the time required for rumination and to reduce the large particle sizes in the rumen (Zebeli et al., 2007). This process can be slowed further if dietary protein is limited, restricting growth and activity level of microbes in the gut (DeRamus et al., 2003). Typically, greater feed intake is a result of higher feed quality and a higher rate of organic matter degradation directly affecting the rate of passage through the rumen (Okine, 1989). With this shorter time spent in the rumen there, is less time for ruminal fermentation, less H_2 production and less time for methanogens to produce CH_4 (Kumar et al., 2009). If maximum DMI and efficient energy use are achieved, CH_4 emissions (% GEI) will be reduced (Beauchemin and McGinn, 2006) and animal productivity will be increased. Cattle are able to adapt, to some extent, to adverse dietary N conditions by conserving AA and recycling N in times of limited supply (Waterman, 2014) or excreting rumen degradable protein if in excess as shown in Figure 1.

1.3.2 Animal response to low CP

Previous research suggests protein supplementation of low quality forage diets will decrease CH_4 emissions. In a study by Bernier et al. (2012), beef cows were fed low quality forages supplemented with dried distillers' grain for diets with CP levels of 6.0% (deficient), 8.7% (sufficient) and 11.6% (excessive). The excessive CP diet decreased CH_4 by 18.5% GEI

compared to cows consuming the CP deficient diet. DeRamus et al. (2003) monitored CH_4 emissions from cows and heifers continuously grazed or intensively grazed on low quality pastures (< 7% CP) supplemented with protein molasses blocks, cottonseed meal and corn or urea and corn during the winter. Greater CH_4 emissions corresponded to hay with lower digestibility. However, the intensively managed pasture yielded a 22% reduction in CH_4 kg of beef gain^{-1} compared to the continuously grazed pastures.

In contrast, a study by Wilson et al. (2010) did not show a decrease in CH_4 emissions when pasture quality was improved. In that trial, steers were grazed on pastures with a split or single application of hog manure at rates of 70 ± 6 kg available N ha^{-1}and 142 ± 20 kg available N ha^{-1}, respectively, and no manure application as a control. After fertilization, the CP content of the pastures were 58.6% and 53.7% higher than the control pasture for the split and single application pastures, respectively. The authors noted that manure application ensured animal minimum CP requirements were met or exceeded by grazing. The CP content of forage without manure application was 9.5% which is considered deficient for a 325 kg steer gaining 1 kg d^{-}, however, blood serum urea nitrogen (BUN) levels were adequate (2.5 ± 0.4 mmol L^{-1}). Forage GE did not change with manure application suggesting that the protein: energy ratio increased with increasing manure application. Methane emissions ranged from 6.0 to 6.4% GEI with no significant difference observed among steers on the three pastures. The lack of response and relatively low CH_4 emissions can be attributed to the large amount of standing biomass which allowed animals to selectively graze for plants that are most nutritious without additional energy expense (Ominski et al. 2006).

Microbial protein synthesis and ruminal degradation is largely dependent on the concentration of ammonia (NH_3) N in the rumen fluid. Rumen NH_3-N concentrations of less than 50 mg L^{-1} are

considered low and therefore microbial growth would benefit from protein supplementation (Satter and Slyter, 1974). Inadequate rumen NH_3-N can be caused by protein deficient diets or when dietary protein is poorly degraded and therefore less accessible to the animal.

1.3.2.1 *Recycling urea*

To compensate for low dietary intake or diets with high proportions of rumen undegradable protein, ruminants are able to recycle NH_3 by converting it to urea in the liver and circulating it back into the rumen via blood or saliva (Hammond, 1997). The amount that can be recycled depends on the concentration of N in the blood and the amount of saliva secreted (Bailey, 1961; Nolan and Leng, 1972). In a study by Nolan and Leng (1972), it was shown that of the 14.2 g N d^{-1} entering the ruminal NH_3 pool of sheep, 4.3 g N d^{-1} or 30%, was recycled. The amount of saliva secreted greatly depends on the basal composition of the diet, increasing with longer fibre lengths that are typical in forage diets (Kay, 1966). In ruminants, a decreased BUN concentration is related to low protein intake causing a draw of urea out of the blood for N recycling (Säkkinen, 2005). Houpt (1959) injected sheep with urea while feeding low protein diets. Of the injected urea not recovered after basal requirement and urine excretion were accounted for, 22% was used in the rumen for the low protein diet with typical carbohydrate concentration and 52% was used for the low protein carbohydrate supplemented diet. Therefore when additional carbohydrates are available the animal is able to recycle greater amounts N. Conversely, if excess protein is fed or the degradation process occurs at a greater rate than microbial synthesis, NH_3-N will accumulate in the rumen and blood (MacDonald et al., 2002).

1.3.3 Response to excessive CP

1.3.3.1 *Nitrogen excretion*

Proteins typically breakdown faster than carbohydrates, resulting in high levels of NH_3 in the rumen (NRC, 1996) shortly after feed consumption. Whenever there is excess NH_3 in the rumen relative to energy, the unused NH_3 will be absorbed, transported to the liver and converted to urea to prevent NH_3 toxicity (AFRC, 1993), as depicted in Figure 1. The liver also receives NH_3 to be converted to urea from post-ruminal deamination and from tissue (muscle) breakdown. Sheppard and Bittman (2011) estimated typical N excretion levels of steers to be 95 – 210 g N $animal^{-1}$ d^{-1} when maintained on pastures, in corrals, feedlots or barns in Canada. The goal of diet formulation should be to supply rumen degradable protein that matches available energy. If this is accomplished, unnecessary N excretion will be avoided, reducing environmental implications and unnecessary feed costs (AFRC, 1993).

Low feed digestibility can also cause increased excretion of nutrients. A study by Kennedy et al. (1982) showed cold-exposed sheep fed forage diets excreted 20-23% more unused dietary N when N digestibility was reduced by 5-7%. Yan et al. (2007) examined dietary N utilization efficiency in 286 beef cattle from 14 digestibility studies (1984 to 2003) for mean fecal N: N intake (g/g) ratio was 0.321 and the urinary N: N intake (g/g) ratio was 0.460. This study also found that increasing dietary quality, in this case metabolizable energy, reduced N excretion. Nitrogen excretion as a proportion of N intake was reduced proportionately by 0.048 with each 1 MJ increase in metabolizable energy per kilogram of diet DM of diet. They reported the increased dietary energy improved the ratio of fermentable N and organic matter available to the

rumen microbes, leaving less unused NH_3-N that was absorbed into the blood stream and excreted in urine or feces as urea.

It should be noted that environmental conditions and physiological state of an animal can change animal requirements. Therefore the protein: energy ratio should be monitored closely as it will impact rumen microbial fermentation efficiency associated with feeding lower quality diets in the backgrounding stage of production.

1.4 **Sources of energy for ruminants**

Most ruminant bacteria use carbohydrates as a primary energy source for microbial growth and amassing microbial protein as they cannot use protein, fat or ash as an energy source (Russell et al. 1992). Methane is considered an energy loss for ruminants and therefore, from a production perspective, is an inefficient outcome of rumen fermentation (Hungate, 1975, Johnson and Johnson, 1995).

1.4.1 Volatile fatty acid pathways

Ruminants use volatile fatty acids (VFA) derived from rumen fermentation of carbohydrates for energy (Russell et al., 1992). Volatile fatty acids are not commonly used as substrates for methanogenesis because their conversion into CO_2 and H_2 is a long process and often is incomplete before VFAs are absorbed or moved out of the rumen to the lower gastrointestinal tract (Hook, 2010). The VFAs produced by rumen fermentation include predominately; acetate, butyrate and propionate (Van Soest, 1982) with smaller amounts of valerate, caproate, isobutyrate, isovalerate, 2-methylbutyrate and traces of other acids as microbial fermentation end

products (France and Dijkstra 2005). Gases including CH_4, NH_3, CO_2 and H_2 gas are produced during the fermentation process which also creates heat (NRC, 1996). This heat can be helpful for cold stressed animals to maintain their body temperature but overall is considered lost energy and is not accounted for in metabolizable energy (NRC, 1996).

A general description of fermentation in the rumen can be displayed as;

$$C_6H_{12}O_6 + NH_3 \rightarrow \text{microbes} + CH_4 + CO_2 + \text{VFA}$$

Volatile fatty acids are generated through a number of pathways as outlined below (Van Soest, 1982):

Pathway 1. $C_6H_{12}O_6$ (glucose) + $2H_2O \rightarrow 2C_2H_4O_2$ (acetate) + $2CO_2$ + 8H

Pathway 2. $C_6H_{12}O_6 \rightarrow C_4H_8O_2$ (butyrate) + $2CO_2$ + 4H

Pathway 3. CO_2 + $8H \rightarrow CH_4$ (methane) + $2H_2O$

Pathway 4. $C_6H_{12}O_6$ + $4H \rightarrow 2C_3H_6O_2$ (propionate) + $2H_2O$

Of the VFAs produced, acetate leads to the greatest production of H^+ (Pathway 1) and therefore results in the greatest production of CH_4 (Pathway 3). Butyrate also produces H^+ but to a lesser extent than acetate. Propionate (Pathway 4) is electron accepting as $C_6H_{12}O_6$ is reduced to propionate and protons (H^+) are reduced to H_2, decreasing the availability of H^+ for methanogenesis.

McAllister et al. (1996) reported that, although CH_4 production in the rumen can be reduced by shifting the pathway of fermentation toward propionate production, it cannot be eliminated without negative effects on rumen fibre digestion. Methanogens do not degrade fibre; however they can enhance fibre digestion from other rumen microorganisms by preventing the build-up of

H_2 and reduced nucleotides like NADH (Joblin et al., 1989). Removing methanogens entirely therefore would inhibit fibre digestion.

The ratio in which VFA are produced is dependent on the type of substrate that is fermented (Moss et al., 2000), with acetic acid generally accounting for 60 to 70%, propionic acid 15 to 20% and butyric acid 10 to 15% of the total VFA produced (Immig, 1996). Volatile fatty acids can make up 50-70% of the animal's digestible energy intake and are mainly absorbed through the rumen wall, oxidized in the liver and are then used to meet the animal's carbon needs (France and Dijkstra, 2005).

In addition to the type of feedstuff, other factors also impact the proportion of each VFA produced. Increasing digestibility of feed by decreasing particle size of forage tends to increase the proportion of propionate within the fermentation end products (Janssen, 2010). Increased rumen fill and increased feeding frequency have also been shown to increase the proportion of propionate produced as the greatest production of propionate occurs just after feeding and becomes less as the feed is digested (Janssen, 2010). Higher passage rates are associated with fermentation pathways that produce more propionate and less H_2 (Pathway 4) per unit of feed because of the limited time for methanogens to generate CH_4, as discussed above, (Johnson and Johnson, 1995) lowering the acetate: propionate ratio (Kumar et al., 2009).

1.4.2 Consequences of energy deficiency in the rumen

There is potential for a reduction in CH_4 emissions when the correct protein: energy ratio promotes efficient rumen microbial activity. If the rate of protein degradation is greater than the rate of carbohydrate fermentation, N can be absorbed from the rumen as NH_3 and excreted as

urea N (Russell et al., 1992). If the amount of available protein is too low to sustain microbial growth, the rate of carbohydrate fermentation may decline (Russell et al., 1992).

Energy can be retained as either tissue or fat (NRC, 1996) and as energy intake increases above maintenance requirements, protein synthesis will be limited and excess energy will be deposited as fat (NRC, 1996). When energy is not limiting for growth, the empty body will gain smaller proportions of protein and larger proportions of fat and the animal is considered "chemically" mature when added BW contains little protein (NRC, 1996). If net energy intake is less than energy requirements, energy stores like fat deposits, and eventually muscle, will be used (Weiss, 2007) for maintenance which will negatively impact the animal's weight or body condition.

1.4.2.1 *Tissue utilization*

A study by Torbit et al. (1985) examined the starvation response of mule deer in terms of protein and fat catabolism. It was observed that this response can be characterized by three phases. The initial phase marks a small decrease in body protein for maintenance of energy homeostasis. If energy supplies are still deficient, fat reserves and small amounts of protein will be used to meet energy requirements. The last phase is characterized by large losses of both fat and protein supplies.

Blood serum urea nitrogen can indicate the N available to the animal via dietary protein. However, high BUN levels not only indicate a high protein intake but they can also indicate excessive mobilization of muscle (Chimonyo et al., 2002). A study by Chimonyo et al. (2002) considered acceptable BUN concentration for working draft cattle to be 2.5 mmol L^{-1} and showed that those unsupplemented cattle had increasing BUN levels (1.0 to 9.0 mmol L^{-1}) with

decreasing body condition. This relationship implied that the animals utilized tissue reserves to supplement deficient dietary protein levels.

In order to prevent energy wastage and additional environmental GHG implications, CH_4 mitigation strategies are desirable. This may be achieved a number of ways including the inhibition of H_2 producing reactions or promotion of the VFA pathways that use up H_2 (Boadi et al. 2004) and protein supplementation to optimize rumen fermentation efficiency (Bernier et al., 2012). The latter can be accomplished by improving animal N status.

1.5 **Measuring animal nitrogen status**

An animal's N status is an important indicator of nutrient intake and overall nutrient utilization. Physical indicators, like body condition score or BW can suggest if an animal has had an adequate or deficient plane of nutrition over time, however without specific data regarding energy and N intake, these measures are of limited value (Hammond, 1997).

As rumen NH_3 is required for microbial growth, sufficient rumen NH_3 levels should indicate appropriate animal N status. Satter and Slyter (1974) recommend maintaining a level of 5.0 mg NH_3 100 ml^{-1} of rumen fluid for optimal microbial growth. Levels below 2.0 mg NH_3 100 ml^{-1} are considered deficient and limit microbial growth but levels over 5.0 mg NH_3 100 ml^{-1} do not have a further positive or negative affect.

Nitrogen status can also be measured by BUN which has a high positive correlation to rumen NH_3 (Hammond, 1997). Increased dietary N or N solubility increases the rumen NH_3 concentration and therefore BUN levels increase in tandem. Hammond (1983) fed growing steers isocaloric diets with dietary protein concentrations of 6 to 18% DM yielding BUN

concentrations of 0.9 mmol L^{-1} to 4.0 mmol L^{-1} respectively (Hammond, 1983). Some literature suggests appropriate ranges of BUN to be as wide as 2.1 to 7.9 mmol L^{-1} (Kodak Diagnostics, 1991), where other literature suggests that BUN levels for growing steers (where maximum rates of gain were 1.1 to 1.3 kg d^{-1}) should be between 3.9 and 5.4 mmol L^{-1} (Byers and Moxon, 1980) or lower at 2.1 to 3.6 (Ndlovu et al., 2007).

1.5.1 Nitrogen status as affected by extreme cold

As stated earlier, environmental conditions can affect the nutritional requirements of animals and cold temperature can be a major influence, especially on the Canadian prairies. A review by Young (1981) discusses overwintering cattle on the Canadian prairies (Manitoba, Saskatchewan and Alberta) where cattle are subject to temperatures between -10 to -20 °C and dropping below -30 °C for up to 90 days each winter (Young, 1981). Effect of cold can depend on the age and size of the animal, where smaller, younger animals are more affected by cold and temperature change (Christopherson, 1976). However, regardless of age in extreme cold conditions, the rate of passage through the rumen is increased due to increased reticulorumen activity resulting in reduced time in the rumen and time for microbes to degrade feed (Kennedy and Milligan, 1978; Young, 1981; Gonyou et al 1979). Increased passage rates from the rumen due to cold exposure results in more dietary protein escaping degradation and 5-13% more non-NH_3 – N entering the small intestine (Kennedy et al., 1982; Kennedy and Milligan, 1978). Cold exposure has also been shown to increase blood flow to the rumen (Thompson et al., 1978), this increase in blood flow is thought to decrease VFA concentration in the rumen via increased VFA uptake by the blood (Kennedy et al., 1976; Kennedy and Milligan, 1978). Greater absorption of both N and VFA from the rumen decreases the concentrations of those compounds within the rumen and

triggers the body to increase urea recycling. Therefore, cold-adapted animals are able to utilize nutrients more efficiently (Kennedy and Milligan, 1978). In fact, Kennedy et al. (1982) showed that, for sheep offered bromegrass pellets, urea recycling increased by 30% while exposed to temperatures of 1 to 5°C compared to those housed at 22 to 25°C (7.3 g N d^{-1}). Further, rumen NH_3 concentration decreased by 12-20%, while transfer of plasma urea N to the rumen NH_3 pool increased (9.5 g N d^{-1}) for cold sheep compared to warm sheep.

If cattle have access to feed ad libitum while in cold conditions, feed intake and therefore nutrient intake will increase (Baile and Forbes, 1974). With an increase in intake comes increased digestion of non-NH_3 N and an increased protein: energy ratio in cold exposed animals (Christopherson and Kennedy, 1983). The amount of non-NH_3 N gained from endogenous sources increased from 3.9 to 4.7 g d^{-1} ($P < 0.05$) when sheep were exposed to cold (Kennedy and Milligan, 1978) but the concentration of rumen NH_3 was lower in cold sheep ($P < 0.05$) than warm sheep. In a study by Adamczewski et al. (1994), mature Hereford cows compensated for low-quality forage diets by increasing intake, exceeding requirement recommendations from the National Research Council during cold temperatures. Therefore, cold-stressed cattle and sheep should be able to utilize lower protein diets more effectively than thermal neutral animals since intakes increase and nutrients are utilized more efficiently (Ames and Brink, 1977; Ames et al. 1980). However, if forage quality slows the rate of passage to the extent that increased intake is not possible, efficiency benefits will be void.

1.6 **Current modelling methods**

These differences in climate, diet composition and utilization can all have an impact on CH_4 emissions from cattle. As a result of the diverse production systems and environmental conditions under which we raise cattle, it is difficult to accurately predict emissions from cattle ruminant livestock.

1.6.1 Intergovernmental Panel on Climate Change

The intergovernmental panel on climate change (IPCC) was developed by the United Nations Environment Programme and the World Meteorological Organization (IPCC, 2013a). This international (195 countries) voluntary panel does not conduct research or collect climate data but it does review the most current and relevant data on the subject.

The Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories: CH_4 Emissions from Enteric Fermentation (Gibbs et al., 2000) describes two models to predict CH_4 emissions - Tier 1 and Tier 2.

1.6.1.1 *Tier 1 Methodology*

Firstly, CH_4 estimation using the Tier 1 method requires population data of livestock which are generally census collected by the local agricultural ministries. If there are no data available from a local source, data are used from the Food and Agriculture Organization (FAO, 1999). The Tier 1 approach uses a default conversion rate which is a predetermined constant for the rate at which feed energy is converted to CH_4. The conversion rate is typically based on feed quality but if these data are not available, standard values are used. For developed countries conversion rates

of 6.5% and 3% (± 1.0%) are recommended for all cattle and feedlot cattle respectively. When feed has high digestibility and energy values, it is recommended to use the lower bounds of the conversion rate. For developing countries, there are general estimates for dairy, non-dairy and grazing cattle. Because these emission factors are not necessarily based on country-specific conditions, the uncertainty of this method can be high.

Emission factors are based on feed intake and conversion rates of feed energy to CH_4. A country is characterized by the annual livestock population structure, weight classes, rate of gain and production yield. These values are used to estimate the energy requirements for maintenance and production.

Using Tier 1 methodology, the North American dairy herd is characterized as one that utilizes high-quality forage and grain, resulting in the production of 6 700 kg hd^{-1} yr^{-1} of milk on average. The beef cow herd is characterized as mainly grazing with seasonal supplements and fast growing steers and heifers finished in feedlots on grain with a production rate of 118 and 47 kg hd^{-1} yr^{-1} used for the dairy and non-dairy beef herd respectively (Gibb et al. 2000).

1.6.1.2 *Tier 2 Methodology*

Tier 2 methodology is more robust in that more factors that can influence CH_4 are considered including annual average populations of each animal type (mature dairy, mature non-dairy and young cattle), average daily feed intake (MJ d^{-1} DM and kg d^{-1} of DMI) to estimate GHG production. This method is suggested for countries with large ruminant populations because management practices can vary greatly within a country. Cattle are characterized as described for Tier 1, but feeding situation information is also included; animals are confined or are grazing

over large areas. The greatest challenge of the Tier 2 method of prediction is the availability of sound data regarding diet characterization and intake (Gibbs et al. 2000).

1.6.2 BUN as a predictor of methane

As information regarding diet quality and intake are often limited, prediction estimates would benefit from more direct animal measurement. If we consider that increased N may increase rumen efficiency and therefore decrease CH_4 emissions, we might consider using an indicator of animal N status to predict CH_4 emissions. Blood serum urea nitrogen is a measurement of N in an animal's blood and it is determined by a blood test that can be taken on- farm.

1.6.3 Meta-analysis for model development

In order to determine if BUN can accurately predict CH_4 emissions, a data set can be created by compiling several CH_4 studies that contain BUN as a model variable for a meta-analysis. Meta-analyses compare and combine treatment effects of individual studies (Viechtbauer, 2010) and can also be used to explore between-study variability or heterogeneity of the treatment effects (Duffield et al., 2008). This technique assumes that the studies selected are a random sample of the entire population of studies so that any findings can be generalized beyond the studies included (Hedges and Vevea, 1998).

One type of meta-analysis involves a regression process called backward elimination (Gill, 1978). This technique begins with a set of variables that have been chosen as most likely to impact the model, being as inclusive as possible, and also considering possible interactions. Variables corresponding to the lowest t-statistic are removed from the model. The estimates are

again computed and the process is repeated until all variables correspond to a t-statistic that is larger than the critical value. During this process, interaction terms are considered for elimination before individual terms. This is logical because if there is a significant interaction, the individual terms that make up that interaction should be included in the model, even if they are not significant alone.

1.7 **Conclusion**

Based on the current status and production practices of the Canadian cattle industry, there is reasonable cause for concern that backgrounding cattle may not be receiving adequate dietary protein when fed forage diets. Dietary deficiencies can lead to inefficient rumen digestion contributing to CH_4 emission inventories and increased feed costs via wasted feed energy. Feed production practices and feeding strategies should be closely considered to ensure animals receive adequate nutrient. Further, the concept of offering more than adequate CP for improved rumen efficiency should be explored to improve environmental sustainability and productivity.

Currently, feed quality inventory data from cattle operations are sparse and inconsistent. Without a sound data set characterizing feed quality and animal consumption, it is very difficult to predict CH_4 emissions using the available Tier 2 model (IPCC, 2007). The validity of CH_4 prediction models would be improved with a more direct measure of animal efficiency like animal N status.

2. RESEACH HYPOTHESES AND OBJECTIVES

2.1 Hypotheses

Backgrounding cattle offered forage diets containing CP concentrations greater than that required for microbial and animal growth will improve rumen efficiency and lower enteric CH_4 emissions. If by increasing dietary CP to correct a protein deficiency, animal nitrogen status (BUN) will increase and more N will be available for microbial activity in the rumen. Further, an increased rate of fermentation resulting from an increased protein: energy ratio will increase DMI and animal productivity, thereby decreasing CH_4 emissions (% GEI) and the number of days required for backgrounded animals to achieve targeted gains. A decrease in the number of days in the backgrounding phase will decrease overall emissions from forage-fed animals.

Blood serum urea nitrogen will be highly correlated to dietary CP and will be a valid tool for estimating not only the N status of cattle but also rumen efficiency and resulting CH_4 emissions. The relationship between BUN and CH_4 production will be non-linear whereby beyond some concentration of dietary CP, no further improvement in efficiency (and therefore reduction in CH_4) will occur with additional dietary CP. The relationship between BUN and CH_4 will be quadratic, increasing linearly at low levels and plateauing once the microbial community has reached maximum efficiency at some point above 2.1 mmol L^{-1}.

A meta-analysis and backward elimination regression method will determine BUN as a significant variable for CH_4 emission prediction that can be easily measured on-farm to remediate the downfalls of current emission models which lack consistent and reliable diet and intake data required for prediction.

2.2 **Research objectives**

The overall objectives of this study were to assess enteric CH_4 emissions from cattle offered forage diets to determine if increased dietary CP will improve the efficiency of the rumen thereby decreasing CH_4 emissions and to develop a model to predict CH_4 emissions that can be easily measured on-farm without the need for detailed information regarding feed quality and quantity inventories.

The specific objectives of the first manuscript were to: 1) observe the impact of dietary CP on gain in backgrounding cattle, 2) to create groups of cattle differentiated by N status by offering diets varying in CP concentration and measuring N status via BUN levels, and 3) to observe the impact of dietary CP on rumen metabolic efficiency as measured by CH_4 emissions.

The specific objectives of the second manuscript were to: 1) examine the relationship between BUN and enteric CH_4 emissions from cattle to determine the validity of a prediction model based on on-farm BUN measurements, 2) to observe CH_4 emissions from cattle with inadequate BUN concentrations (< 2.1 mmol L^{-1}) and determine if those cattle produce more CH_4 as a % GEI, and 3) to determine the BUN concentration which is most efficient, resulting in reduced CH_4 emissions (% GEI).

3. MANUSCRIPT 1

Enteric methane produced by backgrounded beef steers as affected by dietary crude protein content of forage diets fed during extreme cold

3.1 **Introduction**

Methane production has a significant impact on the Canadian cattle industry as it contributes to the national GHG inventory, accounting for 3% of Canadian annual emissions (Environment Canada, 2013). Not only do these emissions negatively affect the public's perception of the industry, they decrease production efficiency because every liter of CH_4 equates to a loss of 36.2 kJ of energy. Of the gross energy (GE) consumed by ruminants between 3% from grain diets (Beauchemin and McGinn, 2005), and up to 11% from forage diets (Ominski et al., 2006), can be emitted as methane. Increasing rumen fermentation efficiency can decrease methane production and improve production efficiency and therefore potential profitability of beef producers in Canada.

When dietary CP concentrations are below 7%, rumen microbial efficiency is compromised (Van Soest, 1982). It has been shown that enteric CH_4 emissions of mature cows increase by 18.5% (GEI) with decreased forage CP concentration from 11.6 to 6.0 % CP (Bernier, 2011) due to compromised rumen fermentation. Therefore, it was hypothesized that increasing forage CP concentrations, to meet or exceed rumen microbial requirements, would increase efficiency of rumen fermentation and lower enteric CH_4 emissions in growing cattle. A further increase in forage CP concentrations to meet or exceed animal CP requirements would improve animal metabolic efficiency and growth rate, thus reducing enteric emissions associated with weight gain. Rumen energetic efficiency was determined by measuring CH_4 emissions (% GEI) with the expectation that greater forage CP concentration results in a more efficient rumen microbial community thereby lowering CH_4 emissions and increasing BUN concentration. Blood serum urea nitrogen may be used as an indicator of N status of the animal and N availability to the microbial community.

The objectives of this study were: 1) determine the impact of dietary CP content on gain in backgrounding beef steers 2) to create groups of cattle differentiated by N status by offering diets varying in CP concentration and measuring N status via BUN levels and 3) to observe the impact of dietary CP on rumen metabolic efficiency as measured by CH_4 emissions.

3.2 **Methods**

3.2.1 Feeding and animal management

Sixty Red Angus cross steers (321 ± 14 kg) were housed (15 animals per pen) at the University of Manitoba, Glenlea Research Station. Steers were vaccinated with Vista Once SQ, Covexin Plus (Merck Animal Health, Kirland, QC) and given a supplemental vitamin A, D and E injection (Dominion Veterinary Laboratories Ltd. Winnipeg, MB), treated with Noromectin pour-on solution (Kane Veterinary Supplies Inc, Edmonton, AB) and ear tagged for identification purposes prior to the trial. Each pen received one of four diets fed ad libitum to achieve 10% orts for the duration of the trial. Each of the four pens was equipped with a heated watering bowl and the pens were partially enclosed by an open-faced shed and bedded with flax shives. Vitamin A, D and E booster injections (Dominion Veterinary Laboratories Ltd. Winnipeg, MB), were administered on day one. Following a 14- d feed and environmental adaptation period in which steers were fed trial diets, performance, BUN and enteric CH_4 emissions were measured during three, 21- d consecutive periods from January 22 to March 25. Each of the fours pens was equipped with four GrowSafe feeding system nodes (GrowSafe Model 4000E feed monitoring system, GrowSafe Systems Ltd., Airdrie, Alberta) to enable the collection of individual animal DMI.

The first diet was formulated to have a borderline adequate to low (L) CP concentration for rumen microbial growth at 7% CP (Van Soest 1982). The second diet, 9% CP, was developed to be adequate for rumen microbes (ARM) but inadequate for muscle growth (NRC, 1996). The third diet was formulated to 11% CP for adequate microbial and muscle growth (AMG) in a thermal neutral animal. Finally the fourth diet was formulated to have a 13% CP content, which exceeds (E) the requirements for a 330 kg steer gaining 0.76 kg d^{-1} (NRC, 2000). A full description of the diet ingredients can be found in Table 1.

Grass hay was pre-chopped and mixed with a Jaylor vertical mixer (Jaylor Fabricating, East Garafraxa, ON). Feed was offered five times daily to ensure constant access to feed.

Data collection within this study was approved by the Animal Care and Use Committee of the Lethbridge Research Centre according to the guidelines established by the Canadian Council on Animal Care. The research staff did not control direct handling and care of the animals in this study.

3.2.2 Feed sampling and analysis

All diet components were sampled at the beginning of the trial including hay and straw sampled via bale cores. The corn silage was sampled weekly to monitor the DM content and diets were adjusted accordingly as a consequence of changes in moisture.

All diet and ingredient samples were frozen at approximately -23°C until processing for nutrient analysis. The samples were dried in a forced air oven at 60°C for at least 48 hr to determine DM content, ground through a 1 mm screen. All analyses were analysed in duplicate. The ANKOM

200 automated fibre analyzer (ANKOM Technology, Macedon, NY) was used to measure acid detergent fibre (ADF), NDF values and the fat content of the ingredients (AOAC version 1/30/09). The modified AOAC (1990) 968.08 and 935.13A method was used to determine Ca and P. The starch fraction was measured with an YSI 2700 SELECT Biochemistry Analyser (YSI Incorporated Life Sciences, Yellow Springs OH).

The balance of the diet analyses were performed by DairyOne Forage Laboratory, Winnipeg, MB. The CP content of diets and components was measured by following an AOAC 990.03 method on a Leco FP-528 (LECO Corporation, St. Joseph, MI).

Total digestible nutrients were calculated by DairyOne Forage Laboratory using CP, NDF, fat, ash, lignin, acid detergent insoluble crude protein and neutral detergent insoluble crude protein values and a hybrid equation combining calculations from Weiss et al.(1992), Weiss (1993), Weiss (1995) and Stern et al.(1995).

3.2.3 Blood sample collection and analysis

Blood samples were collected before the trial began (day -19 of trial or -5 days of diet adaptation began) to establish a baseline BUN level and once per period (day 10 in each period) during the trial. Serum separator vacutainers (10 ml), containing gel and a clot activator, were used to collect the blood via tail vein or jugular puncture if necessary. Blood urea nitrogen was analyzed from serum samples using a colorimetric test with a Vitros 250 (Ortho Clinical Diagnostics Inc., Pub. No. MP2-9, Rochester, NY) by Veterinary Diagnostic Services (Manitoba Agriculture, Food and Rural Development, Winnipeg, MB).

3.2.4 Enteric methane collection and analysis

Enteric CH_4 emissions were collected using the sulphur hexafluoride (SF_6) tracer gas technique (Boadi et al., 2002) for 2 d in each of 3 period (total of 6 days). Stainless steel permeated tubes (12.5 x 40 mm) were filled with SF_6 gas and weighed weekly (14 weeks) to determine the rate at which SF_6 was escaping the tube. Perm tubes were selected for use in the trial based on: tube minimum half-life for expiration, tube minimum SF_6 flow rates of 388 ng min^{-1} and a maximum flow rate deviation from the mean of 3%. The SF_6 release rates averaged 612.8 ng min^{-1}. The tubes were inserted orally to the rumen of each steer using a speculum, on -8 days of the trial, prior to initial CH_4 gas collection to allow the SF_6 gas release from the perm tube to reach steady state in the rumen. The CH_4 collection was performed using pre-evacuated stainless steel canisters (130 mm diameter) connected to 900 mm capillary tubing (128 μm internal diameter) with a 15 μm filter and flexible nose piece mounted onto nylon cattle halters to collect the exhalation from the mouth and nose of each steer. During the collection period, steers were released back into their assigned feedlot pens while wearing the collection system. Four collection systems were also placed throughout the feedlot, three on the fences between pens so the steers could not reach them and one on the feed bunker adjacent the pens to collect background CH_4 and SF_6 samples.

The canisters collected gas for 24 hr on all animals for 360 attempted observations. Upon removal of the collection apparatus, visual observation determined if there was any damage to the collection devise that would impair sampling (broken hose, hose blockage etc.). Once the collection apparatus was removed from the steers they were pressure checked to ensure no leaks or blocks in the system that were not apparent from the visual check. Samples with canister pressures of between 160 and 500 mm Hg (21 and 66 kPa) were considered acceptable; those

outside this range were discarded due to a high likelihood of a leak or blockage causing a misrepresentative sample. The canisters were then pressurized to 3.38 mm Hg (0.48 kPa), with a pressurized N gas canister and pressure gauge, to prevent contamination of the gas sample before analysis. Using a Varian CP-3800 gas chromatograph (Varian Inc., 2004), CH_4 was quantified using flame ionization and SF_6 was quantified by electron capture detector (Varian, Maple Creek, CA). This instrument was calibrated with prepared standards (100 ppm CH_4 scotty gas – Aire Liquide Canada Inc., Winnipeg, MB 20 ppt SF_6 Scott-Marin gas – Air Liquide Canada Inc. Winnipeg, MB) and the peak area and retention time was used to determine the concentration of gases in the samples. The following equation was used to determine the proportion of enteric CH_4 in each sample after background CH_4 and SF_6 have been removed.

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

When SF_6 levels contained in the canister where similar to background SF_6 levels the sample was discarded. Further, if the ratio of SF_6 (ppt) to CH_4 (ppm) was not between 1:1 and 2:1 the sample was discarded. Once CH_4 data were compiled within the appropriate gas ratios, samples were removed if CH_4 values were greater than three standard deviations higher or lower than the mean CH_4 value.

3.2.5 Statistical analysis

Intake was assessed by calculating a test statistic for daily DMI as compared to the critical value based on the number of observations collected (Gill, 1978). Those intake values above or below three standard deviations of the mean intake during the days when the collection apparatus was put on or taken off were removed. Further, the period of time chosen for intake measurement as

corresponding with CH_4 production for the CH_4 (% GEI) calculation was chosen by comparing regression analyses using the SAS 9.3 TS Level 1M2 program (SAS, 2010) from three options: 1) intake during time while the animal was wearing the collection apparatus and adjusted to a 24-hr period. 2) Intake during the 24- hr day beginning at midnight of the day the apparatus was put on the animal and ending at the time the apparatus was removed from the animal, adjusted to a 24- hr period. 3) Intake during the 24- hr day beginning at midnight of the day the apparatus is put on the animal averaged with the 24- hr day ending at midnight after the animal has had the apparatus removed. The latter option being the chosen based on the regression analysis results of each of the proposed DMI interval to CH_4 emission data sets.

The statistical model used for the analysis of these data was $y_{ijk} = \mu + d_i + p_j + dp_{ij} + e_{ijk}$ where d = the effect of the i^{th} diet, p = the effect of the j^{th} period, dp = the interaction effects of the i^{th} diet and the j^{th} period and e = the error deviation of the k^{th} steer of the i^{th} diet in the j^{th} period. In this study i = one to four (dietary % CP of 6.9, 10.3, 12.1 and 13.6), j = one to three (three consecutive 21 day periods) and k = one to 60.

The Bonferronni test for mean separation was used to determine significant differences among diets by DMI, ADG, BUN and CH_4 L d^{-1}. Where Bonferronni found a significant interaction but no significant mean separation, estimate and contrast statements were used to differentiate the treatments. These analytical procedures were carried out in SAS 9.3 TS Level 1M2 (SAS, 2010).

3.2.6 Results

Diet DM decreases with increasing proportions of silage; 47.2, 49.7, 59.3 and 68.7% DM for diets L, ARM, AMG and E corresponding to 74.8, 68.9, 51.3 and 34.1% corn silage in the diet (Table 1).

Table 1: Ingredient and nutrient composition (DM basis) of total mixed ration fed to steers during trial

Ingredient, %	L	ARM	AMG	E
Corn Silage	74.8	68.9	51.3	34.1
Grass hay, (CP 8.89%)	14.8	-	-	-
Grass hay, (CP 10.59%)	-	29.2	38.1	18.9
Grass hay, (CP 13.06%)	-	-	6.8	41.6
Straw, barley	9.8	-	-	-
Canola meal	-	1.4	3.4	4.9
Mineral*	0.1	0.1	0.1	0.1
Salt*	0.5	0.4	0.4	0.5
Nutrient Composition				
DM, %	47.2	49.7	59.3	68.7
CP, %	6.9	10.3	12.1	13.6
NDF, %	47.9	47.9	49.9	52.1
ADF, %	28.8	28.3	29.5	30.2
TDN, %	64	67	65	65
Ca, %	0.29	0.32	0.36	0.46
P, %	0.23	0.28	0.30	0.32
Starch, %	18.5	17.3	13.4	9.6
Fat, %	1.51	1.50	1.52	1.69
CP : TDN	0.11	0.15	0.19	0.21

* Loose pre-mixed mineral containing 16% Ca and 16% P. Loose salt containing 99% salt and trace amounts of I and Co.

The forage diets L, ARM, AMG and E fed to the steers contained 6.9%, 9.3%, 11.2% and 13.3% CP respectively. Ingredients varied in order to ensure that fibre content (NDF and ADF) and energy levels (TDN) were as consistent as possible across all diets. Therefore diets were considered isocaloric with TDN values of 64, 67, 65 and 65 for diets L, ARM, AMG and E,

respectively. A 1:1 mineral was included to meet macro and micro mineral requirements of steers.

Corn silage was the ingredient contributing the largest amount of starch at 24.3% DM and decreasing proportions of corn silage in the diets decreased starch content to 18.5, 17.3, 13.4 and 9.6 for diets L, ARM, AMG and E respectively. Fat content increased slightly with increasing proportions of canola meal with a 0.18% increase from diet L to diet E. With increasing CP and constant TDN, the CP: TDN ratio increased incrementally between diets from 0.11, 0.15, 0.19 to 0.21 for diets L, ARM, AMG and E respectively. Dry matter intake for steers ranged from 5.76 to 7.89 kg d^{-1} (SE ± 0.14) across the four diets with average period intakes of 7.06, 7.01 and 7.27 kg d^{-1} (SE ± 0.08) for periods 1, 2 and 3, respectively (Table 2).

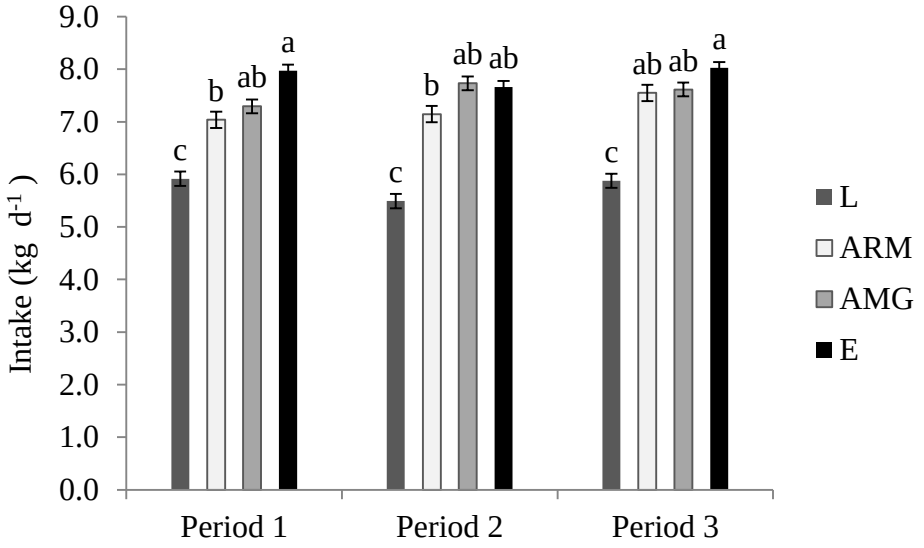
Table 2: Diet crude protein, BUN, intake, productivity and methane emission data for steers fed forage diets

	Diet						Period				P-Values		
	N	L	ARM	AMG	E	SE	1	2	3	SE	D	Pd	DxPd
Diet CP, %		6.9	10.3	12.1	13.6								
DMI, kg d^{-1}	179	5.8	7.2	7.5	7.9	0.1	7.1	7.0	7.3	0.08	<0.01	<0.01	<0.01
DMI%BW	179	1.8	2.1	2.1	2.1	0.04	2.1	2.1	2.0	0.02	<0.01	<0.01	<0.01
CPI, g d^{-1}	360	472	870	944	1124								
BUN, mmol L^{-1}	180	0.8	1.8	3.1	3.5	0.1	2.5	2.0	2.4	0.1	<0.01	<0.01	<0.01
ADG, kg d^{-1}	58	0.1c	0.7bc	0.8b	1.0a	0.1					<0.01		
Days to reach target gain, d*		409	76	65	57								
CH_4, L d^{-1}	154	199.0 ± 5.7c	245.3 ± 5.6b	252.6 ± 5.4ab	259.4 ± 5.4a		237.3 ± 4.5	240.0 ± 4.6	234.6 ± 4.8		<0.01	0.71	0.64
CH_4 cumulative, L		81 399	18 261	16 288	14 788								
CH_4, % GEI	145	7.5	7.3	7.1	6.9	0.2	7.4	7.4	6.7	0.2	0.24	<0.01	0.04

*Based on a target gain of 54 kg. Diets were formulated for a 0.80 kg d^{-1} rate of gain. SE values when equal for all for treatments are listed in the SE column. When SE differs by treatment, values are listed next to mean value.

A significant diet by period interaction (P<0.0001) was observed whereby steers offered the E diet had a significantly higher intake than diet ARM and L in period one but was not significantly different from ARM in the other two periods. Intake for steers fed diet L was significantly lower than intake of all other diets during all three periods, as depicted in Figure 2.

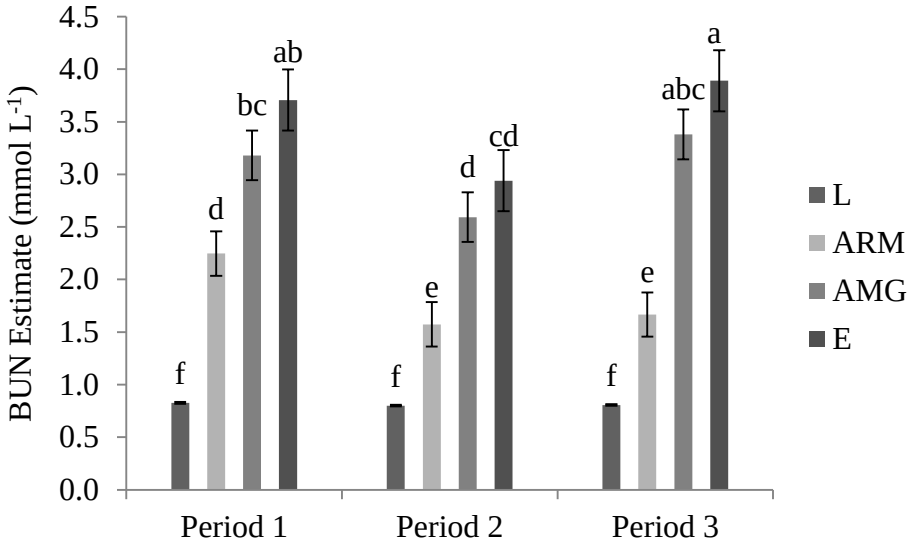
Figure 2. Dry matter intake for backgrounding steers fed forage-based diets for three, 21- d periods



Dry matter intake as a percent of body weight was lowest for diet L (1.8%) whereas the other three diets were consistent at 2.1%. Crude protein intake (CPI, g d^{-1}) was 472, 870, 944 and 1124 for diets L, ARM, AMG and E respectively.

Average baseline BUN for steers' pre-trial was 2.94 mmol L^{-1}. The diets offered during the trial resulted in BUN levels of 0.8, 1.8, 3.1 and 3.5 (SE ± 0.11) mmol L^{-1} for diets L, ARM, AMG and E, respectively, with a significant diet by period interaction (Figure 3).

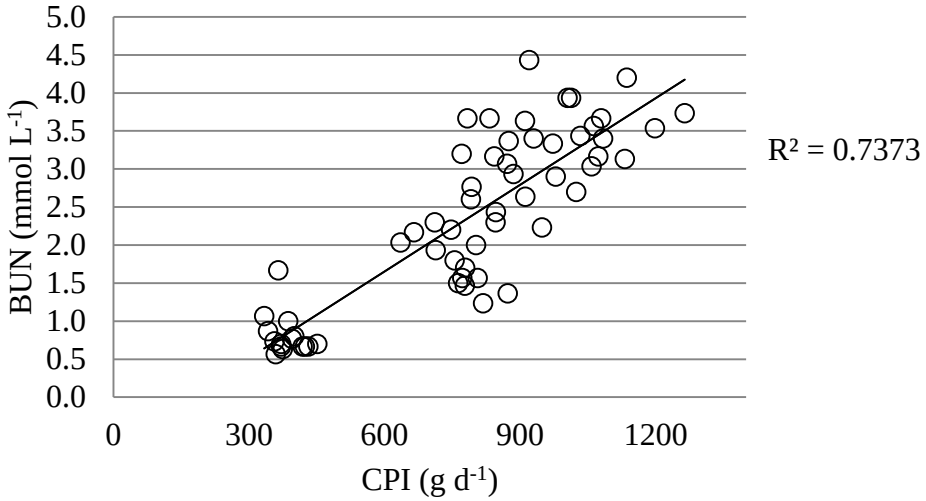
Figure 3. Steer blood serum urea nitrogen levels resulting from four forage diets fed for three, 21- d periods



All but one observation of steers offered diet L were below the acceptable minimum BUN of 2.1 mmol L^{-1} (Kodak Diagnostics, 1991). Similarly diet ARM caused six steers in period one, 13 in period two and 14 in period three to have BUN concentrations at a lower than acceptable level. Both diets AMG and E provided overall adequate N as only two observations from each diet were considered inadequate. All four of the inadequate BUN observations from diet AMG and E occurred in period 2. Two of the low BUN observations corresponded to animals with low intakes the day of blood sampling. The remaining two observations corresponded with animals having normal intakes and ADG (kg d^{-1}) and therefore, the cause of these low BUN concentrations cannot be explained. As depicted in Figure 3, there was a diet by period interaction ($P < 0.001$) where BUN levels decreased in period two for all diets except diet L. In period three, BUN concentration for steers fed diet AMG and E returned to period one levels or higher (Figure 3). Blood serum urea nitrogen (mmol L^{-1}) had a significantly positive ($P< 0.001$)

correlation to CPI (g d^{-1}) with an R^2 value of 0.74 as depicted in Figure 4. Methane (L d^{-1}) was correlated with BUN (mmol L^{-1}) with $R^2 = 0.26$ ($P<0.05$).

Figure 4. Relationship between BUN and CPI of backgrounding steers fed forage diets

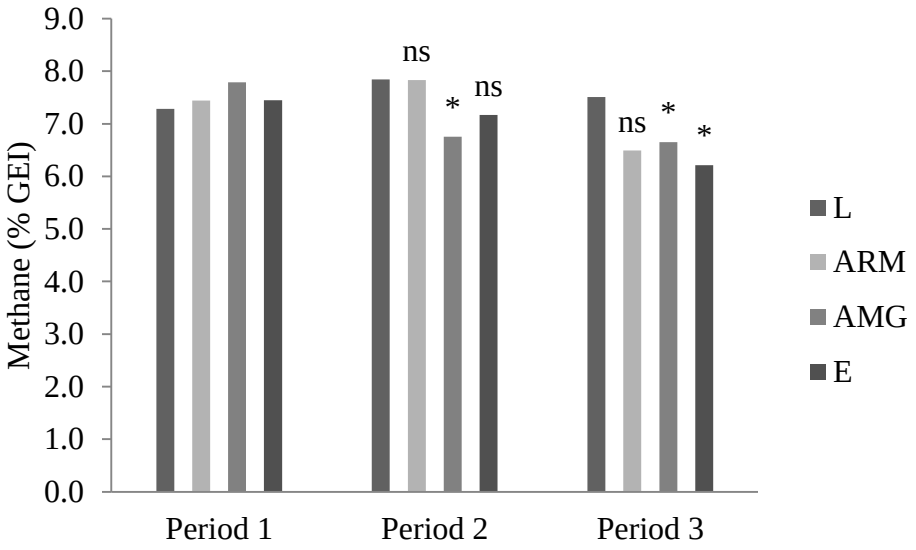


Average daily gains were calculated for the 65- d growth trial and significantly increased with increasing forage CP; L- 0.13a, ARM- 0.71b, AMG- 0.84bc and E- 0.95c (kg d^{-1}, SE ± 0.103). The slower rate of growth of steers fed diets with lower CP concentrations indicates that these animals will require more days on feed to reach a targeted gain of 54 kg.

Methane (L d^{-1}) emissions were 199.0 ± 5.7c, 245.3 ± 5.6b, 252.6 ± 5.4ab and 259.4 ± 5.4a for L, ARM, AMG and E diets respectively (<0.01) with no diet by period interaction (N = 154) nor a period effect. However, an interaction was observed when CH_4 was expressed as % GEI with

values of 7.5, 7.3, 7.1 and 6.9 (% GEI, SE ± 0.2) for diets L, ARM, AMG and E respectively (N = 145), as indicated in Figure 5.

Figure 5. Methane (% GEI) emissions from steers fed forage diets over three periods



However the Bonferronni test found no significant difference among mean separations therefore, means were assessed using contrast and estimate statements within SAS (SAS, 2010). The relationship of emissions from diet L to emissions from other diets in the first period was compared to the relationship of emissions from diet L to emissions from other treatments in the following periods to determine if emissions are decreasing over time with additional dietary CP. The difference in emissions between diet L and diet ARM in period one wass not significantly different than the relationship between emissions from those two diets in period two (P = 0.79) or period three (P = 0.07). The difference in emissions between diet L and diet AMG in period one was significantly different than the relationship between emissions of those two treatments

in period two (P = 0.01) and it was also significantly different than the relationship between emissions from diet L to diet AMG in period 3 (P = 0.04). When comparing the emission difference of diet L to diet E in period that relationship is found to be not significantly different than that same relationship in period two (P = 0.21) but it is found to be significantly different to that relationship in period 3 (P = 0.02).

Although condition were extremely cold through all periods, with average maximum daily temperatures of -17.5°C and average minimum daily temperatures of -28.1°C during period one, they were consistently cold. The same can be seen in period three with moderately higher average temperatures of -4.5°C daily maximum and -17.6°C daily minimum. The second period had an average minimum and maximum temperatures of -21°C and -14°C, but also experienced a 33°C fluctuation in temperature over the course of 10 d.

3.3 **Discussion**

Diets offered in this trial were manipulated in order to observe the effects of CP content of forage diets on CH_4 emissions. By offering 6.9% CP in diet L, microbial digestion is limited (Van Soest, 1982) and expected rate of passage slows increasing CH_4 emissions. The purpose of diet ARM at 10.3% CP was to provide the rumen microbes with adequate N but to be inadequate for animal growth (NRC, 1996). The AMG diet with 12.1% CP is expected to be more than adequate CP for rumen microbes and animal growth allowing for efficient degradation of feed and less CH_4 emitted compared to the two lower CP diets. The E diet with the highest CP concentration at 13.6% may be considered an excessive amount of dietary protein with the

purpose of discerning the upper limit to which CP can decrease CH_4 emissions via improved rumen efficiency.

Silage as a diet ingredient may have an impact on emissions because ensiled forages have been shown to emit less CH_4 than dried forage (Moss et al., 2000). This can be attributed to the increased proportion of butyrate and reduced proportion of actetate produced during digestion of grass silage as compared to grass hay (Shingfield et al., 2002). Benchaar et al. (2001) modelled a 32% reduction of CH_4 per unit of GE intake for alfalfa silage than for alfalfa hay. Corn silage also contains the largest source of starch of the ingredients used, with diets containing 9.6-18.5% starch. Starch content in diets can cause an increase in propionate production which would decrease the CH_4 (% GEI) emissions (Boadi et al., 2004). However, contrary to previous research, an increase in CH_4 emissions (% GEI) was observed with increasing levels of starch. Therefore the dietary effect on emissions cannot be attributed to starch content of the diets.

The range of diet fat content for each diet was 1.51, 1.50, 1.52 and 1.69 (%) for L, ARM, AMG and E, respectively. Inclusion levels were lower than required to show emission reductions according to previous studies with inclusion rate of at least 3.5% fat (Boadi et al. 2004).

The rations were formulated to offer diets with increasing protein content but consistent fibre and energy content in order to avoid confounding impacts on CH_4 emissions. With uniform energy and increasing protein content the CP: TDN ratios achieved were 0.11, 0.15, 0.19 and 0.21 for L, ARM, AMG and E diets respectively. Those levels are consistent with the lower range of forage quality data from the Saskatchewan forage survey (Gov. Sask. Min. Ag., 2013) where CP: TDN ratios ranged from 0.11 to 0.32.

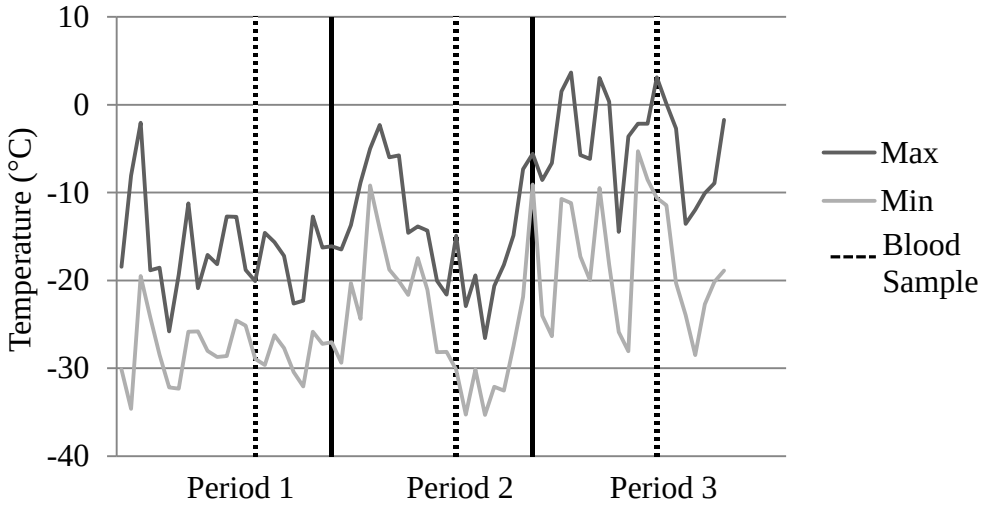
The expected DMI for animals consuming forage diets with these energy levels is 7.73 kg d^{-1} to 9.68 kg d^{-1} (NRC, 2000). Our data showed that a forage-based diet containing 6.9% CP and 67% degradable CP resulted in a low DMI relative to diets that had dietary CP levels sufficient to support microbial activity in the rumen. Suppression of ruminal fermentation rate can reduce rate of passage, rumen capacity and DMI. There was a 0.40 kg d^{-1} increase in DMI between periods two and three for diet ARM however in the third period diet ARM was no longer significantly different from diet E. This increase in DMI can be attributed to the animals growing over time leading to an increased capacity to consume feed. Diet average DMI (% MBW) is low for diet L at 1.8 and is adequate at 2.1 (NRC, 2000) for the three other diets (SE ± 0.04).

Daily CPI (g d^{-1}) was 472, 870, 944 and 1124 for L, ARM, AMG and E diets, respectively. There was a significant correlation between dietary CPI and BUN concentration with an R^2 value of 0.74 as shown in Figure 3. The trial was successful at creating an N limiting scenario for steers offered diet L where only 2% of observations measured within the acceptable range (2.1 to 7.9 mmol L^{-1}, Kodak Diagnostics, 1991) of BUN concentration and an average concentration of 0.8 mmol L^{-1}. As was expected, diet ARM was also inadequate for animal growth with an average BUN concentration of 1.8 mmol L^{-1} and only 36% of observations within acceptable range. Both the higher CP diets, AMG and E, produced adequate BUN levels of 3.1 and 3.5 mmol L^{-1} respectively, with 96% of observations falling within range for both diets.

A diet by period interaction was observed for BUN where concentration of the three higher CP diets dropped in the second period. During period 2 there were also large fluctuations in temperature as indicated in Figure 6. The drop in BUN values in the second period may be indicative of an increase in N excretion caused by the change in temperature. In a study by

Kennedy and Milligan (1978), rumen NH_3-N in sheep declined from 100 mg N L^{-1} to 78 – 82 mg N L^{-1} when closely shorn sheep were exposed to 2 to 5 °C for 35 days. Further, plasma urea-N transfer to the rumen NH_3 pool was greater in cold sheep than in warm (22 to 25°C) sheep at 9.5 g N d^{-1} and 7.3 g N d^{-1} respectively. The difference in ruminal NH_3-N levels was attributed to increased N excretion. It has also been shown that cold acclimated cattle are subject to a decrease in microbial degradation which suggests an increase in nutrient excretion (Kennedy, 1976, Christopherson and Kennedy, 1983).

Figure 6: Maximum and minimum daily temperatures during trial at experiment location, Glenlea, Manitoba (Jan – Mar)



It is possible that the observed decrease in BUN seen in the second period of the present study is due to an increased flow of urea N to the rumen from the blood or by increased N excretion. The greatest effect on BUN levels were seen in AMG and E suggesting that the higher levels of CP

concentration lead to the greatest urea recycling and N excretion. However the information from Kennedy and Milligan (1978) is based on acute cold exposure and not long term exposure where animals may have a chance to acclimate to the cold. Although the animals in the present study had been cold acclimated it is unclear if the temperature increase in the second period was enough to nullify the benefits.

Steers offered diet L and restricted by low CPI had a lower ADG (0.13 kg d^{-1}) compared to those receiving higher CP diets which gained 0.71, 0.84, and 0.95 (kg d^{-1}) for ARM, AMG and E diets, respectively (SE ± 0.103, $P<0.0001$). This was expected as diet L did not contain enough CP for animal growth or muscle synthesis. As the animals fed diet L grew slower than the animals offered the higher CP diets, they required more days to reach the target gain of 54 kg during the backgrounding phase, and therefore these animals had much lower production efficiency.

Methane measurements showed that only diet L produced significantly lower CH_4 emissions (L d^{-1}) compared to the other three diets. This is perhaps expected due to the extremely low N concentration available to the rumen microbes, slowing the rate of fermentation and lowering DMI, producing less CH_4 as a result. However, CH_4 (L d^{-1}) is only descriptive of the rumen output, not rumen efficiency. Methane measured as % GEI describes the amount of energy lost as CH_4 as a proportion of energy consumed providing an indicator of efficiency. Methane (% GEI) in this trial was slightly higher at 6.9 to 7.7% GEI than the conversion factor used by IPCC methodology of 6.5% (IPCC, 2013). The range of CH_4 emission (% GEI) from this study is also slightly higher than a trial by Ominski et al. (2006) where growing steers were offered all-forage diets over winter and emitted CH_4 levels of 5.1 to 5.9 (% GEI). However the forage diets offered in that trial (Ominski et al., 2006) contained dietary CP concentrations that were higher than the present trial (13.6 to 16.7% CP).

Steers entered the backgrounding phase at 321 kg and on average required 54 kg of gain to exit the backgrounding phase of production at a typical target weight of 375 kg. The animals offered L diet were gaining 0.13 kg d^{-1} and therefore required 409 days to reach the target weight and exit the backgrounding phase. These steers were emitting 199.0 ± 5.7 L d^{-1} and in the time required to reach the target gain, they will emit 81 399 L of CH_4. In contrast, the animals offered the E diet gained 0.95 kg d^{-1} and required 57 days to reach the target gain. Although the high CP diet results in higher emission per day (259.4 ± 5.4 L d^{-1}) the cumulative emissions while in the backgrounding phase were much less (14 788 L of CH_4).

It is important to note that although increased dietary CP content decreased CH_4 emissions (% GEI) in this study, it should not be assumed that this relationship will continue with excessive levels of CP content. If rumen NH_3-N exceeds 167 mg 100 ml^{-1} of rumen fluid NH_3 rumen toxicity could occur (Payne, 1977). More likely the body would excrete the excess N as urea (Huntinghton and Archilbeque, 1999), potentially leading to other environmental implications.

Urea can hydrolyze within one or two days of excretion making it available for further transformations and ultimately creating an important source of N_2O (Dijkstra et al. 2013). Based on a study by Yan et al. (2007), the ratio of urine N (g) to intake N (g) is 0.46. Nitrogen intake in the present study averaged 76, 139, 151 and 180 (g d^{-1}) for diets L, ARM, AMG and E respectively. Using this information, steers were estimated to excrete 35, 64, 69 and 83 (g N d^{-1}) in urine from diets L, ARM, AMG and E respectively. The relationship described by Dijkstra et al. (2014) clearly explains that the higher CP diets would result in increased urinary N excretion.

The estimated N_2O emissions (CO_2-eq d^{-1}) in Table 3 were calculated based on a urinary N excretion to N intake ratio of 0.46 and a N fecal excretion to N intake to ratio of 0.32 (Yan et al.

2007). Dijkstra et al. (2013) suggests volatilization rates can range from 3 to 15% based on field experiments and up to 4 to 52% based on enclosure measurements from single urine patches influenced by urinary N composition, soil type, moisture, temperature and wind speed. Methane and estimated N_2O emissions were converted to CO_2-eq using global warming potential values of 28 and 265 respectively (IPCC, 2013b). Although increasing CP content of forage diets may decrease CH_4 emissions it will also increase N_2O emissions via urine and fecal excretion (Table 3).

Table 3. Actual dietary CP: TDN ratios, N intake and CH_4 emissions (CO_2-eq d^{-1}) and estimated N_2O (CO_2-eq d^{-1}) emissions from steer fecal and urine excretion

Diet	CP: TDN	N Intake (g d^{-1})	ADG (kg d^{-1})	CH_4 (CO_2-eq kg $gain^{-1}$)	Low Range N_2O* (CO_2-eq^{-1} kg $gain^{-1}$)	High Range N_2O* (CO_2-eq^{-1} kg $gain^{-1}$)
L	0.11	76	0.13	39.78	0.02	0.34
ARM	0.15	139	0.71	7.00	0.25	4.29
AMG	0.19	151	0.84	6.32	0.31	5.33
E	0.21	180	0.95	5.18	0.46	7.94

* Excretion values calculated based on urinary N excretion to N intake ratio of 0.46 and N fecal excretion to N intake to ratio of 0.32 (Yan et al., 2007). Volatilization rates calculated based on field conversion rates of 4% (low) to 52% (high) (Dijkstra et al., 2013).

In order to determine a recommended level of CPI for forage backgrounding diets, it is important to balance potential environmental implications of CH_4 emissions and N_2O volatilization. Figure 7 demonstrates that when CH_4 emissions and potential for N_2O emissions are compared using common units (CO_2-eq), N intake reaches an optimum level at approximately 155 g d^{-1} when volatilization rates are high by minimizing both CH_4 and N_2O emissions (CO_2-eq). Diet AMG resembles the optimum level of N intake according to this example providing 151 g d^{-1} with a CP: TDN ratio of 0.19 when volatilization conditions are favourable and conversion rates reach

52%. As discussed above, animals exposed to acute cold may increase N excretion rates, increasing N available for volatilization.

Yan et al. (2007) also suggested that N excretion as a proportion of N intake can be reduced with increased metabolizable energy by 0.048 for each 1 MJ of energy per kg of DM of diet. Therefore diets should be scrutinized based on CP: TDN ratio rather than dietary CP alone. However, if energy intake increases above an animal's maintenance requirement, protein synthesis will become most limiting if CP concentration is not increased in tandem and excess energy will be deposited as fat (NRC, 1996). Fat deposition is not desirable in backgrounded animals where the objective is to grow young animals to an appropriate frame size before adding fat. The diets in this trial were isocaloric and further investigation would be required to determine optimum energy levels should they be greater than requirements as defined by NRC (2000).

Another important consideration for determining optimum dietary CP and CP: TDN ratio is animal productivity. Slower growing animals require a greater number of days on feed and therefore produce more total CH_4 as described above. Figure 8 shows the relationship between ADG and CH_4 kg gain^{-1} (CO_2-eq) as a function of N intake where faster growing animals produce less CH_4 overall. The shaded area of Figure 8 depicts the level of N intake required to provide maximum gain with minimum CH_4 emissions, suggesting N intake should be 140 g d^{-1} or higher, and therefore backgrounding forage diets should contain at least as much CP as diet AMG (12.1% CP).

The observations from this study suggest that an increase in dietary CP content from 6.9 to 13.6% can reduce CH_4 emissions on average by 8%. Previous research has shown more drastic

reductions in CH_4 emissions with dietary modifications; up to 33% with dietary supplementation of fat (Mathison, 1997) and by 20-25% when feeding ionophores (Johnson and Johnson, 1995). However, emissions from cattle fed ionophores only periodically decreased because of supressed intake and returned to baseline levels within two weeks of feeding as rumen microbes adapted to the supplement and animal intakes became normal (Johnson and Johnson, 1995). Bras (2013) observed a 33% reduction in CH_4 emissions when grain concentrate levels were increased from 0 to 60%. However, it is important to recognize production constraints of backgrounding operations regarding the use of high energy diets options. For example, supplementing forage diets with significant quantities of grain or with fat may reduce CH_4 emissions but alter the CP: TDN ratio resulting in deposition of fat rather than lean tissue.

Figure 7. Methane emissions and volatilization potential of N_2O express by CO_2-eq from backgrounding cattle

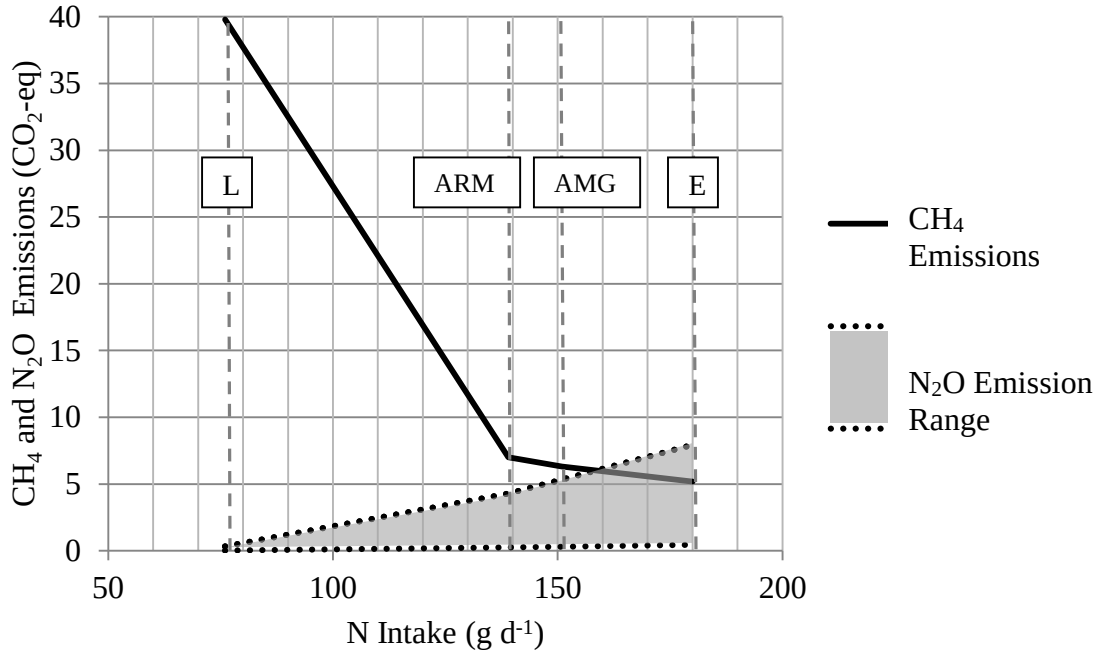
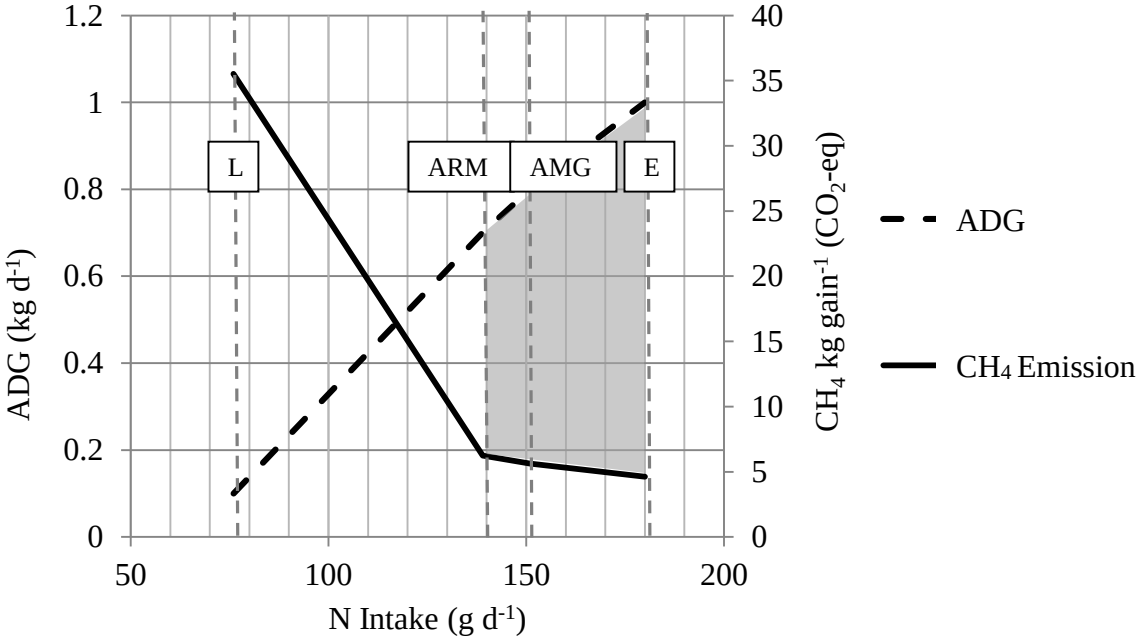


Figure 8. Methane emissions and gain based on protein intake in backgrounding cattle



3.4 **Summary**

It was observed that backgrounding animal productivity as measured by DMI and ADG increased with increasing diet CP concentration (6.9 to 13.6% CP). As expected, N status, as measured by BUN level increased with increased diet CP concentration.

Further, when CP concentration of forage was below rumen microbial requirements, CH_4 (L d^{-1}) was less than for animals fed higher CP forage diets. However the slower growing L diet animals required 333, 344 and 352 more days on feed than the ARM, AMG and E diets respectively, to achieve desirable gains during the backgrounding phase. Therefore during the time required for steers fed diet L, they produces 78 – 82 % more CH_4 (L) than the higher CP diets. When CH_4 is measured as % GEI, there is a 9% - 17% decrease in emissions with increased CP (6.9 – 13.6% CP) over time.

Based on the findings of the present study, there is merit in ensuring higher CP forage diets for the reduction of CH_4 (% GEI) especially for production situations where increased dietary energy or feed additives are not feasible. This study also shows potential for the use of BUN as an indicator of animal N status to act as tool for the prediction of ruminal energetic efficiency as measured by CH_4 emissions.

4. MANUSCRIPT 2

Modelling methane emissions of cattle by diet and animal parameters including blood serum urea nitrogen

4.1 **Introduction**

Decreased CH_4 emissions (% GEI) have been observed with increased dietary CP concentrations (as described in Manuscript 1), which suggests that models to predict CH_4 emissions should contain dietary CP. The challenge is that although many models developed to predict CH_4 emissions, such as IPCC Tier 2 (IPCC, 2007) require accurate and consistent feed quality data. Much of this information is not currently available (Johnson and Ward, 1996; Gibbs et al., 2000).

It is, therefore, necessary to explore other methods of determining feed quality and the nutrient status of the animal. It is known that rumen NH_3 increases with increasing dietary CP and that rumen NH_3 is highly correlated to BUN (Hammond, 1997). Therefore, it may be helpful to use BUN to measure an animal's N status and indirectly its rumen fermentation efficiency and CH_4 emission potential. In this way, a direct blood sample could be taken from a sample of a farm herd and used to estimate CH_4 emissions, thereby avoiding the need for feed quality or intake data.

To satisfy the objective of creating a robust model, many animal types; mature and growing and feed qualities; high and low CP levels, from many different studies should be included in the analysis. A meta-analysis can be used to combine treatment effects of several studies (Viechtbauer, 2010), by assuming that the studies selected are a random sample of the entire population (Hedges and Vevea, 1998), in order to increase the sample size.

We hypothesized that CH_4 emissions of cattle can be modelled using diet and animal variables including BUN for the purpose of on-farm CH_4 emission evaluation. The objectives were 1) to examine the relationship between BUN and enteric CH_4 emissions from cattle to determine the validity of a prediction model based on on-farm BUN measurements 2) to observe CH_4

emissions from cattle with inadequate BUN concentrations (< 2.1 mmol L^{-1}) and determine if those cattle produce more CH_4 as a % GEI and 3) to determine at what BUN concentration animals are most efficient, producing less CH_4 (% GEI).

4.2 **Methods**

4.2.1 Data selection for meta-analysis

The intent of the selection process was to include many CH_4 studies with a range of cattle types, feed types and CP levels for the development of a model for CH_4 emissions where the variables can be measured on-farm. Five data sets collected over nine years were included in the regression analysis (Table 4). When multiple years of data were collected in a study, each year was considered a unique data set to account for the variation due to differences in diet and environment. All data were collected by the University of Manitoba since data containing both CH_4 and BUN samples in western Canada were unavailable from other institutions.

Two animal classes were included with 181 observations from cows and 296 observations from steers, for a total of 477 observations. Parameters measured for each animal included CH_4 production as average output per day (L d^{-1}) and as expressed by a percentage of their daily intake (% GEI). Methane production was the variable of interest in a multiple regression based on animal and group-level variables. These included, CP (% DM), GE (MJ kg^{-1}), NDF (% DM), MBW (kg $^{0.75}$), ADG (kg d^{-1}), DMI (kg d^{-1}) and BUN (mmol L^{-1}).

Table 4. Trials included in meta-analysis data set for development of a methane emission prediction model

Authour	Trial	Year	Animal Type	Feed Type	N
Blair	1	2014	Steers	TMR	60
Donohoe	2	2011b	Cows	TMR/ BG	62
	3	2011a	Cows	TMR/ BG	61
Bernier	9	2009	Cows	TMR	29
	10	2008	Cows	TMR	29
Bouchard	4	2007	Steers	Silage/ Hay	40
	5	2006	Steers	Silage/ Hay	40
Wilson	6	2006	Steers	Pasture	39
	7	2005	Steers	Pasture	57
	8	2004	Steers	Pasture	60
Total Obs.					477

TMR = Total mixed ration, BG = Bale grazing, a, b = data collected over two periods during the same winter (2011, Jan- Mar).

Interactions were also included in the initial regression analysis including; MBW (kg $^{0.75}$) x trial, ADG (kg d^{-1}) x trial, DMI (kg d^{-1}) x trial and BUN (mmol L^{-1}) x trial. Animal variables occurred as a result of the trial treatment and diet variables were manipulated for trial design within each trial. Therefore interactions between trial and diet variables were not included in the model.

4.2.2 Statistical analysis

The data were tested for normal distribution determining the skewness and kurtosis values. Outliers were declared as ± three standard deviations from the mean CH_4 value and were removed from the data set in the same manner as the steer trial data previously described. In the

cow (L d^{-1}) regression, two data points were removed leaving 105 total cow observations after editing. One data point was removed in the cow (% GEI) data set leaving 82 observations after cropping. Four data points from the steer (L d^{-1}) data set and 4 data points from the steer (% GEI) data set were also removed, leaving 263 and 262 total observations remaining, respectively.

The data was analyzed using the MIXED procedure followed by a regression analysis process called backward elimination (Gill, 1978) within SAS (SAS, 2010). Models were developed for cow CH_4 L d^{-1}, cow CH_4 % GEI, steer CH_4 L d^{-1} and steer CH_4 % GEI (Table 5). The variables included in a regression analysis should be as independent as possible as stated earlier (Quinn and Keough 2002); see Table 6 for correlation coefficients.

The statistical model used for the analysis of these data was $y_{ij} = \mu + t_i + b_1v_{1ij} + b_2v_{2ij} + b_3v_{3ij} + c_1x_{1ij} + c_2x_{2ij} + c_{2i}x_{2ij} + c_3x_{3ij} + c_4x_{4ij} + c_5x_{4ij}^2 + e_{ij}$ where y_{ij} = a measurement of methane on the j'th animal in the i'th trial and t_i is the effect of the i'th trial. Where b_1 indicates the diet variables used in the regression and are identified by v where, v_1 is the effect of CP, v_2 is the effect of GE concentration and v_3 is the effect of NDF concentration of the j'th animal in the i'th trial. Animal variables used in the regression are indicated by c and differentiated by x where x_1 is MBW of an animal, x_2 is its ADG, x_3 is its DMI and x_4 is BUN effect of the j'th animal in the i'th trial. The error deviation is indicated by e_{ij} representing the error deviation from the j'th animal in the i'th trial.

Table 5: Methane L d $^{-1}$ and % GEI prediction models and characteristics for cows and steers

Model Cow Log CH_4 (L d^{-1})

Variable	Num DF	F Value	Pr > F	Slope	Partial R^2
Trial	3	22.28	<0.01		30.8
Diet GE (MJ kg^{-1})	1	9.82	<0.01	-0.04	4.5
MBW ($kg^{0.75}$)	1	23.27	<0.01	0.01	10.7
BUN Effect	1	1.52	0.22	-0.01	0.7
BUN Effect * Trial	3	6.91	<0.01	-0.03 – 0.03	9.5
Residual	95				43.7

Model Cow Log CH_4 (% GEI)

Variable	Num DF	F Value	Pr > F	Slope	Partial R^2
Trial	3	22.82	<0.01		33.3
Diet CP (%)	1	8.54	<0.01	-0.03	4.2
Diet NDF (%)	1	12.55	<0.01	-0.03	6.1
DMI (kg d^{-1})	1	30.25	<0.01	-0.02	14.7
BUN Effect	1	11.71	<0.01	-0.03	5.7
Residual					36.0

Model Steer CH_4 (L d^{-1})

Variable	Num DF	F Value	Pr > F	Slope	Partial R^2
Trial	5	41.56	<0.01		41.7
ADG (kg d^{-1})	1	8.79	<0.01	20.53	1.8
BUN Effect	1	0.54	0.46	-1.09	0.1
BUN Effect * Trial	5	6.26	<0.01	-11.86 – 15.10	6.3
Residual					50.2

Model Steer CH_4 (% GEI)

Variable	Num DF	F Value	Pr > F	Slope	Partial R^2
Trial	5	7.24	<0.01		9.3
Diet NDF (%)	1	4.60	0.03	0.10	1.2
DMI (kg d^{-1})	1	60.42	<0.01	-1.59	15.5
DMI (kg d^{-1}*Trial)	5	7.73	<0.01	0 – 1.78	9.9
Residual					64.0

Table 6: Correlation coefficients of prediction variables included in cattle emission (% GEI) models

Cow (% GEI)	Diet NDF (%)	DMI (kg d^{-1})	BUN (mmol L^{-1})
Diet CP (%)	-0.712 (<0.001)	0.030 (0.763)	0.281 (0.004)
Diet NDF (%)		0.014 (0.890)	-0.086 (0.381)
DMI (kg d^{-1})			0.229 (0.019)

Steer (% GEI)	Diet NDF (%)	DMI (kg d^{-1})
Diet CP (%)	0.520 (<0.001)	-0.258 (<0.001)
Diet NDF (%)		-0.409 (<0.001)

P-values are shown as values in parenthesis.

4.3 Results

One of the objectives of the analysis was to ensure a wide range of variables for a robust model (Table 7). Because the basis of this model is the inclusion of CP in forage diets for rumen efficiency, it was imperative to analyze a wide range of dietary CP levels (4.9 – 26.4 % CP) which, as expected, resulted in an extensive range of BUN levels (0.5 – 9.9 mmol L^{-1}).

Figure 9 depicts the distribution of CH_4 data, after outliers were removed, separated by animal type and CH_4 measurement units. Significant CH_4 emission prediction models are presented in Table 5, with data separated in the same manner.

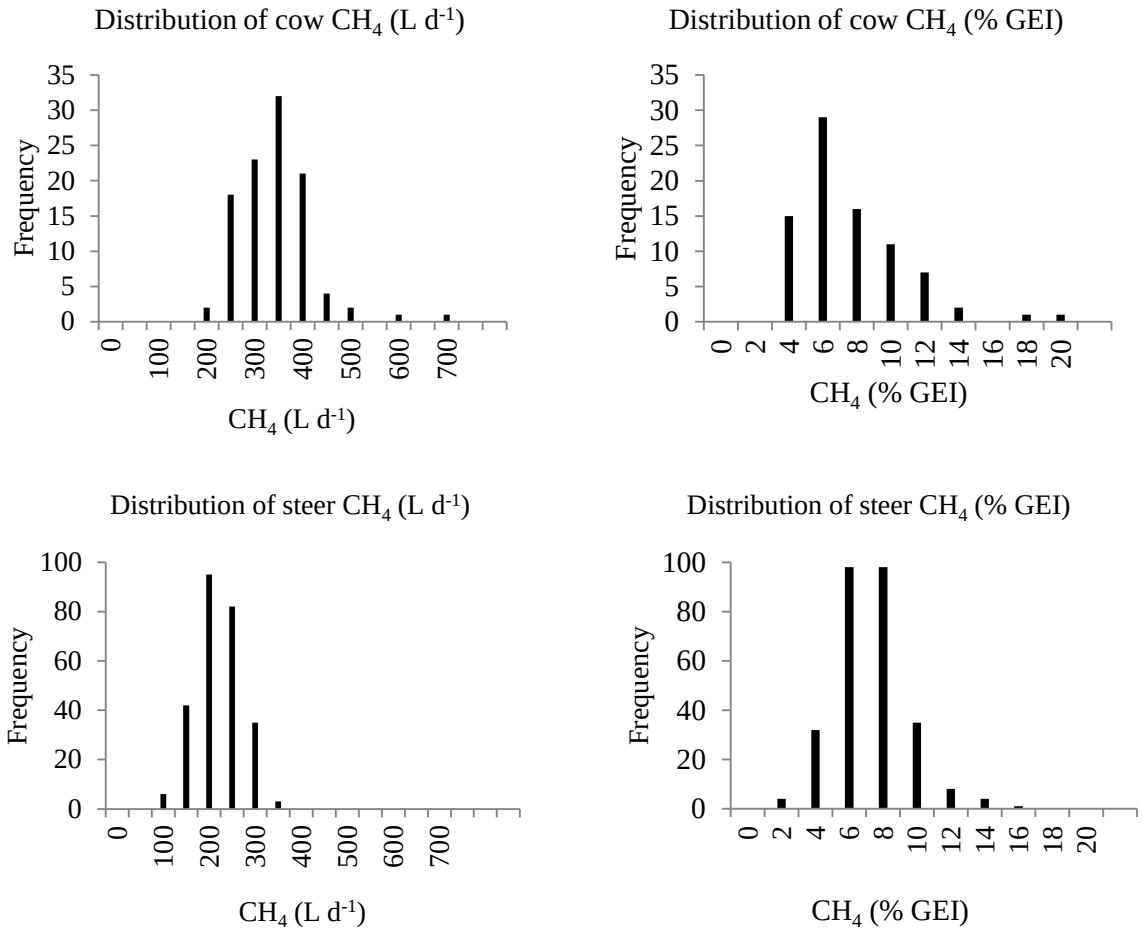
Table 7: Range of variables included in the model to predict methane emissions from individual beef cattle observations with outliers removed

Variables	Unit	Range
Methane	CH_4 (L d^{-1})	51.5 – 556.0
	CH_4 (% GEI)	1.1 – 14.5
Diet Variables	CP (%)	4.9 - 26.4
	GE (MJ kg^{-1})	16.8 - 20.0
	NDF (%)	40.9 - 67.7
Animal Variables	MBW ($kg^{0.75}$)	63.8 - 150.5
	BUN (mmol L^{-1})	0.5 - 9.9
	DMI (kg d^{-1})	2.0 - 23.4
	ADG (kg d^{-1})	-2.3 – 4.2

* Variable ranges listed after outliers have been removed from data set.

The blood serum urea nitrogen effect (BUNX) variable was found to be significant in only the cow CH_4 % GEI (Table 5). For both the cow and the steer CH_4 L d^{-1} models, there was a BUNX by trial interaction. Dry matter intake was significant in the % GEI but not in the CH_4 L d^{-1} models. In all cases, except steer CH_4 % GEI, trial has the largest portion of the partial R^2 other than the residual. The large residual term is typical of animal trials and has been observed in the literature at 25 – 26% (Boadi et al., 2002), however the residuals observed in the present study are much larger, between 36.0 and 64.6% possibly attributed to the inclusion of several trials.

Figure 9: Plots of cow and steer methane data (L d^{-1}) and (% GEI) to determine if normally distributed



4.3 **Discussion**

A group level factor differentiates between conditions within each experiment that have not been accounted for by another variable (weather, ambient temperature, animal variation, breed etc.). All animals in a given trial share the same environmental conditions therefore trial is a group factor. Other group-level variables include characteristics of the diets offered to the animals.

These measures included CP, (% DM), GE (MJ kg^{-1}), and NDF (% DM). The remaining measures are individual animal measurements.

Metabolic body weight (MBW) is an individual animal measurement used in the model rather than total body weight as MBW is a measure of body size since larger animals need more energy to survive and under normal conditions will consume more feed but will use less feed per unit of BW than smaller animals for metabolic activities. The relationship of intake to animal size is non-linear (Schmidt-Nelson 1984) however MBW ($kg^{0.75}$) produces a linear relationship which is necessary for the modelling process which involves the linear regression of significant variables. Further, MBW is common in other intake to output efficiency studies like residual feed intake work (Fitzsimmons et al., 2014, Savietto et al., 2014). The large range of MBW values is a product of the inclusion of both cow and steer cattle classes.

Data from trials 2, 3, 9 and 10 contained some negative ADG values. Trials 2 and 3 included pregnant cows and therefore with their growing fetus should have shown weight gain if maintaining body condition. However, it is possible that due to severe weather these animals may have been sacrificing their own body condition for the growth of the fetus as has been shown in other studies (Kelln et al., 2011). Trials 9 and 10 (Bernier, 2011) used open, dry cows that were not fed for weights gain. Average daily gain values were calculated from a short interval of 16 days and could have been influenced by gut fill as the animals were not fasted when weighed. The highest CH_4 (% GEI and L d^{-1}) values, are from Trial 2 and 3 data sets, and deemed reasonable because similar values were seen throughout that trial and therefore were not removed.

It was also important to consider BUN and BUN^2 as variables in the model allowing for both a linear and a quadratic relationship. Using polynomials in a regression can cause problems with multicolinearity (Quinn and Keough, 2002). To avoid this, deviations from the mean of BUN and BUN^2 were used to show the effect of these terms without a confounding effect between terms.

Similarly, correlation coefficients between variables included in prediction model development exist between 1 and -1 but should be low (<0.5, >-0.5) to ensure no confounding relationships affecting the prediction model. For both the cow and the steer data, diet CP and diet NDF are highly correlated (Table 6). The inverse relationship in the cow data can be attributed to the nature of forage diets where; typically the lower CP, the higher the NDF content (Fales and Fitz, 2007). However, the steer correlation between diet CP and diet NDF is still strong but positive. The cow data included in the study had a much smaller range of diet quality (5.6 – 12.5% CP and 57.8 – 67.7% NDF) than the steer data (4.9 – 26.4 CP% and 40.9 – 62.5% NDF). This larger range in CP content without a corresponding magnitude of change in NDF content may have been the cause of the positive diet CP to diet NDF correlation in Table 6.

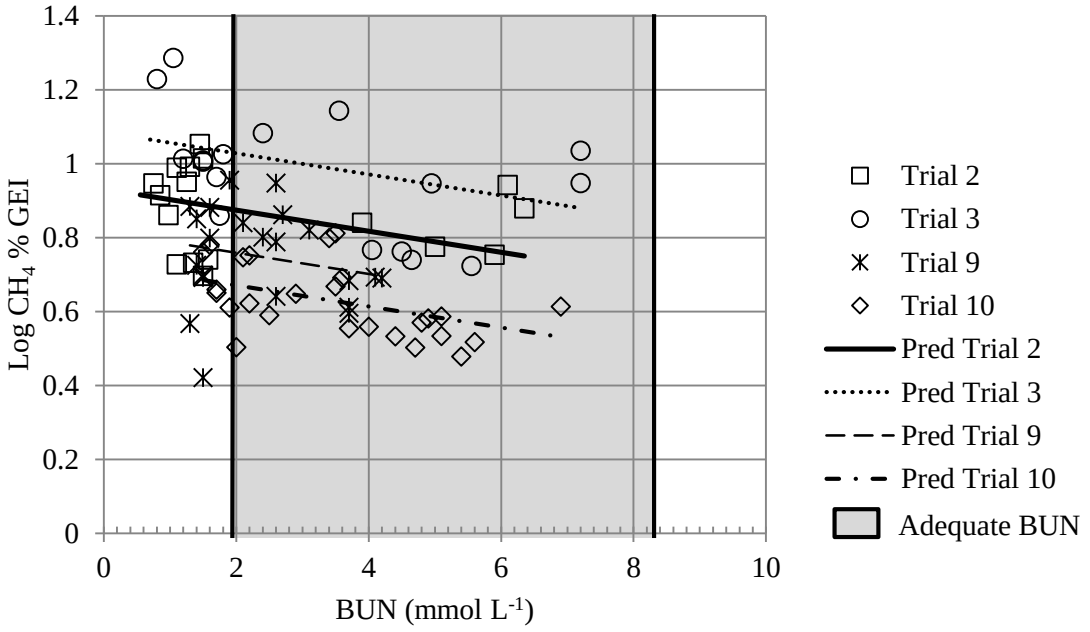
A histogram of the CH_4 data was plotted to check for normality. Skewness values of 1.42 and 2.37 and kurtosis values of 2.22 and 9.67 were found for cow L d^{-1} and % GEI data sets respectively. Normally distributed data sets will have a skewness value of between -1 and 1 and a kurtosis value of between -3 and 3 and therefore the cow data are slightly skewed to the right as depicted in Figure 9. However, skewness values of 0.74 and 0.13 and kurtosis values of 1.44 and 0.71 for steer L d^{-1} and % GEI data sets respectively, indicate that the data are approximately normal (Figure 9). Due to lack of uniformity regarding normality between the steer and cow

data the data sets were not analyzed together. Data from the cow trials were log transformed to remediate the skewed distribution but the steer data were not.

In the models developed (Table 5), trial is considered a categorical variable and therefore the degrees of freedom (df) are equal to the number of trials included in the data set minus one. All other variables have one df because they are continuous variables. The F-values are representative of the magnitude of a variable compared to the error. The effect of trial is 21.85, 21.75 and 41.56 times greater than the error in the cow CH_4 L d^{-1}, cow CH_4 % GEI and steer CH_4 L d^{-1} models. The trial effect is markedly smaller (7.99 times smaller) for the steer CH_4 % GEI model. The slope estimate describes the CH_4 increase that occurs when the corresponding variable is increased by one unit. Furthermore, the slopes presented in Table 6 are the individual slopes of those variables and the cumulative slopes as CH_4 emission prediction lines for the entire data set are demonstrated in Figure 10 and 11. It should be noted that the slope values for the cow CH_4 L d^{-1} are substantially lower than the steer CH_4 L d^{-1} because of the log transformation.

In Manuscript 1 it was shown that dietary CP and BUN concentrations were highly correlated. However, the relationship between BUN and CH_4 production was not found to be quadratic as hypothesized. Further it was hypothesized that there would be some concentration of BUN above which there would be no further improvement in efficiency or decrease in CH_4 emissions. We expected the plateau in the BUN to CH_4 relationship would occur at or above 2.1 mmol L^{-1} where steers are receiving adequate dietary protein (Kodak Diagnostics, 1991). However, neither cow nor steer CH_4 prediction models were a quadratic relationship observed (Figure 10 and Figure 11).

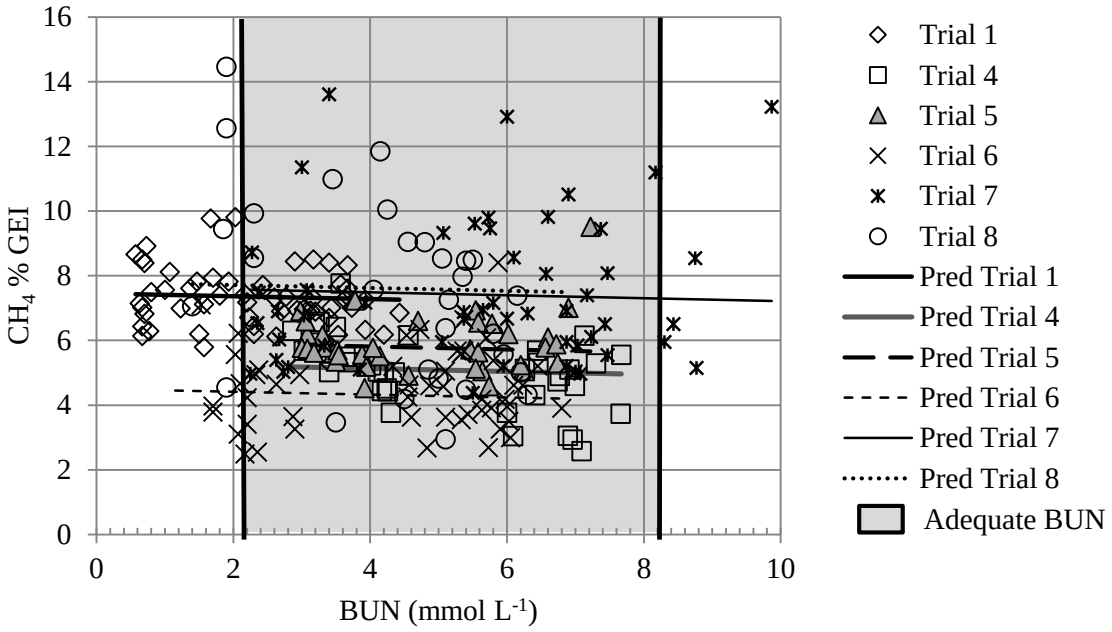
Figure 10: Relationship between log transformed cow enteric methane emissions (% GEI) and BUN (mmol L^{-1})



Emission prediction line equations as shown above; Pred Trial 2: y = -0.03x + 0.93, Pred Trail 3: y = -0.03x + 1.08, Pred Trial 9: y = -0.03x + 0.81 and Pred Trial 10: y = -0.03x + 0.73

The compiled data sets were plotted to determine if there were common trends across the trials included (Morris, 1999). Figure 10 shows the raw data plotted as the relationship of the $\log_{10}$ of CH_4 (% GEI) to BUN for cows and the prediction lines which were developed using the corresponding models listed in Table 5 . Trial 2 and 3 were conducted during two periods of the same winter with the same group of animals. Trial 9 and 10 also used the same group of animals although different from 2 and 3 and sampling occurred in two different years. Cows in trial 2 and 3 were pregnant cows and cows in trial 9 and 10 were open dry cows. There was no interaction in the cow CH_4 (% GEI) model, therefore, the prediction lines as graphed in Figure 10 had the same slope.

Figure 11: Relationship between enteric steer methane emission (% GEI) and BUN (mmol L^{-1})



Emission prediction line equations as shown above; Pred Trial 1: y = -0.05x + 7.50, Pred Trail 4: y = -0.05x + 5.31, Pred Trial 5: y = -0.05x + 5.99, Pred Trial 6: y = -0.05x + 7.67, Pred Trial 7: y = -0.05x + 4.51 and Pred Trial 8: y = -0.05x + 7.80.

Figure 11 shows the relationship between CH_4 (% GEI) and BUN for steers from trials 1, 4, 5, 6, 7, and 8 which were conducted using different groups of steers in different years. It is important to note for Figures 10 and 11, the raw data is plotted and therefore no variables, like DMI, are included. However, these plots are a useful tool describing the relationship between the studies. More specifically, that trial has a major effect on CH_4 % GEI.

One of the objectives of this study was to develop models using variables which can be measured on-farm more easily than required from the IPCC Tier 2 model. However, for all models except the steer CH_4 L d^{-1}, diet variables are significant and therefore require detailed feed information, in the same manner as the Tier 2 methodology. Furthermore, both the cow and steer CH_4 % GEI models found DMI as a significant term which is rarely measured accurately on-farm at the

individual animal scale. Technology exists for precise measurement of DMI for example, the GrowSafe feeding system (GrowSafe Model 4000E feed monitoring system, GrowSafe Systems Ltd., Airdrie, Alberta).

This technology is costly but is becoming more widely used in larger backgrounding/feedlot operations where animals are fed in confinement. However there is still no accurate and logistically feasible method available for measuring intake on pasture.

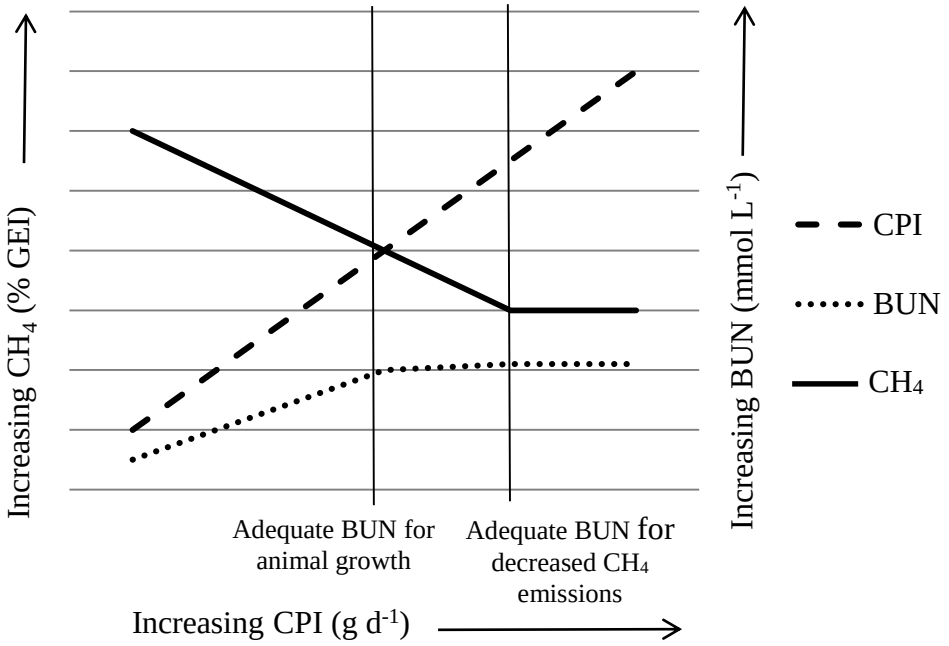
It is not surprising that DMI was a significant variable in both the cow and steer (% GEI) models since DMI is known to have a significant impact on CH_4 emissions (Benchaar et al., 2001, Beauchemin and McGinn, 2006). Since CH_4 L d^{-1} is a direct output measure of CH_4, DMI would be expected to be significant however, a multiple regression will remove variables that are highly correlated to other variables to avoid confounding results (Quinn and Schmidt 2002). This could be the cause of DMI being not significant in the L d^{-1} models.

In general, trial has the highest partial R^2, except for in the steer (% GEI) and excluding the residual, therefore the variation attributed to trial is still very large despite the multiple variables considered in the present models. This suggests that significant variables have not been accounted for in this model including breed, ambient temperature, weather patterns etc. Including these types of trial differences would decrease the residual contained in the trial variable. It is important to note when considering the models developed that all of the initial variables are accounted for and if a term is not in the final model it means that it was found to be not significant in predicting CH_4. Discovering which terms do not aid in the prediction of CH_4 is as noteworthy as those that do.

Based on the above findings CH_4 emissions can be modelled using diet and animal variables including BUN except for steer CH_4 % GEI, however only a very small portion of the variability can be explained by these models. Further, all prediction models except steer L d^{-1} include diet information. Therefore the same issue regarding lack of diet quality information arises with this method as with the existing IPCC Tier 2 method (IPCC, 2007) when predicting CH_4 emissions of cattle in Canada

The second objective of this study was to determine if animals with BUN levels deemed as inadequate (< 2.1 mmol L^{-1}) have greater CH_4 (% GEI) emissions than animals with adequate BUN. The expected relationship between BUN and CH_4 (% GEI) is depicted in Figure 12, where BUN concentrations increase with increasing CPI until some level of adequate BUN concentration, at which point the animal would begin to excrete more N causing BUN levels to plateau. Methane emissions were expected to decrease with increasing BUN concentration as increasing levels of N are available to the rumen resulting in more efficient microbial degradation. When microbial efficiency reaches a maximum, CH_4 is expected to reach a minimum. Based on the raw data relationship (Figure 10 and Figure 11) there is no obvious increase in CH_4 emissions (% GEI) for those animals receiving < 2.1 mmol L^{-1}. Contrary to the relationship depicted in Figure 12, as CPI and BUN increase CH_4 overall decreases only slightly with no indication, or plateau, from the current data set of which concentration is most efficient for the rumen.

Figure 12. Relationship hypothesized between dietary CPI, BUN and CH_4 (% GEI) emissions from beef cattle



The data included in the meta-analysis for this study were separated by animal type based on normality indicators preventing the analysis of the data as one group. However, based on the BUN diet x period interaction observed in Manuscript 1 (Figure 3) and the severe temperature fluctuation occurring in tandem (Figure 6), the analysis may benefit by further separating the data set based on ambient temperature or season. The goal of this additional analysis would be to determine to what extent and in what time frame temperature fluctuations can impact an animal's cold acclimation and therefore the influence of weather on BUN concentrations. A better understanding of this relationship is imperative to interpreting the relationship between BUN and CH_4. The data set used in this study did not allow for this division of data due to a lack of observations but this concept should be considered for future investigation.

The third objective of this study was to determine the minimum BUN concentration at which microbial activity in the rumen is most efficient, producing the lowest amount of CH_4 from forage diets. It was predicted that BUN concentrations would increase with increasing dietary CP intake until BUN concentration reached an adequate concentration for animal production again at which point it may plateau as the animal begins to excrete more N (Figure 12). In the Manuscript 1 BUN concentrations increased from 0.8 to 3.5 mmol L^{-1} with increasing dietary CP from 7.8 to 14.1 g d^{-1}, however BUN values do not plateau at or above 2.1 mmol L^{-1}. Further it was expected that CH_4 emissions would decrease with increasing BUN and plateau at a higher BUN concentration where N is available for improved efficiency of rumen microbes and muscle synthesis. This was not observed in the present study as shown in Figure 10 and Figure 11 where the range of adequate BUN concentrations depicted by the grey area to not appear do not appear to plateau at any concentration. Further, BUN was found to explain very little to none of the CH_4 emissions as predicted (Table 6). As explained above, the relationship between CH_4 and BUN may be better understood with the inclusion of ambient temperature or weather data. This may help determine the dynamics of BUN and under what circumstances animals are able to use additional N for efficiency and what circumstances they may be excreting additional N due to environmental factors.

4.4 **Summary**

A series of models were created for cow and steer cattle groups for the prediction of CH_4 emissions as measured by L d^{-1} and % GEI. All models except the steer (% GEI) model required BUN as a significant prediction variable. However, the partial R^2 attributed to the BUN effect was very small (0 to 5.7) and both the trial effect and the residual terms accounted for large

portions of the partial R^2 at 9.3 to 41.7 and 33.3 and 30.8% for steer (% GEI), steer (L d^{-1}), cow (% GEI) and cow (L d^{-1}) models respectively. Based on the limited capability of this model to predict emissions and the significance of dietary variables in the model, this method does not improve the system which currently exists by the IPCC as was the initial objective.

Contrary to the relationship expected between BUN and CH_4 (% GEI) emissions, the meta-analysis data set in this study showed no obvious impact of BUN concentrations < 2.1 mmol L^{-1} on CH_4 emissions. Nor was there any apparent BUN concentration at which efficiency, as measured by of decreased CH_4 emissions, was improved.

5. GENERAL DISCUSSION

5.1 Current state of forage preservation in the Canadian cattle industry

Many producers in the cattle industry in Canada have adopted low- cost production methods to stay profitable in the last two decades. These production systems include feeding strategies that avoid the need for substantial labour and machinery usage as required for feeding TMR, ensiling forages, ensuring all native forage hay is preserved early in the season etc. Low cost and labour-saving forage preservation and feeding strategies like swath or bale grazing forages can have negative implications on nutrition and animal efficiency if hay is not preserved at a high quality. Most beef cattle operations in Canada harvest forage after full head or full bloom (Sheppard et al., 2014) resulting in mature forage with increased fibre content and decreased energy and protein content. In addition to increased maturity, other factors that may result in low CP forage include environmental conditions such as weather delaying or degrading harvest. Small profit margins in the cattle industry of recent years may have exacerbated economic constraints that could also impact forage quality such as lack of labour or machinery to preserve forage quickly and limited fertilization of forage stands. Without a feeding strategy that includes supplementation, backgrounding cattle may not receive the required nutrients when poor quality forages are the primary feed ingredient.

5.2 Inadequate dietary protein as it impacts methane emissions

It is reported that feeding low-quality forages to beef cattle may lead to increased CH_4 emissions. Methane emissions negatively impact the environment by contributing to the GHG inventory but also production whereby CH_4 emitted is a waste of feed energy which could instead be used for

gain. If nutrient deficiencies of forage diets can be corrected, with improved CP: TDN for example, CH_4 emissions (% GEI) may be reduced.

Feeding low-quality forage characterized by low dietary protein concentrations or by large undegradable protein fractions where protein is less accessible to microbes can cause rumen N deficiencies resulting in inadequate rumen NH_3. Undegradable protein fractions can also be increased via inadequate preservation practices causing molding or aerobic fermentation, heating and spoilage.

Further forage diets, typical of backgrounding operations, tend to degrade slower because of the time required to reduce the large particle sizes in the rumen (Zebeli et al., 2007) and made slower still if dietary protein is lacking, restricting growth and activity of microorganisms (DeRamus et al., 2003). The slower the degradation process the more time available for ruminal fermentation, more H_2 production and more time for methanogens to produce CH_4 (Kumar et al., 2009). Feed containing a higher degradable protein fraction or will degrade more quickly and if DMI can be maximized CH_4 emissions (% GEI) will be reduced (Beauchemin and McGinn, 2006).

Not only does decreasing particle size increase rate of passage but it also tends to increase the proportion of propionate leading to a further decrease in CH_4 production (Janssen, 2010). Again the option for processing feed to offer diet of smaller particle size can depend on labour and machinery resources. As well, propionate production increases just after feeding and therefore CH_4 emissions decrease by increased feeding frequency (Janssen, 2010 and Johnson and Johnson, 1995), which is also dependant on the type of feeding strategy used.

5.3 **Decreasing methane emissions by improved rumen efficiency**

In Manuscript 1, it was observed that increasing dietary CP levels from 6.9 to 13.6% increased the N available for microbial activity improving fermentation efficiency and increasing animal N status (BUN) from 0.8 to 3.5 mmol L^{-1}. A diet by period interaction was observed regarding CH_4 emissions (% GEI) where steers with higher N status emitted less CH_4 (% GEI) by the end of the trial than at the beginning of the trial.

The benefits of increased dietary CP were also evident when relating ADG and CH_4 emissions to steer days on feed. Steers receiving diet L with 6.9% CP required 409 days on feed and produced 81 399 L of CH_4 to reach the same target gain as steers fed diet E containing 13.6% CP for 57 days and producing 14 788 L of CH_4; only 18% of that produced from steers receiving diet L.

Increasing dietary CP in backgrounding diets is only beneficial if animals are removed from the backgrounding phase once they reach the targeted weight. However, if the goal is not to reach a target weight but to instead retain calves over the winter so that they can be grazed during the following pasture season then the number of days the animal stays in the background phase is not reduced and additional dietary CP will increase CH_4 (L d^{-1}) during a constant period of backgrounding. In this way, it is very important that diet recommendations take into account management schemes.

5.4 **Diet nutrient recommendations for rumen efficiency**

Increased dietary CP from 6.9% to 12.1 and 13.6% CP decreased total CH_4 emissions (L) from the backgrounding phase by 80 and 82%. However, it should not be assumed that this

relationship will continue by further increasing levels of CP content. If rumen fluid concentrations of NH_3-N become excessive, the animal will begin to excrete excess N as urea to prevent NH_3 toxicity (Huntinghton and Archilbeque, 1999). Increased N excretion can lead to more N available for volatilization and N_2O emissions (Dijkstra et al. 2013). In fact, N_2O has a greater global warming potential than CH_4 at 265 and 28 respectively (IPCC, 2007) and therefore excessively increasing CP concentrations for the reduction of CH_4 may cause a greater overall contribution to GHG inventories by increased N excretion and N_2O emissions. Therefore dietary CP concentration recommendations should balance potential environmental implications of CH_4 emissions and N_2O volatilization.

Cattle backgrounded in Canada are often exposed to extremely cold and adverse weather conditions which can influence N utilization. Acute cold can cause ruminants to excrete higher concentrations of urea N (Kennedy and Milligan, 1978). Cold-adapted animals use N more efficiently by increasing the N recycled in the body and requiring less dietary N. The time required for animals to become cold acclimatized or de-acclimated however is unclear. Therefore N excretion based on dietary intake is difficult to predict in cold environments.

Increasing metabolizable energy will decrease N excretion as a proportion of N intake by a factor of 0.048 for every 1 MJ $kg^{-1}$of diet (Yan et at. 2007). However if energy intake increases above an animal's maintenance level requirement, protein synthesis rate will become most limiting and excess energy will be deposited as fat (NRC, 1996). The backgrounding phase is meant to grow young animals to an appropriate frame size before adding fat and therefore diets should be formulated based on CP: TDN ratio rather than dietary energy or CP alone.

In Manuscript 1, isocaloric diets were fed and therefore it is not possible to discern the appropriate CP: TDN ratio as described by Yan et al. (2007). With the intent to identify optimal CP recommendations, Dijkstra et al. (2013) suggested that the rate at which urea N, excreted as urine or feces, is converted to N_2O can range from 3 to 15% and can reach as high as 52% depending on volatilization conditions. The data from Manuscript 1 suggests that diet AMG provides an optimal level of CPI at 151 g d^{-1} and a CP: TDN ratio of 0.19 to achieve a balance between CH_4 and potential N_2O emissions.

Animals receiving inadequate levels of dietary CP have a lower ADG and require more days on feed producing more CH_4 during their lifetime. Examining the relationship between ADG and CH_4 kg $gain^{-1}$ (CO_2-eq) shows that CPI levels should be approximately 115 g d^{-1} or higher for efficient gains and therefore forage diets should contain at least as much CP as diet ARM (10.3% CP), which is in line with recommendations from NRC (1996).

An objective of Manuscript 1 was to determine if increased dietary CP concentration of forage diets was a valid tool for reducing enteric CH_4 emissions. This study demonstrated that an increase in dietary CP content from 6.9 to 13.6% could reduce CH_4 emissions by 8% as a portion of GEI. This method falls short of previously documented emission reductions associated with fat supplementation (Mathison, 1997) or grain concentrate diets (Bras, 2013) by 33%. However the inclusion of fat or high portions of grain may not cause an imbalance of the CP: TDN ratios required for lean muscle growth in backgrounding diets.

Although increasing CP concentrations in forage diets does not reduce CH_4 emissions as drastically as some other mitigation methods, like fat or grain supplements, it is clear from Manuscript 1 that dietary CP and therefore BUN does act as an indicator of rumen efficiency.

5.5 **Prediction of enteric methane emissions**

A constraint for using the Tier 2 (IPCC, 2007) model for CH_4 prediction is the lack of information regarding diet quality and intake. Nutrient analysis to determine diet quality exists however it is underutilised. Accurate DMI is more difficult to measure especially at an individual animal scale and although the technology exists it is still cost prohibitive for most farm operations. Therefore, prediction of enteric CH_4 emissions could be improved if a method could be developed that did not require specific diet quality or quantity detail.

Manuscript 1 showed that BUN served as an indicator of N status resulting from CPI, thus incorporating both diet quality and intake factors to one value. Blood serum urea nitrogen is collected via a blood sample taken on-farm with potential to act as a direct measure of individual animal emissions. One of the objectives of Manuscript 2 was to develop a model containing variables that could be measured easily on-farm as a more effective tool than the IPCC Tier 2 model. However not only were separate models required for steer and cow groups, but diet variables were found to be significant in three of the four models (no significant diet variables found in the steer CH_4 L d^{-1} model), therefore detailed dietary information obtained via feed tests was still necessary in the same manner as the Tier 2 methodology.

Although many diet and animal variables were included in these models, trial accounted for large portions of the partial R^2 (9.3 to 41.7%) in all four models. To enhance the effectiveness of the prediction models, additional information to differentiate between trials is necessary. Variables that have not yet been accounted for in this model, that may help describe the differences among trials are; breed, ambient temperature, weather patterns etc. Including these types of trial differences individually would decrease the residual that falls under the trial term.

It is important to note that if a term has been removed, it has been found to be not significant in predicting CH_4 emissions and therefore is just as notable as those that remain.

5.6 **Adequate range of blood serum urea nitrogen relating to methane prediction**

One of the objectives of Manuscript 2 was to determine the optimal BUN concentration resulting in minimum CH_4 emissions. An animal with inadequate dietary CP will have a low BUN concentration (< 2.1 mmol L^{-1}) and therefore, based on the above discussions, would produce greater CH_4 emissions caused by inefficient rumen degradation. Furthermore, by regressing BUN concentrations with CH_4 (% GEI) emissions, the range of BUN concentration resulting in decreased CH_4 emissions should be apparent. However, no relationship was found in either the steer or cow CH_4 (% GEI)-BUN to suggest a level of BUN for improved efficiency. Therefore no recommendation for adequate range of BUN can be made from the observations in this study. It is possible, based on the lack of response to low BUN concentrations, that the current standard for low BUN (< 2.1 mmol L^{-1}) is too high. Further investigation of factors influencing both BUN and rumen efficiency should be considered as explained below.

5.7 **Future research**

The reduction of CH_4 emissions as a result of increased dietary CP in forage diets observed in Manuscript 1 suggests that correcting diet deficiencies in backgrounding cattle may play an important role in CH_4 mitigation. Although cattle may not be receiving adequate dietary CP, caution should be taken in making recommendations to increase CP content without an upper

limit. Future work to decipher optimal CP: TDN in forage diets in terms of balancing CH_4 and N_2O emission in cold environments is necessary before producer recommendations can be made.

Although Manuscript 1 demonstrated that CH_4 was reduced with increased dietary CP, and it is known that BUN and dietary CP are strongly correlated, BUN only accounted for 0 to 5.7% of the variation associated with the prediction models. Therefore BUN was not able to predict CH_4 with any more accuracy, nor were the variables required any simpler to derive, than those used in the IPCC Tier 2 model (IPCC, 2007). However the issue remains that accurate and consistent diet and intake data do not exist on a national scale and therefore more research is needed to develop a method of on-farm measurement of efficiency to improve the current system of enteric CH_4 prediction.

The selection and development of such an indicator first requires a better understanding of how extreme cold and temperature fluctuations impact the N status and rumen efficiency of cattle. The time required for animal acclimatization or de-acclimatization to cold is unclear and therefore so is the relationship between weather patterns, BUN and N excretion and therefore rumen efficiency and CH_4 emissions. Future research should concentrate on the dynamics of weather and N status before another potential CH_4 indicator can be chosen.

6. Conclusions and management implications

It can be concluded that:

- Inadequate CP in forage diets resulted in increased CH_4 emissions presumably due to insufficient protein for growth and optimal activity of the rumen microbial community.
- Increasing CPI of steers fed forage diets increased N status as measured by BUN (mmol L^{-1}).
- Increased CP of forage diets from 6.9 to 12.1 and 13.6% decreased the number of days required for backgrounding cattle to achieve a target liveweight and therefore the total amount of CH_4 (L) emitted by 80 and 82% respectively.
- Although increased CP is a valid option for lowering CH_4 emissions it does not have as large of mitigation impact as some feeding strategies previously explored for other cattle classes; supplementation with fats or high starch concentrates.
- Blood serum urea nitrogen is a significant variable in predicting CH_4 emissions in all models except for the steer CH_4 (% GEI) however in all cases BUN accounts for very little of the partial R^2. All prediction models also contained significant diet variables and therefore the models developed in this study are of no greater benefit than the models currently used by the IPCC Tier 2 methodology (IPCC, 2007).
- Although BUN and CPI are highly correlated and in Manuscript 1 and increased CPI resulted in decreased CH_4 (% GEI), BUN explained very little of CH_4 emitted and therefore the ideal BUN concentration for efficient rumen fermentation could not be determined in this study. Nor was there any indication that BUN concentrations below 2.1 mmol L^{-1} resulted in increased CH_4 emissions.

It can be recommended that:

- Producers managing backgrounding operations emphasise the importance of harvesting hay for nutritional values rather than biomass in order to meet the nutritional requirements of growing animals in cold climates.
- Producers should analyze forage feed for nutrient content in order to make sound decisions regarding feeding strategies to ensure animals are receiving adequate nutrients for efficient rumen digestion.
- The purpose of the backgrounding stage on individual operations should be considered before CP content of forages is determined. Only if animals will be removed when they have reached a target weight should CP content be increased above animal requirement in forage diets as CH_4 (L d^{-1}) increases with increased dietary CP.

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