Mitigating enteric methane production and nitrogen excretion from forage-fed ruminants

by

Isaac Asante Aboagye

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in
Animal Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

ABSTRACT

Sustainability of animal agriculture requires efficient use of energy and nitrogen (**N**) by ruminants fed high forage diets. Therefore, there is a need to decrease enteric methane (**CH_4**) emissions and prevent excessive N release from beef cattle into the environment. Thus, this thesis focused on quantification and mitigation of CH_4 emissions and N excretion from beef cattle fed corn silage (**CS**) and alfalfa based diets. In western Canada, production of corn with short growing season (≤ 2600 corn heat unit, **CHU**) for silage is increasing due to its potentially high nutritive value, while alfalfa is a forage of choice because of its high crude protein (**CP**) and total digestible nutrient contents. The objective of the first study was to determine the variability in nutrient content, degradability of dry matter (**DM**) and neutral detergent fiber (**NDF**), and CH_4 production of short-season whole plant corn hybrids grown and adapted in two locations of Alberta (**AB**; central or southern) and harvested before or after a light frost (-1.5ºC). Harvesting southern AB hybrids after frost did not affect starch content but NDF degradability increased. Harvesting central AB hybrids after frost increased starch content and reduced CH_4 emissions, but had limited effects on DM and NDF degradabilities. The objective of the second study was to determine the long term effects of hydrolyzable tannin **(HT**) alone and in combination with condensed tannin (**CT**) at low [0.25%; chestnut (**CN**) or CN and quebracho mix (**CNQ**)] and high (1.5%; CN or CNQ) doses on N use and CH_4 emissions in growing beef steers fed an alfalfa silage-based diet. Tannin, irrespective of type or dose, decreased ruminal ammonia-N concentration. Tannin type and dose did not affect daily CH_4 production but 1.5% CNQ tended to decrease CH_4 yield (CH_4 per kilogram of DM intake) compared with control. The objective of the third experiment was to determine the effects of different forms of HT, including 2% CN, 1.5% tannic acid (**TA**), or a sub-unit (gallic acid, **GA**)] on CH_4 production, N utilization, and diet

digestibility in beef cattle fed a diet mainly containing alfalfa silage. Both TA and CN decreased CP digestibility and shifted N excretion from urine to feces, while GA decreased CH_4 production and decreased the proportion of urea N in urinary N. In conclusion, for short-season CS, it is possible to select hybrids adapted for their use in central AB based on their CHU rating to reduce the carbon footprint of animal agriculture without affecting animal performance. Also for alfalfa silage based diets supplemented with tannin, the simplest unit or metabolite of the tannin has the potential to lower CH_4 emissions and N excretion from beef cattle.

ACKNOWLEDGEMENT

This thesis is the aresult of a long and challenging voyage and many people have contributed greatly to its successful end. First and foremost, I would like to thank God for protecting my life and granting me good health through my academic journey. Secondly, I would like to thank my supervisors Drs. Masahito Oba and Karen Beauchemin. Dr. Masahito Oba accepted me as a student at U of A when I needed a supervisor. I am grateful for his effective criticism and scientific knowledge on my research. Dr. Oba has made my PhD a pleasant experience. Literally, Dr. Karen Beauchemin brought me to Canada and trained me as a student from my MSc to PhD. The rapport she established with me as a supervisor is the best a student could wish for himself or herself. I am extremely grateful for her patience, motivation, enthusiasm, scientific expertise and motherly love shown me over the past 6 years. Karen as she is affectionately called is an exceptional mentor and a true scientist.

Again, I would like to appreciate Dr. Vern Baron who is a member of my supervisory committee for providing insightful discussion and vital input on my research. It was a pleasure to work with you. In addition, I would like to express my gratitude to Dr. Kris Johnson who agreed to be my external examiner. I greatly appreciate the time and effort that Dr. Kris Johnson invested in reviewing my thesis. Special thanks go to Dr. Karen Koenig for her scientific input as co-author of some published chapters of my thesis. I would also like to acknowledge the financial support from Agriculture and Agri-Food Canada that made this research and my stay in Canada possible.

Furthermore, I would like to express special gratitude to all members of Drs. Beauchemin, Koenig and Baron Labs and the barn crew of the Lethbridge Research and

Development Center. This research project was a real teamwork effort and would not have been possible without many helping hands! I also would like to thank Holly Horvath (Graduate Program Administrator at the University of Alberta) for her invaluable help throughout my program. Lastly, I would like to thank my wife Mrs. Eunice Aboagye and my baby boy Ayeyi Aboagye, family and friends here in Canada and back home in Ghana and for their unconditional support throughout my program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A:P	Acetate: propionate ratio
AB	Alberta
ADF	Acid detergent fiber
ADG	Average daily gain
BUN	Blood urea nitrogen
BW	Live body weight
CH_4	Methane
CHU	Corn heat unit
CN	Chestnut
CNQ	Chestnut and quebracho mix
CO_2	Carbon dioxide
CO_2e	CO_2 equivalent
CoM	Coenzyme M
CP	Crude protein
CR	Clearance rate
CS	Corn silage
CT	Condensed tannin
CV	Coefficient of variation
dH_2	Dissolved hydrogen
DE	Digestible energy
DM	Dry matter
DMD	Dry matter degradability

DMI	Dry matter intake
GA	Gallic acid
G:F	Gain to feed ratio
GE	Gross energy
GHG	Greenhouse gas
GP	Gas pressure
GWP	Global warming potential
H_2	Hydrogen
HT	Hydrolyzable tannin
LeRDC	Lethbridge research and development center
MP	Metobolizable protein
N	Nitrogen
N_2	dinitrogen
N_2O	Nitrous oxide
NADH	Nicotinamide adenine dinucleotide
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber degradability
NDSC	Neutral detergent soluble carbohydrate
NEg	Net energy for gain
NH_3	Ammonia
NH_3-N	Ammonia-nitrogen
NH_4^+	Ammonium
NO_3^-	Nitrate

OM	Organic matter
PUN	Plasma urea nitrogen
Q	Quebracho
SD	Standard deviation from the mean
SEM	Standard error of the mean
SF_6	Sulphur hexafluoride
TA	Tannic acid
TMR	Total mixed ration
UNE	Urine nitrogen excreted
VFA	Volatile fatty acid
WPCH	Whole plant corn hybrid

CHAPTER 1 – Literature review

1.1 Introduction

Globally, the livestock industry (including animals, manure, feed, machinery, and land use change) contributes 14.5% of anthropogenic greenhouse gas (**GHG**) emissions (Gerber et al., 2013). Greenhouse gas (carbon dioxide, **CO_2**; methane, **CH_4**; nitrous oxide, **N_2O**) emissions increase the warming effect of solar and thermal radiation on the earth's surface and atmospheric temperature and consequently cause climate change (IPCC, 2006). The CH_4 emission, which is mainly from ruminant production systems, also accounts for 2 to 12% of energy intake loss in ruminants (Johnson and Johnson, 1995) representing a potential inefficiency. Also, there is inefficiency in dietary nitrogen (**N**) utilization by ruminants that are fed forage diets high in soluble protein, because an increase in N intake results in an increase in the N excreted in feces and urine (Dijkstra et al., 2013). The negative effects of CH_4 emission and N excretion on both the environment and animal performance warrants cohesive efforts by scientists to find mitigation strategies that are economically feasible. Strategies related to animal nutrition can be economical and effective methods of reducing CH_4 per unit of animal product, but lack of knowledge hinders their successful implementation. Some of the strategies could be used for forage based diets where CH_4 losses are high and N utilization is often low. These include use of starch-containing forages and tannins as extracts or tannin-containing forages.

Alfalfa is widely used for ruminant production because of its nutrient quality. Alfalfa has high soluble protein content and negligible tannin concentration (Berard et al., 2011). Therefore, protein degradation in the rumen of alfalfa diets is extensive and increases rumen ammonia-N (**NH_3-N**) concentration leading to excretion of high levels of N into the environment. It is possible that feeding tannins to ruminants fed alfalfa based diets may help reduce N losses.

Tannins have the ability to form complexes with carbohydrates and proteins, which may reduce enteric CH_4 emissions (Beauchemin et al., 2008) and reduce N excretion in urine (Grainger et al., 2009). Thus, by using tannins, N utilization may be improved while reducing emission of CH_4 from cattle that consume alfalfa based diets.

It is also known that feeding diets containing high concentrations of structural carbohydrates, as is the case with low quality forages, results in greater energy lost as CH_4 than when feeding diets containing grains, which contain starch. However, it is unclear whether the CH_4 reduction associated with feeding starch also occurs for forages such as corn silage (**CS**), that contain high concentrations of both structural and non-structural carbohydrates. Corn is a warm-season crop and growing it in a cold environment can be challenging. Nevertheless, early maturing corn hybrids with corn heat unit (**CHU**) rating ≤ 2600 have made CS production achievable in short-season areas of western Canada (Gabruch and Gietz, 2014; Guyader et al., 2018). Factors such as variety (hybrid), physiological maturity, season, and time of harvest cause variation in nutritive value of forage and affect the energy lost as enteric CH_4 by ruminants. These factors are not well understood for short-season CS.

1.2. Ruminant Production, Environment and Climate Change

1.2.1. Environmental Impacts of Ruminant Production

As the world's population is estimated to increase from 7 billion to more than 9 billion people in 2050 concern about food security is on the rise (UN, 2017). The issue of food security especially in rural areas could largely be addressed by the increase of animal products in human diets (FAO, 2011), which would require an increase in the intensity of livestock farming. The rise in meat production due to intensive livestock farming is projected to come from chicken,

followed by pigs and to a lesser but growing extent from ruminant (cattle, sheep and goat) production, especially in developing countries (FAO, 2011). Ruminants provide not only meat but also milk in human diets, which together are the highest quantity of animal protein in human diets (Eisler et al., 2014). Again, compared with other livestock, ruminants occupy the largest area of land worldwide and are efficient in using fibrous feeds that cannot be used as human food. However, the environmental sustainability of intensive ruminant production systems has been highly criticized (FAO, 2006).

Globally, there are vastly different ruminant production systems due to the different ecological zones, animal breeds, management practices and traditions. However, production systems can be largely categorized as grazing, integrated crop-animal production (mixed), and feedlot (concentrated animal feeding) systems (Gerber et al., 2015). The grazing system for ruminant production uses more than 90% of dietary dry matter (**DM**) from rangelands, pastures, and annual forages (Sere and Steinfeld, 1996). Integrated crop-animal production systems use greater than 10% of the DM fed to animals from forages and by-products from cereal grain and other crops (e.g., stubble or straw) or more than 10% of the value of production comes from non-livestock farming activities (Sere and Steinfeld, 1996). It is estimated that about 34% and 63% of cattle produced globally are raised under grazing and mixed systems, respectively. These systems cover the largest land areas across all climatic zones and supply the majority of the world's beef (Table 1.1; Figure 1.1; Gerber et al., 2013). In contrast to grazing and mixed systems, feedlots or concentrated animal feeding systems are on the rise, mainly because these systems are more efficient and use less land. Beef cattle that are raised in feedlots are usually fed purchased grain (70% to 95% dietary DM), and thus have high animal performance in terms of

average daily gain (**ADG**) and feed conversion ratio (Pelletier et al., 2010; Capper, 2012; Gerber et al., 2015).

There are environmental benefits (ecological function) associated with raising animals under the various production systems (Table 1.2). For instance, grazing and mixed systems provide ecosystem services (increased rangeland productivity, nutrient cycling, soil fertility enhancement, and carbon sequestration), and preserve wildlife and other forms of biodiversity (Gerber et al., 2013). Also, the manure from ruminants may contribute to about 35% of soil organic matter (**OM**), which helps maintain soil structure, water retention, and drainage capacity for plant growth (Steinfeld et al., 1996). However, intensive grazing and clearing lands for feed production and pasture establishment to increase animal productivity can cause significant negative environmental impacts (Table 1.2). The millennium ecosystem assessment estimated that 10 to 20% of all grassland is degraded due to overgrazing (FAO, 2009). The degradation of lands in turn, leads to soil erosion, degradation of vegetation, release of carbon from soil OM, reduction in biodiversity, and impaired water cycles (FAO, 2015). Also, feedlots can have negative impacts on water resources, air quality, and animal welfare (FAO, 2015).

Regardless of production system, ruminant production contributes to GHG emissions, which are implicated in climate change. Some of the implications of climatic change include an increase in the frequency and intensity of storms, an increase in areas affected by drought, and hotter and more frequent heat waves (IPCC, 2013). Again, climate change may affect ruminant production systems themselves indirectly (e.g. lack of good quality and quantity of forage) or directly (animal health and production; Henry et al., 2018). Poorly managed production systems can also increase N released into the environment, directly or indirectly through manure or fertilizer application. The N released into the environment can leach into ground water, streams,

and rivers, which can kill aquatic life or cause nitrate (NO_3^-) poisoning in drinking water (Follett and Hatfield, 2001). Also, the N released can enter the atmosphere as N_2O and consequently lead to the negative effects of climate change.

Table 1.1. Global cattle population and beef production by the production system in 2010. Sourced from (Gerber et al., 2013).

Production system	Cattle population ($\times 10^6$)	Beef production[1] ($\times 10^6$)
Grazing	506	22
Mixed[1]	911	39
Feedlot	29	5
Total	1446	66

[1]Under the mixed production system dairy and beef herds contributed 28% and 31%, respectively, to global beef production.

Figure 1.1. Estimated cattle population in grazing, mixed and feedlot systems. (Source: Gerber et al., 2013)

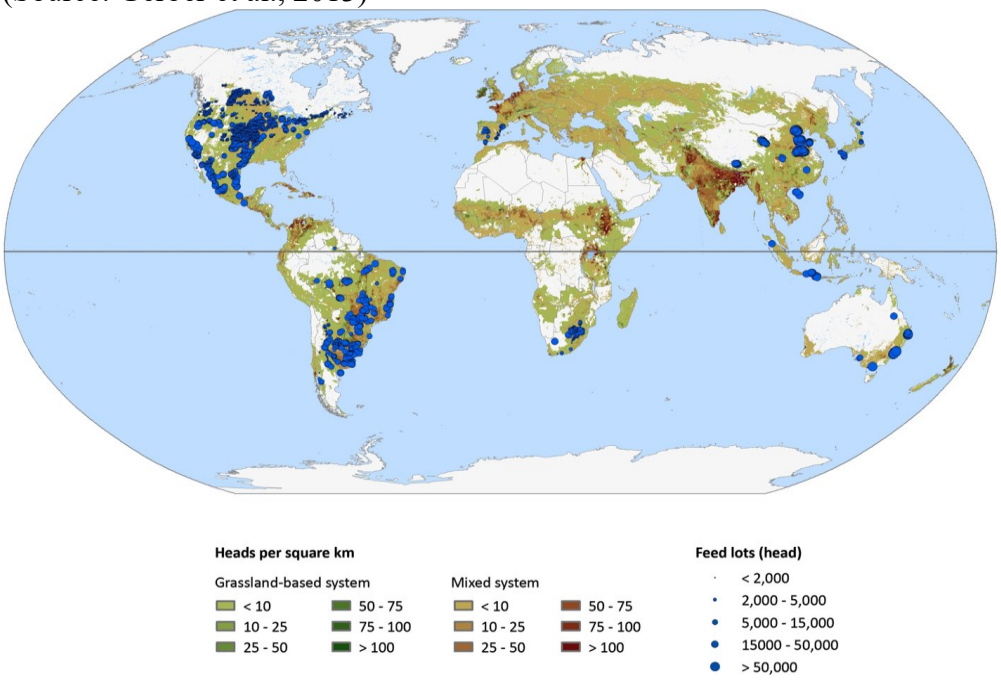


Table 1.2. Summary of major environmental interactions and improvement opportunities. Source (Gerber et al., 2015)

	Environmental Categories							
	Land and water (feed)		Nutrient Cycle		Biodiversity		GHG emission	
System of Production	Interactions	Opportunities for improvement	Interactions	Opportunities for improvement	Interactions	Opportunities for improvement	Interactions	Opportunities for improvement
Grazing	(-) Large occupancy, possible degradation. (+)Management in open space allowing multiple usages. (+/-) Animal trampling	Improved range and pastureland management, (rotational grazing). Development of feeding operation in integration with crop for stubbles or by-product. Increase productivity through animal health and genetics.	(+/-) Fertility transfer from grazing site to manure deposition site.	Manure storage and recycling could be improved.	(-) Overgrazing and land degradation. (+) Habitat creation and maintenance.	Range management with biodiversity (open spaces). Improved rangeland and pastureland management to avoid land degradation and land conversion	(-) Relatively high GHG intensity (GHG per product).	Legume introduction in pasture. Improved range and pastureland management through mobility, rotational grazing to control land conversion.
Mixed	(+) Use of crop residues and improvement of agricultural productivity, which results in higher land use efficient.	Feeding balanced rations, providing feed supplements and health to increase feed efficiency.	(+) Fertilization.	Improve collection rates and processing of manure for recycling.	(-) Grassland abandonment or intensification.	Land use planning to maintain a mix agro-ecosystems. Manure management and, using integrated pest management to avoid bad effects of intensification.	(+/-) Moderate to low intensity.	Feeding balanced rations, feed supplements and good health to reduce emission intensities. If possible reducing age at first calving and of breeding overhead to decrease intensities.

Feedlot (concentrated animal feeding)	(-) Competition with plant-based food product. (-) Withdrawals and water pollution related. (+) Use of agricultural crop residues	Minimize usage and pollution of water. Precision feeding, good health and animal management to improve feed efficiency.	(+/-) Fertilization/Pollution.	Frequent manure collection and processing to minimize pollution. Precision agriculture to optimize nutrient use efficiency.	(-) Nutrients and pesticides pollution.	Minimize water usage and pollution. Through precision feeding and animal management feed conversion efficiency is improved thus spare land for conversion.	(+) Relatively low GHG intensity.	Improve energy use efficiency e.g. farm equipment and transport. Manure management and processing.

1.2.2. Greenhouse Gas Emissions from Ruminants

Undoubtedly, the livestock industry contributes to a significant amount of GHG into the atmosphere. These gases are known to cause of climate change by increasing the warming effect of solar and thermal radiation on the earth's surface and atmospheric temperature (IPCC, 2006). From 1880 to 2012, the average global temperature increased by 0.85 °C and given current GHG emissions, temperature may exceed an increase of 1.5°C by the end of 2100 above the 1850 to 1900 level (IPCC, 2013). The GHG have different warming effects, so they are expressed based on their global warming potential (**GWP**, a relative measure of how much heat a GHG traps in the atmosphere compared to the heat trapped by a similar mass of CO_2 over a period of time e.g. 20 or 100 years). For instance, the GWP of CH_4 and N_2O is 28 and 265 times larger than CO_2 over a 100-year span, respectively (IPCC 2007; 2013; Gerber et al., 2013).

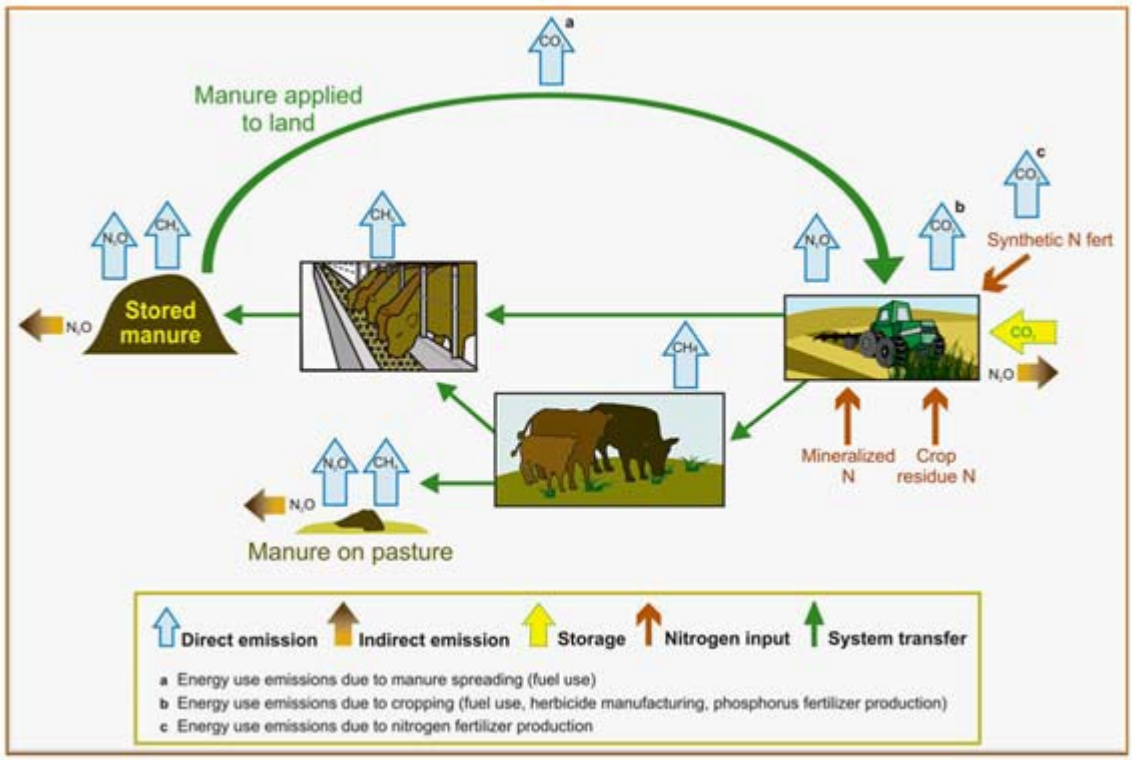
Livestock production systems especially from ruminant production emit multiple gases from different sources in the life of the animal, both directly and indirectly (Figure 2.1). Thus to calculate the quantity of GHG produced throughout the life of the animal, the emissions are usually expressed in CO_2-equivalent (**CO_2-e**), by multiplying the amount of the respective GHG gas by its GWP (IPCC, 2006).

Globally, on the basis of life cycle assessment including land use change, it is estimated that the livestock industry produces about 7.1 Gt of CO_2-e annually, which represents about 14.5% of total anthropogenic GHG emissions (Gerber et al., 2013). Gerber et al. (2013) estimated that CO_2 is the most prevalent GHG from human activities, followed by CH_4 and N_2O (71.6%, 20.7% and 6.9%, respectively); however, the non-CO_2 gases (CH_4 and N_2O) are the most prevalent in the livestock sector (73%). In Canada, based on a 2016 national inventory report, without land use change accounted for, agriculture contributed to 8.5% of the national

GHG emission (704 Mt of CO_2-e; Environment and Climate Change Canada, 2018). The Canadian agriculture sector accounted for 30% and 77% of the national CH_4 an N_2O emitted, respectively. The main source of agricultural emissions was the livestock industry, which contributed about 55% (33 Mt of CO_2-e) mainly as CH_4 and N_2O (based on 2016; Environment and Climate Change Canada, 2018).

Using a whole farm model analysis, Beauchemin et al. (2010) estimated that GHG emitted in western Canada through beef production was on average 21.7 kg CO_2e/kg of beef produced, comprised of 5% CO_2, 68% CH_4 and 27% N_2O. Within the whole farm model analysis, CO_2 produced was from energy-use because carbon loss and storage in soils was assumed to be at equilibrium. For CH_4, 93% was produced from the gastrointestinal tract (enteric) with minor contribution from manure (7%); for N_2O the majority was from manure (85%) with 15% contributed by soil N inputs (applied manure, and fertilizer and crop residue; Beauchemin et al., 2010). The high emission of the non-CO_2 gases (CH_4 and N_2O) from ruminants indicates there is an opportunity to reduce CH_4 emissions from enteric fermentation and N_2O produced from manure.

Figure 1.2 Direct and indirect sources of greenhouse gas emission from ruminant production. Source: Beauchemin et al. (2010).



1.2.3. Ruminal Methanogenesis

Ruminants, like other mammals, do not secrete digestive enzymes that digest structural carbohydrates (cellulose and hemicellulose). Ruminants rely on a consortia of microbes under anaerobic conditions of the rumen to degrade plant structural carbohydrates, proteins and other organic polymers into monomers. The monomers are then fermented to end-products such as volatile fatty acids (**VFA**), NH_3, CO_2, and hydrogen (**H_2**). The VFA (mainly acetate, propionate and butyrate) in turn, are used by the animal as its main source of energy, while CO_2 and H_2 are used by methanogens, through a process known as methanogenesis, to form CH_4.

The majority of enteric CH_4 is produced in the rumen (87 to 92%), however, CH_4 production (8 to 13%) can also occur in the hind gut (Murray et al., 1976; Torrent and Johnson,

1994). Murray et al. (1976) reported that about 98% of total CH_4 produced both in the rumen and hind gut is emitted by eructation through the mouth with the remaining (2%) emitted through the feces. The greater emission through the mouth is because about 89% of the CH_4 produced in the cecum and large intestine (hind gut) is absorbed into the bloodstream and released by expiration.

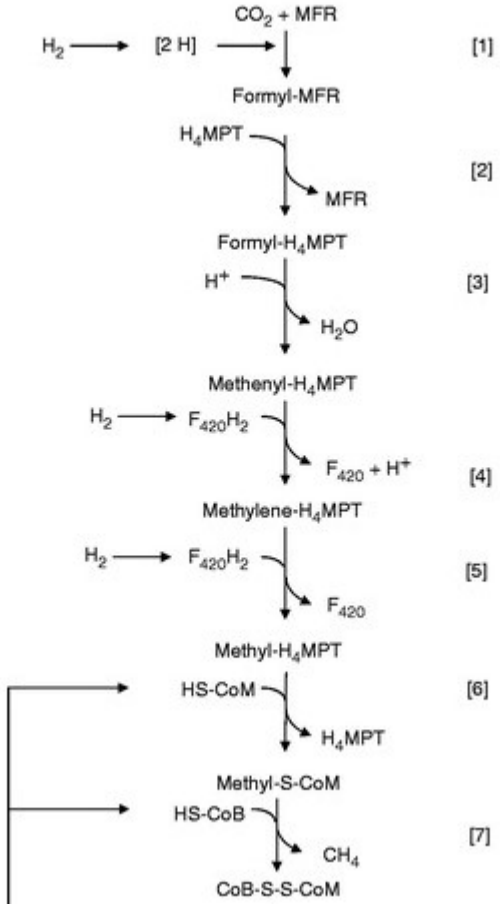
Methanogens belong to the archaea domain and derive their energy by forming CH_4 (Mathison et al., 1998; Morgavi et al., 2010). Archaea are found in different environments but those found in the rumen are strict anaerobes and unlike bacteria, the archaeal membranes contain glycerol di-ethers or glycerol tetraethers and their cell wall lacks peptidoglycan and instead contains pseudomurein (Leng, 2018). Also, methanogens have specific cofactors and enzymes (e.g. F_{420}, methanopterin and coenzyme M [**CoM**]) involved in methanogenesis (Deppenmeier, 2002). In the rumen, the proportion of methanogens is about 0.3 to 4.0% of the rumen microbial mass and they are normally placed within 3 genera, namely *Methanobrevibacte*r (61.6%), *Methanomicrobium* (14.9%), and rumen cluster C (15.8%) (Morgavi et al., 2010).

In the rumen the dominant substrates used for methanogenesis are CO_2 and H_2, but other substrates such as acetate, formate, and methyl compounds (methanol, mono, di and tri-methylamine) can be used to form CH_4 (Thauer, 1998). During glucose fermentation or glycolysis, reduced cofactors such as nicotinamide adenine dinucleotide (**NADH**) need to be re-oxidized to NAD^+ for the fermentation process to continue and enable microbial growth (Hegarty and Gerdes, 1999). After glycolysis, the oxidation of NADH to NAD^+ may be directly coupled to the products formed from pyruvate. Acetate, which is the main VFA formed quantitatively from pyruvate, is not coupled directly with oxidation of NADH and thus NADH is oxidized through H_2 production (van Lingen et al., 2016). However, NADH is

thermodynamically reduced at elevated partial pressure of H_2 in the rumen. Therefore, methanogens utilize H_2 to reduce CO_2 to CH_4 and keep the partial pressure of H_2 at a low level to enable NADH to be re-oxidized to NAD^+ (Hegarty and Gerdes, 1999).

The use of CO_2 and H_2 as substrates for CH_4 production involves a series of chemical reactions that require cofactors and enzymes that direct electron flow through four reductive intermediates (i.e. formyl, methenyl, methylenyl and methyl) with CH_4 as the final product (Figure 1.3; Deppenmeier, 2002). However, in the methanogenic archaea, all other methanogenic substrates use methyl-CoM as the central intermediate with the enzyme methyl-S-CoM reductase for the conversion of methyl-CoM to CH_4 (Deppenmeier, 2002). The use of CO_2 as the terminal user of carbon and a sink for H_2 shows how important methanogens are to rumen microbial fermentation (Morgavi et al., 2010). The removal of H_2 is essential to maintain a high rate of ruminal fermentation, which means methanogens contribute indirectly to plant fiber digestion (Reynolds et al., 2014). However, due to the high energy content in CH_4 (13.3 Mcal/kg), the loss of energy in the form of CH_4 partially offsets the positive effect of methanogens on fiber digestion.

Figure 1.3. Pathway of methanogenesis from CO_2 and H_2 substrates. Source (Deppenmeier, 2002).



[1] formyl-MFR dehydrogenase; [2] formyl-MFR:H_4MPT formyltransferase; [3] N5,N10-methenyl-H_4MPT cyclohydrolase; [4] N5,N10-methylene-H_4MPT dehydrogenase; [5] N5,N10-methylene-H_4MPT reductase; [6] N5-methyl-H_4MPT:CoM-SH methyltransferase; and [7] methyl CoM reductase; where MFR is methanofuran; and H_4MPT is tetrahydromethanopterin.

In the rumen, methanogens also coexist with protozoa intracellularly or extracellularly through interspecies H_2 transfer, and it has been estimated that about 37% of rumen CH_4 production is a result of this symbiotic relationship between protozoa and methanogens (Finlay et al., 1994). In a study that summarized *in vitro* and *in vivo* studies on defaunation (removal of protozoa), CH_4 reduction was 13% (Hegarty, 1999), it was 11% but when only *in vivo* studies

were considered (Morgavi et al., 2010). Clearly, experimental designs including the method used to obtain protozoa-free animals and dietary effects may contribute to variation in protozoa (Morgavi et al., 2010), thus more animal studies are needed to understand the relationship between protozoa and methanogenesis.

1.2.4. Techniques for Measuring Methane

Quantifying enteric CH_4 production accurately is essential in understanding dietary effects on methanogenesis at animal, farm and regional levels. There are several methods of measuring CH_4 emission from ruminants and these have been reviewed broadly in the literature (Goopy et al., 2016; Hill et al., 2016) as indirect (e.g. *in vitro* and modeling) or direct (e.g. sulphur hexafluoride (**SF_6**) technique, respiration chambers, Greenfeed system) approaches. Herein, the *in vitro* gas production, SF_6 and respiration chambers are discussed. See Table 1.3 for a summary of the other methods.

The amount of gas released and the production of VFA are related to the quantity of substrate being fermented in the rumen (Dijkstra et al., 2005). Thus, different systems have been developed for measuring *in vitro* gas production. The extensive use of forage in ruminant diets has led to the use of *in vitro* techniques to select suitable forages based on their fermentation kinetics. The methods described by Menke et al. (1979) for CH_4 measurement have recently been modified by companies such as ANKOM Technology® to enable mechanization. Briefly, the principle of the *in vitro* batch culture system relies on the incubation of a feed substrate with rumen fluid and buffer under anaerobic conditions in gastight culture bottles (Figure 1.4 A). Gas accumulates at different time-points and a cumulative volume is recorded by correcting for gas produced from blanks without substrate (Goopy et al., 2016). Gas is sampled from the headspace and analyzed for composition using gas chromatography to estimate amount of CH_4 produced.

The *in vitro* gas system is an inexpensive and rapid technique for estimating CH_4 production in ruminants; however, it does not account for the effect of animal intake on rumen retention time and the complexity of the rumen environment, which may lead to significant differences in CH_4 production between *in vivo* and *in vitro* systems. *In vitro* systems can also estimate VFA production and substrate DM disappearance (Blümmel et al., 2005).

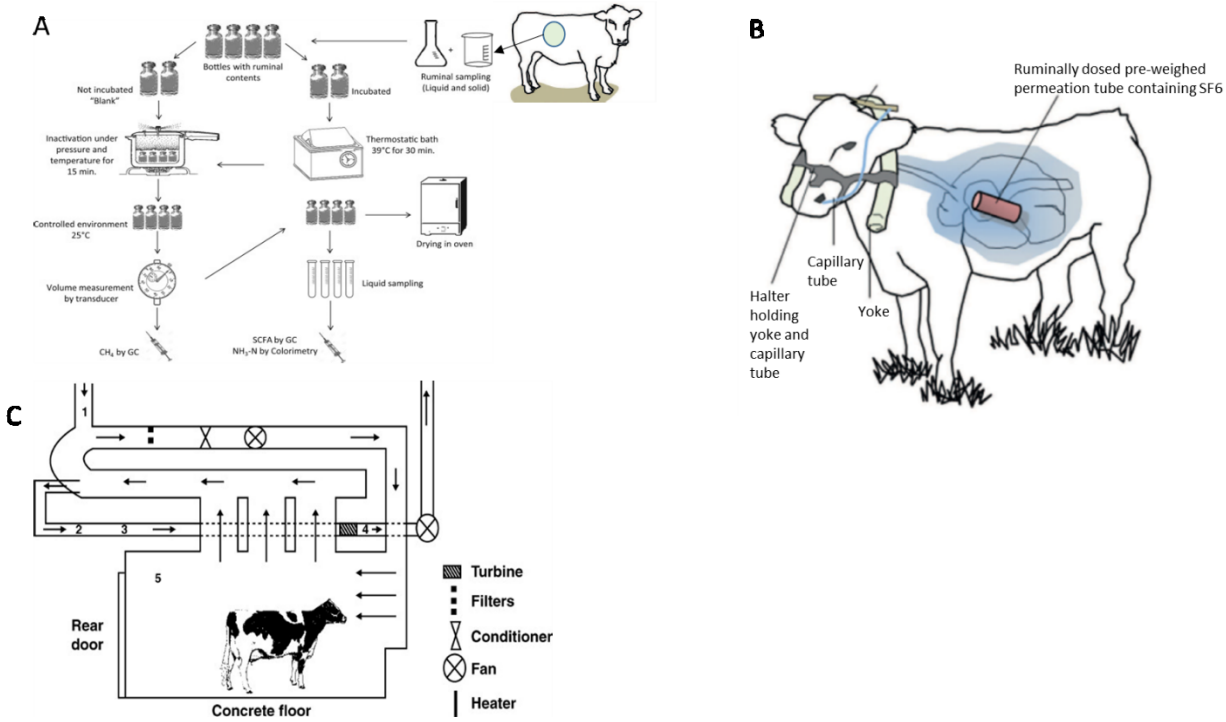
The SF_6 method is a direct method of measuring CH_4 from ruminants that are grazing (Chaves et al., 2006) or kept in individual feeding stalls (Aboagye et al., 2018). The basic principle of the SF_6 technique is that CH_4 production can be measured if the emission rate of a tracer gas, which is safe and inactive and behaves like CH_4, is known. As described in detail by Johnson et al. (1994), a permeation tube containing a predetermined release rate of SF_6 at 39ºC is orally inserted into the rumen. Then an evacuated yoke (canister) connected to a halter and fitted with a capillary tube is put around the neck to collect the air (CO_2, CH_4 and SF_6) that moves passively from the animal's muzzle and mouth (Figure 1.4 B). The capillary tube is designed to deliver half the yoke's volume (about 1.7 and 2.5 L for sheep and cattle, respectively) during the collection period of 24 h. At the end of gas collection, the yoke is pressurized with N gas, and CH_4 and SF_6 concentrations are determined by gas chromatography. Enteric CH_4 production over a 24 h time period, is estimated by multiplying the CH_4 /SF_6 concentration ratio by the known permeation tube release rate, corrected for background CH_4 and SF_6 concentrations (Williams et al., 2011). Given that SF_6 (146 g/mol) is heavier than CH_4 (16 g/mol), these gases may disperse and accumulate differently depending on location, ventilation and the structure of the building (especially for animals kept indoors), and as such, measuring background gas is necessary to improve the value of CH_4 estimated. However, there are variations within (among days) and among animals using the SF_6 technique and thus the technique needs to be conducted

over multiple days and using sufficient numbers of animals to ensure representative emissions are estimated. For instance, Grainger et al. (2007) reported that the coefficient of variation (**CV**) among animals was 19.6% and within animal was 6.1%, and both were 1.8 units greater when compared to respiration chambers. Although the SF_6 method has lower precision and accuracy compared with respiration chambers, it is a very low cost way of measuring CH_4 production. However, it can be less physically robust than chambers due to high equipment failure, and it is also more labor intensive. Considering these sources of variation, the number of repeated measures necessary to give accurate results should be determined before carrying out the experiment.

Respiration chambers can be used to directly measure CH_4 produced from individual animals. The building design of chambers may vary but the basic principle remains unchanged. An animal is housed in an enclosed room with air inlet and outlet (Figure 1.4 C). By running intake and exhaust fans at different speeds, air can be pulled through each chamber with a negative pressure generated inside to prevent escape of air from the chamber (Goopy et al., 2016). However, at Lethbridge Research and Development Centre (**LeRDC**) the respiration chambers are enclosed in a building and so a small positive pressure (2 to 5 Pa) is generated in the chamber to ensure there is no inflow of gases from the hallway to the chamber (McGinn et al., 2004; Beauchemin and McGinn, 2006). Also, the air in the chamber is well mixed with a small positive pressure, air is not drawn into the chamber from the manure trap (K. A. Beauchemin, LeRDC, personal communication). Calibration is essential for this system of CH_4 measurement. This is done by releasing an amount of standard gas of known concentration to calculate recovery values. The results can be influenced by the volume of the chamber, temperature, humidity, pressure, and incoming air composition. Therefore, temperature, relative

humidity, and ventilation are controlled to allow animals to remain in a thermo-neutral zone while gas sampling is ongoing (Goopy et al., 2016). Changes in CH_4 concentrations are measured by sampling incoming and outgoing air, using gas analyzers, infrared photoacoustic monitors, or gas chromatography systems (Grainger et al., 2007; Goopy et al., 2014). One major critique of this technique is that the animal's natural behavior in terms of feed intake or movement is restricted in the chambers. To minimize changes in behavior and decreased feed intake, animals are adjusted to the chambers before the experiment begins (McGinn et al., 2009). Because of the high costs of chamber construction and sensitive instruments (gas analyzers and flowmeter), and the maintenance of chambers, usage is limited. However, models derived to estimate national and global CH_4 emissions from ruminants are often developed from a few data obtained from respiration chambers because of its precision (Johnson and Johnson, 1995). Thus, the chamber system has been used as the standard to determine the accuracy of other techniques for measuring CH_4 production.

Figure 1.4. Different techniques of measuring methane production (modified from Grainger et al., 2007 and Hill et al., 2016).



A, is an *in vitro* gas technique; B is the SF_6 technique; and C is the respiration chamber technique for CH_4 measurement.

Table 1.3. Techniques for measuring methane emission from ruminants. (Source: Goopy et al., 2016)

Method	Description	Suitability	Cost	Accuracy and precision
In vitro incubation	Feed substrate is incubated in airtight bottles/bags to allow gas accumulation, and then gas samples analyzed for CH_4 concentrations.	Used to rank feeds and additives for methanogenic potential under controlled conditions.	Low	May not represent whole-animal (*in vivo*) emissions.
Modeling	CH_4 is estimated from feed intake using models, usually developed from previous experimental data.	Applicable in cases where measurements are not possible. Requires estimates of feed intake, which can be challenging to obtain.	Low.	Based on assumptions and thus equations limit their ability to accurately predict CH_4 production.
Respiration Chambers 	Measures CH_4 concentration within exhaled breath while the animal is in an enclosed chamber.	Not suitable for examining effects of grazing management. Restricts normal animal behavior and movement; may decrease feed intake. Only a few animals can be used for measurement at any one time.	Expensive to construct and maintain. Use is technically demanding. Requires animal training.	Provides most accurate and precise measurements of emissions, including CH_4 from ruminal and hindgut fermentations.

Ventilated hood 	An airtight box is placed to surround the animal's head. Gas exchange is measured only from the head rather than the whole body.	Can be used to assess emissions from different feeds. Restricts normal animal behavior and movement; not suitable for grazing systems	Lower cost than whole-animal chamber. Requires animal training.	Does not measure hindgut CH_4.
SF_6 tracer technique 	A small permeation tube containing SF_6 is placed in the cow's rumen, and SF_6 and CH_4 concentrations are measured near the mouth and nostrils of the cow.	Allows the animal to move about freely; suitable for grazing systems. Can be used to measure large numbers of individual animals. Depends on SF_6 which is a GHG.	Lower cost, but higher level of equipment failure and more labor-intensive than respiration chambers. Animal must be trained to wear a halter and collection yoke.	Less precise than respiration chambers. Does not measure hindgut CH_4.

Polyethylene tunnel 	A large tunnel made of heavy-duty polyethylene fitted with end walls and large diameter ports. The concentrations of air between the incoming and outgoing air are continuously monitored	Suitable for measuring CH_4 emissions under semi-normal grazing conditions. Can be used for individual or small group of animals. Feed intake under grazing is a challenge.	Operation simpler and portable than respiration chambers.	Frequent calibration, provides high CH_4 recovery rate. Tunnel's temperature and humidity are difficult to control
Open-path laser 	Lasers and wireless sensor networks send beams of light across paddocks containing grazing animals. The reflected light is analyzed for greenhouse gas concentrations	Measures CH_4 emissions from herds of animals and facilitates whole-farm measurements across a number of pastures. Emissions cannot be attributed to a single source.	Expensive and technically demanding. Requires sensitive instrumentation to analyze CH_4 concentration and capture micrometeorological data.	Accuracy is highly dependent on environmental factors and the location of test animals. Data must be carefully screened
Greenfeed® Emission Monitoring Apparatus 	Patented device that measures and records short-term (3–7 min) CH_4 emissions from individual cattle repeatedly over 24 h by attracting animals to the unit using a "bait" of pelleted concentrate.	Suitable for comparing effects of feeds or supplements	Patented device; must be purchased from supplier, C-lock Inc. (Rapid City, South Dakota, USA).	Provides comparable estimates to respiratory chamber and SF_6 techniques. Does not measure hindgut CH_4.

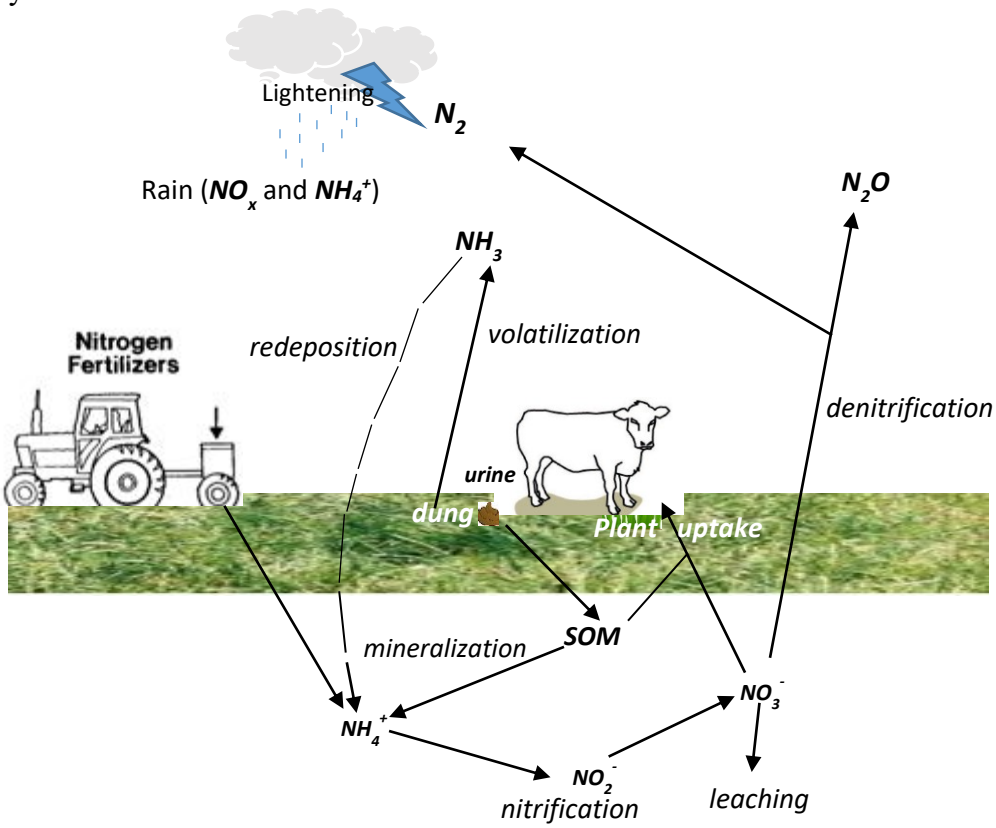
1.2.5. Nitrogen Excretion and Nitrous Oxide Formation

Overfeeding crude protein (**CP**) to ruminants can increase dietary N intake and in turn, increase N excretion in manure (urine and feces). However, feeding low dietary CP to decrease N excretion may lower animal performance (Broderick, 2003; Fanchone et al., 2013), but this may not always be the case (Cyriac et al., 2008; Li et al., 2009). Most dietary N consumed by cattle is excreted, and most of the excreted N (40 to 75%) is in the form of urinary N (Satter et al., 2002; Cole and Todd, 2009). Also, more than half of the N excreted in the urine of cattle is in the form of urea, which upon contact with urease in the soil or feces is rapidly hydrolyzed to CO_2 and volatile NH_3 (Hristov et al., 2011). However, the N in feces is less volatile because it is mainly in the form of indigestible organic N (proteins or nucleic acids; Hristov et al., 2011).

Nitrous oxide emission from the N cycle of livestock production systems is complex (Figure 1.5). Before 1750, the reactive forms of N (e.g. NO_3^-, NH_3) occurred in the soil mostly through conversion of dinitrogen (**N_2**; inactive N) by lightning and through biological N fixation (Ciais et al., 2013). Currently, nitrogenous inputs to the soil have increased from synthetic fertilizer usage, leguminous crop residues and ruminant manure, such that the N input process (mineralization and nitrification) exceeds the N output process that leads to N_2 formation (denitrification; Ciais et al., 2013). The current increase in N input from manure and fertilizers leads to redeposition of volatile NH_3 and direct increase of N_2O in the atmosphere (an intermediate gas formed through microbial processes of nitrification and denitrification; de Klein and Eckard, 2008; Ciais et al., 2013). Nitrification is an aerobic process by specialized microbes in the N cycle where ammonium (**NH_4^+**) is converted to NO_3^-. Nitrate may easily be available for plant uptake, but because of its negative charge, it does not stick to the negatively charged soil, making it vulnerable to loss and polluting water bodies. However, if the NO_3^- concentration

increases due to anaerobic conditions in the soil (wet soil or low oxygen), then denitrification can occur through the reduction of NO_3^- by anaerobic microbes to N_2. Again, when the NO_3^- concentration becomes abundant through run-off and leaching from agricultural soil, then incomplete denitrification will occur by reducing the NO_3^- to N_2O.

Figure 1.5. Overview of nitrous oxide emission from nitrogen cycle in a ruminant production system.



SOM, soil organic matter; NH_3, ammonia; NH_4^+, ammonium ion; NO_2^-, nitrite; NO_3^-, nitrate; oxides of nitrogen, NO_X; N_2, dinitrogen; and N_2O, nitrous oxide. The nitrification and denitrification process via manure and fertilizer application are involved in direct N_2O emission, whereas, indirect emission is through volatilization of NH_3 and the leaching and runoff of NO_3^- from agricultural soils (Ciais et al., 2013).

1.3. Nutritional Methods of Reducing Methane from Ruminants

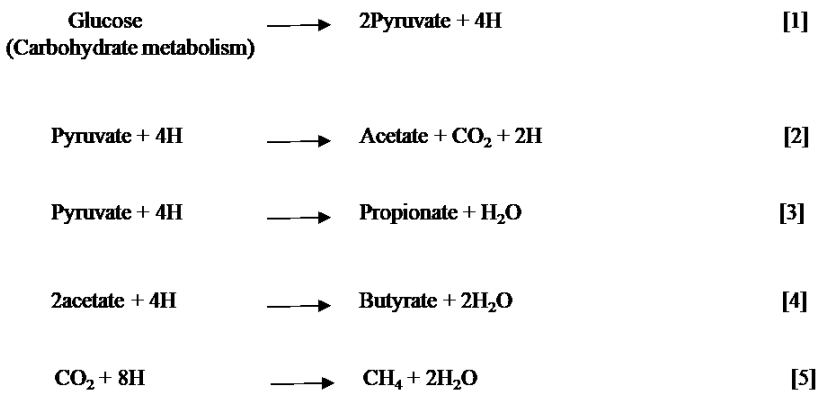
1.3.1. Overview of Nutritional Methods of Reducing Methane Emissions

Many strategies (Figure 1.6) to reduce enteric CH_4 emission have been proposed and tested through a range of experiments as reviewed extensively by Beauchemin et al. (2008), Eckard et al. (2010), and Knapp et al. (2014). Among the strategies, nutritional management is relevant to this thesis, and hence will be discussed with emphasis on forage quality and tannin supplementation.

1.3.2. Type of Carbohydrate

Carbohydrates represent the largest component of ruminant diets and are essential for meeting the energy requirements of the animal. Carbohydrates are usually broadly classified as cell wall and cell content carbohydrates, with the latter being more digestible (Ishler and Varga, 2001). The type of carbohydrate in a ration influences the amount of enteric CH_4 due to microbial species and the proportion of VFA produced during ruminal fermentation (see the equations below; Johnson and Johnson, 1995; Knapp et al., 2014). A regression analysis study by Moe and Tyrrell (1979) reported that the neutral detergent fiber fraction [**NDF**; cellulose, hemicellulose and lignin (non-carbohydrate)] of a diet and its digestibility result in higher CH_4 production than the neutral detergent soluble carbohydrate fraction (**NDSC**; pectin, β-glucans, galactans, starches, sugars, fructans and silage organic acids). The fermentation of NDF favors H_2 production where acetate is the main VFA produced, which promotes CH_4 production (Van Soest, 1994; see equations below). On the contrary, fermented NDSC (especially starch or sugar) increases the proportion of propionate or butyrate in the VFA, which serves as H_2 sink to reduce CH_4 production (Janssen, 2010; Wang et al., 2014; Ungerfeld, 2015). Also ruminal pH decreases

with increasing soluble carbohydrate concentrations, which can inhibit the growth of methanogens and protozoa (Hegarty, 1999).



Within the NDF fraction, the relative proportion of cellulose or hemicellulose may affect CH_4 production differently (Ellis et al., 2007). For instance, in a regression analysis, Moe and Tyrrell (1979) showed that hemicellulose fermentation may only produce 37% of the CH_4 produced by cellulose. However, in the study by Holter and Young (1992), CH_4 production was correlated positively with hemicellulose and negatively with cellulose fermentation. In both data sets, the fermented cellulose and hemicellulose were from combined forage and non-forage feed ingredients. Cellulose and starch are first hydrolyzed to glucose (6-carbon sugar) before being fermented but they produce different VFA proportions and affect CH_4 production differently. Hemicellulose is a mixture of 5- and 6-carbon sugars, and so, the rate of fermentation and utilization of VFA is expected to result in different proportions of VFA and CH_4 production than does starch or cellulose. However, the differences in CH_4 production may not necessarily be due to the effect of different chemical substrate (starch, cellulose and hemicellulose) but the function of the microbial species and the majority of the carbohydrate substrate in the diet (NDF:NDSC; Baldwin and Allison, 1983; Knapp et al., 2014). Forages are high in NDF and favor production of acetate, while grains are high in starch and stimulate the growth and metabolic activity of

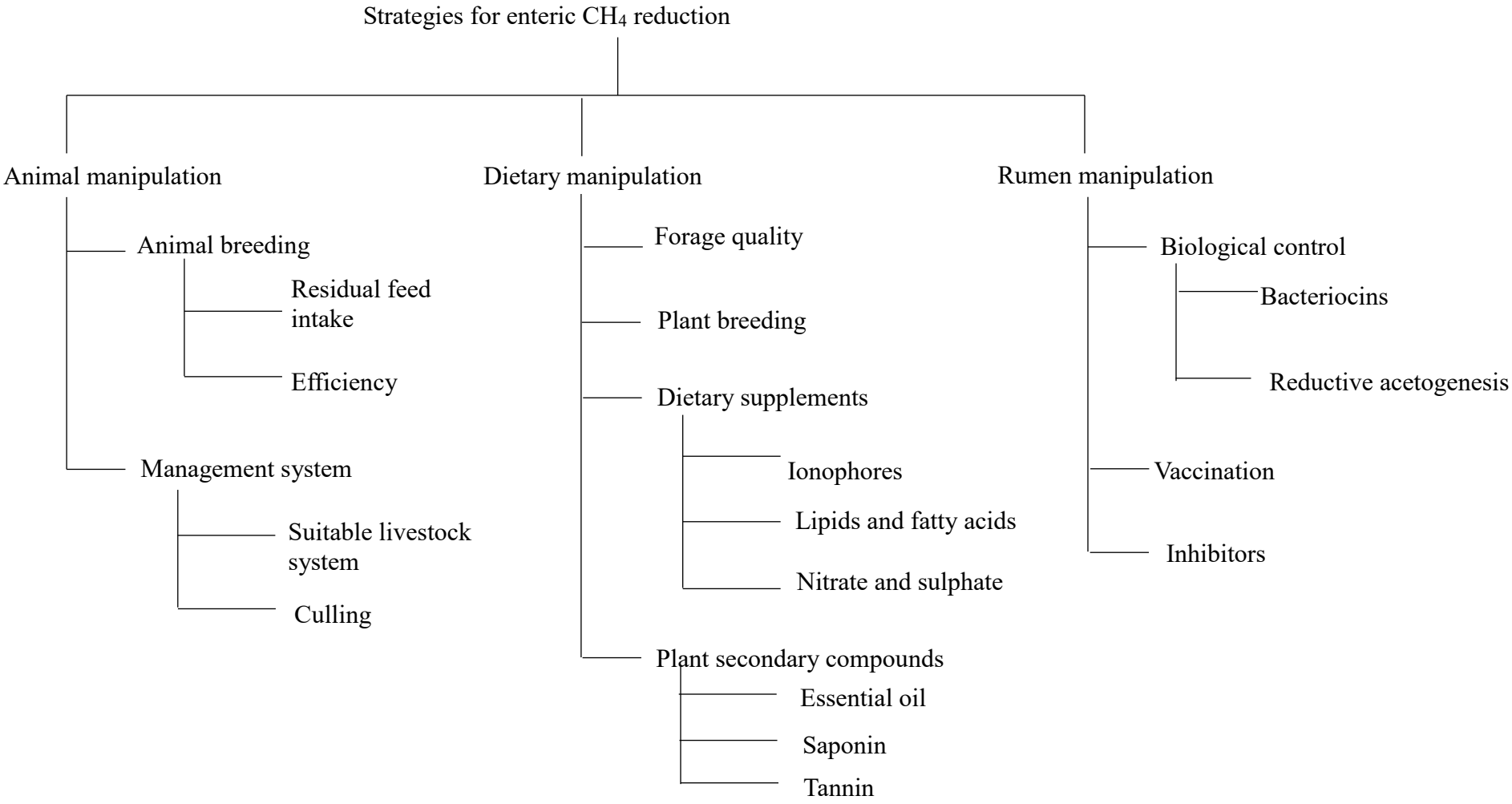
propionate-producing microbes and hence a reduce CH_4 production (Johnson and Johnson, 1995). Thus the proportion of forage:grain in diet may affect CH_4 production differently.

1.3.3. Type of Forage

High grain diets such as those fed in feedlots can improve cattle performance and reduce enteric CH_4 per beef produced (Capper, 2012). However, the majority of the feed used in beef production is forage (Beauchemin et al., 2010), and low quality forages may account for 75% of global ruminant CH_4 emissions (Leng, 1993). Thus, increasing the quality of forage to increase feed efficiency and ADG may decrease CH_4 per animal product (Waghorn and Clark, 2006; Knapp et al., 2014). Forages can be fed fresh (through grazing or harvested and fed outdoors or indoors) or fed as conserved hay, haylage or silage. Quality of forage is influenced by factors such as species, variety, physiological maturity, season, time of harvest and conservation method. However, the quality of a particular forage is determined by its effect on animal productivity through nutrient composition, DM intake (**DMI**) and digestibility. Thus, among forage types, considerable variations exist in their chemical composition, DMI and digestibility; and these sources of variation affect the quantity of CH_4 produced.

With increasing DMI, there is more feed to ferment and so the total CH_4 produced (g or Mcal/d) increases with increasing DMI, but when CH_4 is expressed as a proportion of DMI (CH_4 yield), it usually decreases as DMI increases above maintenance (Blaxter and Clapperton, 1965; Moe and Tyrrell, 1979; Pinares-Patiño et al., 2009). In general, CH_4 reductions for forage diets are correlated with increases in nutrient quality and decreases in digestibility, which are associated with higher intake and passage rate (Knapp et al., 2014).

Figure 1.6. Strategies for reducing methane emissions in ruminants



1.3.3.1. Grain Based Forages vs. Traditional Forages

The impact of increasing the ratio of grain:traditional forages (legumes or grass) on decreasing CH_4 emission in ruminants is generally known (Johnson and Johnson, 1995; Beauchemin et al., 2008). Due to the higher starch content in grain compared with legume or grass, increasing grain in the diet decreases CH_4 yield or CH_4/kg of animal product, provided animal production stays the same or increases (Ferris et al., 1999; Yan et al., 2000). In a meta-analysis study, which comprised 87 experiments with 260 treatments, Sauvant and Giger-Reverdin (2009) concluded that CH_4 yield is decreased in ruminants fed more than 35 to 40% grain in their diet. However, increasing grain in the diet may also negatively affect NDF digestibility (Firkins, 1997; Ferraretto et al., 2013) and potentially decrease animal productivity. In addition, fermentable OM in manure could increase due to a lower digestibility with a possible increase in CH_4 emission from stored manure (Lee et al., 2012). Therefore, finding a balance for grain addition and fiber digestibility by using cereal grain forages, which contain both structural and non-structural carbohydrates could reduce CH_4 emission and improve animal productivity.

Starch-containing forages such as CS (starch content, 182 to 350 g/kg DM; Baron et al. 2014) and small-grain silage (e.g. barley with starch content, 147 to 255 g/kg DM; Zahiroddini et al., 2004; Addah et al., 2011) could be suitable substitutes for traditional forages (legumes and grass) in decreasing enteric CH_4 production (Beauchemin and McGinn, 2008). For instance, in a total mixed ration (TMR), which contains CS and grass silage as the forage components, adding more CS rather than grass silage (75:25 vs 25:75), decreased CH_4 yield or CH_4/kg milk yield by 13% and 6%, respectively (Department for Environment, Food and Rural Affairs, 2010). Similarly, Hassanat et al. (2013) reported that, replacing alfalfa silage with CS (0:100) compared with alfalfa silage replacing CS (100:0) produced 13% less CH_4 yield and 6% less CH_4/kg milk

yield in lactating dairy. On the contrary, replacing grass silage with CS in a total mixed ration (**TMR**; forage:concentrate, 70:30) of growing bulls decreased CH_4 yield by 9% at 5 mo of age but increased CH_4 yield by 14% at 11 mo of age (Staerfl et al., 2012). Also, when CS:alfalfa silage proportion increased from 20:80 to 80:20 in a lactating dairy cow ration, CH_4/NDF digested increased linearly from 190 to 270 g/kg (Arndt et al., 2015). The inconsistencies in these studies show that the quality of CS relative to alfalfa or grass silage may affect the CH_4 response.

Corn, unlike barley, is a warm season crop and so could be a high-risk crop to grow in cold environments like Western Canada. However, early maturing corn hybrids with CHU (accumulated thermal units from planting to grain maturity) rating ≤ 2300 have made CS production attainable in short-season areas of western Canada. For example, in Alberta (**AB**), corn-growing areas have increased by 296% since 2006 to 17,148 acres in 2011 (Statistics Canada Agriculture Census, 2011). The increased rate in corn growing areas in AB relative to barley (the preferred crop) could have been influenced by the greater energy content of CS, due to higher starch content (Beauchemin and McGinn, 2005; Addah et al., 2011) with potential reduction in CH_4 emission and improvement in animal performance (Benchaar et al., 2014). However, there are still inconsistencies in feeding CS on animal production and enteric CH_4 emissions.

Increasing maturity of CS increases DM yield and starch content and reduces NDF content at harvest (Ferraretto and Shaver, 2012). Thus, one would expect CS harvested at later maturity to decrease CH_4 emissions. However, research on the effect of feeding CS with increasing maturity on enteric CH_4 emissions has been inconsistent. In beef (McGeough et al., 2010) or dairy cattle (Hatew et al., 2016) fed CS diets with increasing DM at harvest from 250 to

400 g/kg fresh forage tended to linearly reduce or reduced CH_4, respectively. However, Cammell et al. (2000) and Zom et al. (2012) did not report a linear reduction in enteric CH_4 emissions in dairy cattle fed CS diets with DM at harvest increasing from 230 to 420 g/kg fresh weight. The inconsistencies in the effect of feeding CS on enteric CH_4 emission could be due to differences in chemical composition as a result of hybrid type and growing environment, which influence maturity at harvest. In addition, the effects of adding CS to the diet on CH_4 production may partly depend on feeds that are replaced by CS (grain or forage).

In the cold environments of western Canada, there is insufficient information describing the variability in nutrient content, fiber digestibility, and CH_4 emission potential of short-season corn hybrids grown for silage. However, these hybrids are increasingly grown in different adaptation zones of western Canada for the production of meat and milk. It is therefore important to identify the factors (e.g. genetic differences, adaptation zones, maturity and frost prior to harvest) that affect the CH_4 emission potential of short-season CS hybrids.

1.3.3.2. Legumes vs. Grasses

The relatively low fiber content and the greater DMI and passage rate with an increase in the rate of digestion of legumes account for their lower CH_4 yield of ruminants compared with grasses (Waghorn et al., 2002; Kasuya and Takahashi, 2010; Hammond et al., 2011). McCartney et al. (2012) showed that archaeol (methanogen marker) concentration in the rumen of lactating cows decreased after feeding white clover compared with ryegrass, suggesting a reduction in methanogen population. However, other studies have not confirmed this effect; there was no effect on CH_4 emission (g/d, g/kg DMI, g/kg OM digested) when dairy animals were fed ryegrass silage, red or white clover silages (van Dorland et al. 2007). Furthermore, Chaves et al. (2006) reported that heifers grazing alfalfa produced more CH_4 than did those on grass pasture

(162.8 vs. 113.5 g/d); however, potential differences in DMI were not accounted for. Legumes and grasses can vary in chemical composition and digestibility. Factors, such as plant species, variety, maturity at harvest and preservation can all affect nutrient quality and digestibility (Knapp et al., 2014). However, generally, as the plant matures, structural carbohydrate content increases and this can increase enteric CH_4 emission.

Another factor which usually causes legumes to decrease CH_4 emission compared with grass is the tannin content of some leguminous forages (Beauchemin et al., 2008). Tropical legumes are high in tannins and have lower digestibility and produce less CH_4 per unit of intake than temperate legumes (Archimède et al., 2011). Alfalfa is widely used in North America because of its nutritive quality but has no tannin unlike other leguminous forages such as birdfoot trefoil, which has been shown to reduce enteric CH_4 emissions relative to perennial ryegrass in dairy cattle (Woodward et al., 2004). Thus, by supplementing alfalfa based diets with tannins, it might be possible to lower CH_4 production.

1.4. Potential of Tannins to Reduce Methane and Improve Nitrogen Use by Ruminants

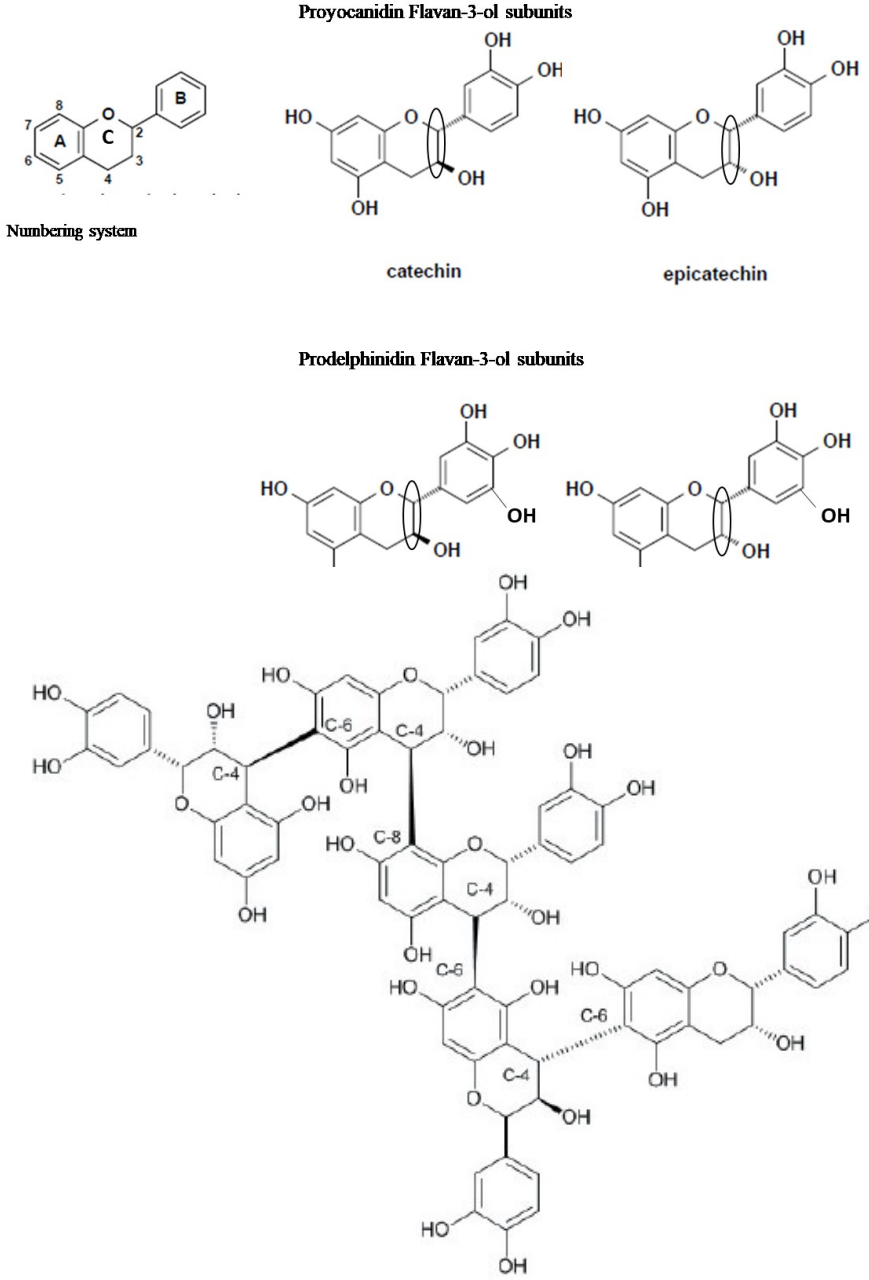
1.4.1. Chemical Diversity of Tannins (Hydrolyzable and Condensed Tannins)

Tannins are a class of polyphenol (hydroxyl attached to aromatic rings) compounds found in the bark of trees, twigs, fruits, flowers and leaves of broad leaf forages. They are commonly grouped under plant secondary metabolites because they are not involved in the biosynthesis, biodegradation and other energy conversion metabolism in the plant (Hagerman, 2011). The large number of phenolic hydroxyl groups enable tannins to form complexes mainly with protein and to a lesser extent with carbohydrates (Patra and Saxena., 2011). Based on the reactivity and structural characteristics of tannins, they are generally grouped as condensed tannin (**CT**) and hydrolyzable tannin (**HT**).

Condensed tannins, which are also referred to as proanthocyanidins, consist of oligomers or polymers of flavan-3-ol subunits (Naumann et al., 2017). The CT have higher molecular weight (1900 to 28000 Da) and their subunits differ due to the hydroxyl groups and the relative stereochemistry of the C-2 and C-3 ring (Figure 1.7; circled). The most common ones are the procyanidin (e.g. catechin and epicatechin, which upon oxidation give rise to cyanidin) and prodelphinidin subunits (e.g. gallocatechin and epigallocatechin, both give rise to delphinidin upon oxidation). The bonding patterns of CT subunits into oligomers and polymers occur mainly through covalent linkages of the C-4 position of the C-ring of one flavan-3-ol to mainly the C-8 and C-6 positions in the C-ring of other subunits (Figure 1.7; B-type linkages, 4→8 and 4→6; Naumann et al., 2017).

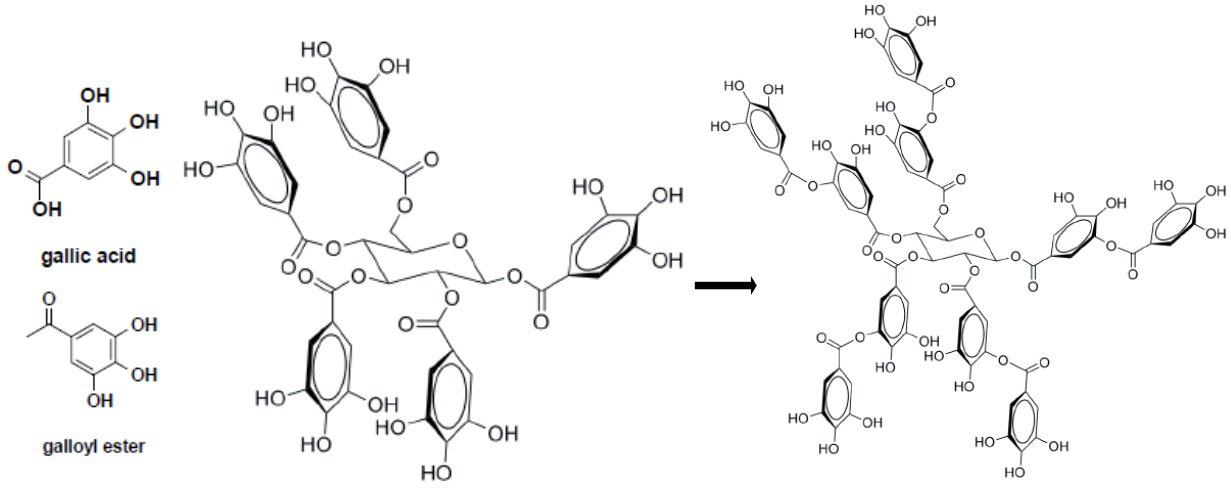
Hydrolyzable tannin is a relatively low molecular weight tannin (500 to 3000 Da) made up of a polyol core, which is usually glucose but may contain other core molecules (glucitol, hammamelose, shikimic acid, quinic acid, and quercitol) with their hydroxyl group esterified with gallic acid (**GA**). Thus, the HT are derivatives of GA; however, through further esterification and oxidative cross-linkages on the galloyl group, more complex HT are formed (Figure 1.8; Hagerman, 2011). Based on the structural features arising from these chemical transformations, HT can be divided into 2 major subclasses: gallotannins and ellagitannins. The gallotannins are formed when more GA units are added to the galloyl groups and this type of HT is commonly referred to as tannic acid (**TA**). Through intramolecular oxidative coupling the galloyl group is dimerized forming ellagic acid moieties. The coupling can be between adjacent GA such as the galloyl groups on glucose C-4 and C-6 (eugeniin) or C-2 and C-3 (casuarictin; also has C-4 and C-6). The casuarictin in turn, may form other intermolecular bonds with itself (e.g. trimer casuarictin) or gallotannins, making HT more complex.

Figure 1.7. Subunit and interlinkage structures of flavan-3-ols occurring in condensed tannins. Source (Hagerman, 2011; Naumann et al., 2017)



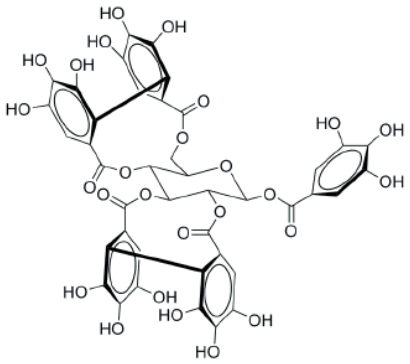
4,8- and 4,6-B-type interflavan linkage in condensed tannin oligomers and polymers

Figure 1.8. Subunit and interlinkage structures of gallotannin and ellagitannin in hydrolyzable tannins. Source (Hagerman, 2011; Naumann et al., 2017)

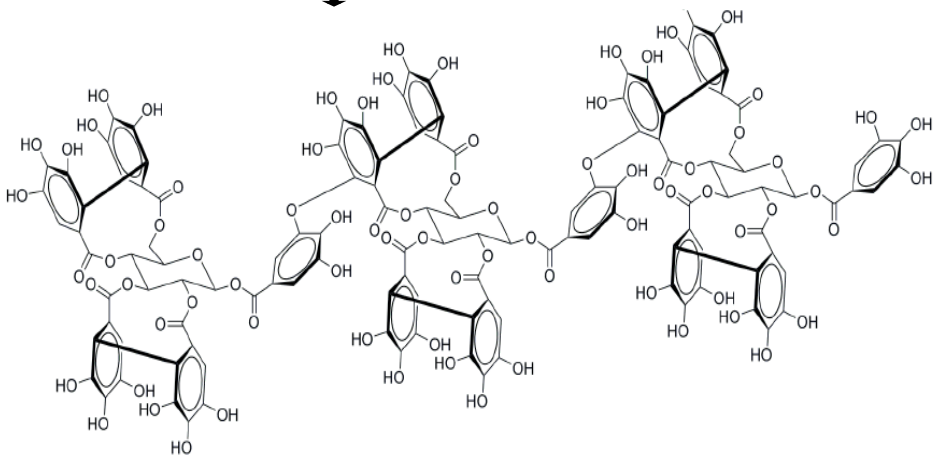


β-1,2,3,4,5,6-Pentagalloyl glucose

Gallotannin (Tannic acid)



Casuarictin (Ellagitannin)



Trimer of casuarictin (Ellagitannin)

1.4.2. Detrimental and Beneficial Effects of Tannins

Tannins were previously considered anti-nutritive when present in feeds because they negatively affected intake, digestion and absorption of proteins, polysaccharides and minerals and subsequently reduced animal performance (Kumar and Singh, 1984). Also, there were reports that tannins may be toxic to the animal by causing ulcer, irritation and desquamation of the intestinal mucosa, liver and kidneys lesions and even death (Reed, 1995). The anti-nutritive and toxicity of tannin are mostly attributed to ingestion of HT because of its lower molecular weight, poorer adsorption to protein and thus easy depolymerization in the rumen to metabolites responsible for cellular damage (Murdiati et al., 1992). However, CT may also affect intestinal organs (Makkar, 2003) and decrease intake and digestibility of proteins such that animal performance may be negatively affected (Grainger et al., 2009). On the contrary, tannin-rich forage can have beneficial effects for ruminants. They may prevent bloat, improve N utilization, act to control endo-parasites, and induce improvements in growth, wool, and milk production (Min et al., 2003; Waghorn, 2008). Tannins are also known to have antioxidant activity, and thus may improve the antioxidant status of animals (Gladine et al., 2007).

The phenolic hydroxyl groups allow tannins to bind with numerous macromolecules, particularly proteins and to a lesser extent with carbohydrates, nuclei acids and metal ions (Makkar, 2003). Thus, the tannin-protein complex is the most important determinant of the nutritional and toxicity of tannins for ruminants. The complex is usually reversible if it is a non-covalent bond (hydrogen and hydrophobic; Jerónimo et al., 2016). The nature of this bond is dependent on the structure of tannin (molecular weight, conformation, flexibility and water solubility) and protein (size, secondary or tertiary structure) as well as the medium (solvent, temperature and pH) in which the interaction takes place (Jerónimo et al., 2016). However, the

effects of tannins for ruminants may depend on the chemical structure, source, concentration in diets, composition of basal diet, and other factors related to the animals, such as animal species and physiological stage (Waghorn, 2008).

1.4.2.1. Intake and Animal Performance

Usually, there is low intake of tannin-containing forages because of a decline in the palatability of feed due to tannin astringency on feed by binding with salivary proteins causing an unpleasant sensation for the animal (Lesschaeve and Noble, 2005). The binding of tannins may decrease fiber digestibility by inhibiting fiber-degrading enzymes or by binding to dietary carbohydrates and in turn, decrease rumen turnover rate, which can impact negatively on intake and animal performance (Makkar, 2003; Waghorn, 2008). On the contrary, feeding tannin-containing forages to animals with high protein requirements may improve performance due to a potential greater supply of metabolizable protein (**MP**, increase in undegradable intake protein) to the lower tract. Under such situations productive response to tannin-containing forages potentially increased by 8 to 38% for ADG and 10 to 21% for milk production, relative to the non-tannin containing forages (Waghorn, 2008).Thus, factors related to the intrinsic characteristics of tannin-containing forages (e.g. type and amount of digestible nutrient and efficiency of use) and the overall diet quality may confound the effects of tannin on animal performance. For instance, in animals fed fibrous and tannin-containing forages, energy may be the first limiting factor for performance and this coupled with fiber intake may decrease digestible energy (**DE**) intake and performance, further. However, feeding tannin-extract as a feed supplement may reduce the confounding effect of tannin-containing forages. This is because free tannin, may bind with salivary proteins (e.g. proline) and allow tannin to pass through the digestive tract in a bound form preventing its degradation, absorption, or interaction with other

dietary or endogenous protein (Alonso-Díaz et al., 2010; Lamy et al., 2011). On the other hand, free tannins may target microbes and disrupt their activities in the rumen (Bhatta et al., 2009) or bind to dietary soluble proteins and increase bypass protein to the lower tract.

1.4.2.2. Rumen Fermentation and Enteric Methane Production

Under anaerobic conditions of the rumen, tannins can be degraded by microbes into other metabolites, which may affect microbial fermentation and subsequently, VFA concentration or proportion in the rumen (Beauchemin et al., 2007; Aboagye et al., 2018). By using tannin-extract from chestnut (**CN**) or quebracho (**Q**) as the only carbon source in a culture technique, microbes such as *Bacillus pumilus*, *B. polymyxia*, *Klebsiella planticola*, *Cellulomonas*, *Arthrobacter*, *Micrococcus, Corynebacterium,* and *Pseudomonas* were identified to produce tannase enzymes to degrade tannins (Deschamps et al., 1980). In the rumen, microbes that can utilize both CT and HT degrade them to into their subunits and subsequently the subunits through the dihydrophloroglucinol and the 3-hydroxy-5-oxohexanoate pathways are converted to either acetate or butyrate (Figure 1.9; Krumholz and Bryant, 1986; Bhat et al., 1998).

Basically, tannin extracts act as rumen modifiers like other nutritional supplements (e.g. inhibitors, electron acceptors) to decrease CH_4 emission (Table 1.4; Hristov et al., 2013; Knapp et al., 2013). However, the main mechanism by which tannins affect methanogensis and decreases CH_4 production is not definitely proven *in vitro* or *in vivo*. There are multiple hypotheses for how tannins decreases CH_4 production: 1) tannins act directly on methanogens (Field et al., 1989, Carrasco et al., 2017); 2) they indirectly affect microbes associated with methanogens such as protozoa (Bhatta et al., 2009); 3) tannins act on fibrolytic bacteria and decrease fiber degradation (Carulla et al., 2005), and 4) lastly, they act as a H sink (Becker et al., 2014). These hypotheses suggest that tannin may either be influencing all, some, or any of the

proposed mechanisms, and this may explain the range (2% to 58%; Cieslak et al., 2012) of CH_4 reductions among researches that evaluated effects of tannin. The mechanisms by which tannin reduce CH_4 may differ with tannin type, source, and concentration.

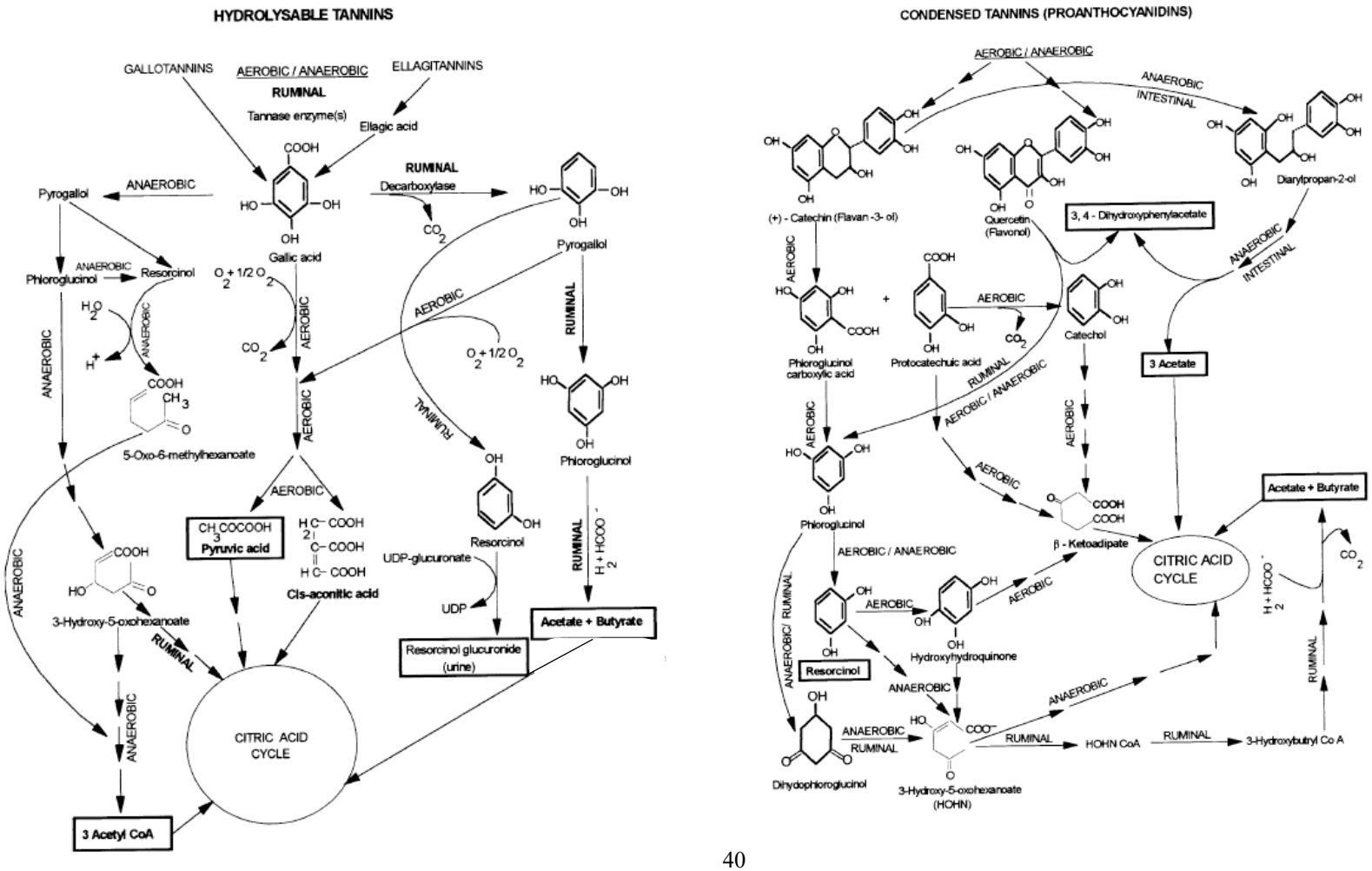
Most of the research work with tannins *in vivo* has focused on CT rather than HT because HT is believed to be toxic to animals due to its lower molecular weight, which influences its hydrolysis in the gut of the animal. However, the effect of HT may influence dietary macromolecules and/or rumen microbes, especially methanogens than the animal itself. Jayanegara et al. (2015) showed that HT has higher binding ability than CT and thus decreased the methanogen population or microbes providing H_2 to a greater extent than CT (Goel and Makkar, 2012). Again, sourcing tannin from a combination of HT and CT may even show a greater potential of decreasing CH_4 than applying HT or CT alone (Bhatta et al., 2009). However, irrespective of the tannin type or source, the level of tannin application is important. A meta-analysis from 30 *in vivo* experiments showed that a linear decrease in CH_4 production was consistent with tannin concentration greater than 2% of the dietary DM, but at this dietary concentration, tannin can negatively affect DMI (Jayanegara et al., 2012). Thus, as little is known about the effect of HT on cattle, more research is needed to understand the effect of HT, alone or in combination with CT, and level of application in terms of decreasing CH_4 production without adverse effect on animal performance.

Table 1.4. Feed additives targeting enteric methane (CH_4) emission mitigation. Source (Hristov et al., 2013).

Category[1]	Potential CH_4 mitigating effect[2]	Long-term effect	Effective[3]	Animal and environmental safety	Recommended
Inhibitors					
BCM and BES[4]	High	?[5]	Yes	No	No
3-nitrooxypropanol	Medium	?	Yes	?	?
Electron receptors					
Nitrate	High	No?	Yes	?	Yes?[6]
Ionophores[7]	Low[8]	No?	Yes?[8]	Yes?	Yes?
Plant bioactive compounds					
Tannins[9]	Low	No?	Yes	Yes	Yes?
Saponins[10]	Low?	No	?	Yes	No?
Essential oils	Low?	No	?	Yes	No
Dietary lipids	Medium	No?	Yes	Yes	Yes?[11]

[1]Applicable to all ruminants.
[2]High, ≥ 30% mitigating effect; Medium, 10 to 30% mitigating effect; Low, ≤ 10% mitigating effect. Mitigating effects refer to CH_4 production percent reduction relative to control based on combination of study data cited by Hristov et al. (2013).
[3]Effectiveness is determined on the basis of CH_4 mitigation potential, effect on feed intake (no negative effect is beneficial), and/or effect on animal productivity (no negative effect or improvement is beneficial); based on combination of study data cited by Hristov et al. (2013).
[4]BCM = bromochloromethane; BES = 2-bromo-ethane sulfonate.
[5]? = uncertainty due to limited research or lack of data, inconsistent or variable results of the effect.
[6]Practicality of use is unknown. Caution must be exercised when feeding nitrate. Animal should be properly adapted and re-adapted if nitrate supplementation is discontinued for a period of time. Access to molasses blocks with nitrate should be limited so that nitrate intake does not poison the animal. Not advisable to use when diets have high N concentrations.
[7]Most data are from monensin. Monensin shows inconsistent direct effect on CH_4 production in dairy or beef cattle. Meta-analyses have shown improvement in feed efficiency in beef cattle and dairy that may reduce CH_4 emissions per unit of product (meat or milk). On this basis, the overall conclusion is that ionophores likely have a CH_4 mitigating effect in ruminants of up to 5% (Hristov et al., 2013).
[8]Through improvement in feed efficiency, especially when diets contain concentrates; usually no effect when pasture is fed as a sole diet.
[9]See text for extensive discussion on tannin supplements. Most data are on condensed tannin.
[10] Saponins are less effective compared to tannin. Results with tea saponins are encouraging but must be confirmed and data for persistence of the effect are lacking.
[11]Generally effective in reducing CH_4 production but may negatively affect feed intake, fiber digestibility, rumen function, milk fat content, and overall animal productivity. Maximum recommended inclusion rate in ruminant diets is 6 to 7% (total fat) of dietary DM. With the lack of financial compensation to reduce CH_4 emissions, the economic feasibility of supplementing diets with edible lipids is questionable.

Figure 1.9. Pathways for biodegradation of hydrolysable tannin and condensed tannin. Source (Bhat et al., 1998).



1.4.2.3. Nitrogen Metabolism and Ammonia Emission

To optimize efficiency of N utilization, it is critical to maximize microbial protein synthesis to provide the animal with essential amino acids for growth and production. Also by supplying rumen undegradable protein, the amino acid composition of the digesta at the lower tract can be manipulated to match the requirements of the animal for production (NRC, 2001). However, proteins from high quality forages such as alfalfa that are commonly fed to ruminants are highly degradable in the rumen increasing NH_3 concentration in the rumen. The excess NH_3 is absorbed from the gut wall and carried through the portal blood to the liver where it is converted to urea. Ammonia is re-introduced into the rumen as urea (via saliva or gut wall) or the urea is removed by the kidney and excreted in urine.

As tannins have high affinity for protein, they may protect dietary protein from extreme degradation in the rumen by forming stable complexes in the rumen environment (Patra and Saxena, 2011). This can increase protein available for absorption in the lower gut of ruminants (Waghorn et al., 1987; Patra and Saxena, 2011). Also, the binding effect of tannins on dietary proteins may decrease CP digestibility and shift N excretion from urine to feces (Grainger et al., 2009). As fecal N is in the form indigestible organic protein, its environmental impact on N_2O emission is minimal relative to the volatile N in urine. As tannins have the ability to form complexes with proteins, ruminants with tannin added to their diet may reduce their inorganic N excretion in urine. However, as mentioned before, most of the research on improving N utilization with tannins has used CT (Patra and Saxena, 2011; Naumann et al., 2017). Because HT is relatively a small molecule and easily hydrolyzed in the rumen and thus, it is assumed to have poor binding capacity. Although, GA (HT subunit), did not affect *in vitro* protein

degradation of alfalfa protein (Getachew et al., 2008), it regulated the pattern of N excretion by increasing fecal N and decreasing urinary urea N in beef cattle fed at maintenance level (Wei et al., 2016). Also, both TA which consists of about 10 molecules of GA and CT extract from Q showed a strong protein protection from *in vitro* ruminal degradation (Getachew et al., 2008), signifying similarity in binding abilities of HT and CT. Thus, differences in unit structure and molecular weight of HT may affect rumen microbes differently. However, little is known about the effect of a subunit and the type of HT on NH_3 reduction in beef cattle.

1.5. Knowledge Gap

As discussed, enteric CH_4 production coupled with losses of manure N, which can lead to formation of N_2O, are major concerns for beef cattle production. Methane and N_2O are not only potent GHG, but are also an indication of energy and N inefficiencies in ruminant production systems (Eckard et al., 2010). Thus, identifying CH_4 and N_2O mitigation strategies that are not detrimental to growth performance would minimize the environmental impact and improve the efficiency of the western Canadian beef cattle industry.

In western Canada, corn with short growing season (≤ 2600 CHU) for silage production is currently on the rise for beef and milk production. While corn hybrids are assessed routinely for yield potential, less information is available on nutritional quality, and no information exists on variability in potential enteric CH_4 production. It is important for producers to consider quality in addition to yield to maximize animal performance per unit land area. Thus, this study seeks to identify the characteristics of CS hybrids grown in AB with greater nutrient contents, but that produce less CH_4. This will enable producers to select short-seasoned CS hybrids based on their location that can reduce enteric CH_4 emissions from cattle while reducing feeding costs and improving animal performance.

Alfalfa continues to be a forage of choice in most mixed diets for the Canadian beef cattle but this can increase CH_4 production and increase soluble protein intake. The high soluble protein intake can increase rumen NH_3 concentration due to high rumen CP degradation and increase N excretion in urine resulting in N-use inefficiency. Adding tannin to the diet may help improve the use of alfalfa by animals, because alfalfa is a non-tannin containing forage. However, most research with tannin for cattle, has focused on CT with little information on HT. Using the appropriate HT type and dose with or without CT will demonstrate whether adding HT to alfalfa based diets can help reduce CH_4 and improve N-use efficiency in beef cattle.

Thus, management strategies to reduce the environmental impact of using short season CS and alfalfa forages by the Canadian beef industry may differ. Thus, for short season CS hybrids, the focus of the thesis research is on CH_4 potential as affected by starch/NDF contents and digestibility, whereas, for alfalfa the focus is on tannin supplementation of diets.

1.6. Thesis Objectives and Hypotheses

The overall objective of this research is to determine the factors affecting CH_4 production of short season CS hybrids and the effects of HT in mitigating enteric CH_4, and improving N utilization in beef cattle fed an alfalfa silage diet. The overall objective will be achieved with the following sub-objectives: 1) using an *in vitro* batch culture technique, to determine the variability in CH_4 production among CS hybrids harvested before or after frost at two locations in AB; 2) determine the effects of different doses of HT with or without CT when added to an alfalfa silage diet on enteric CH_4 emission, animal performance (DMI, ADG, and feed:gain) and N use of beef cattle; and lastly, 3) determine the effects of different sources of HT or subunit of HT on CH_4 production, N utilization, and diet digestibility in beef cattle fed an alfalfa silage diet.

The hypothesis for the various sub-objectives (in the order as pre-numbered) are: 1) harvesting late after the occurrence of frost would increase DM and starch contents and reduce CH_4 emission of CS hybrids adapted to different adaptation zones in AB compared with harvesting before frost; 2) a combination of HT and CT compared with the control (no tannin) at an optimum dose may lower rumen NH_3 concentration (an indicator for N excretion), decrease CH_4 emissions and improve animal performance; and lastly, 3) feeding HT or a component of HT to cattle fed a high protein diet based on alfalfa silage would decrease both urinary N excretion and enteric CH_4 production, and that the response to HT would depend on the source or its sub-unit.

1.7. Literature Cited

Aboagye, I. A., M. Oba, A. R. Castillo, K. M. Koenig, and K. A. Beauchemin. 2018. Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high forage diet. J. Anim. Sci. 96:5276-5286.

Addah, W., J. Baah, P. Groenewegen, E. K. Okine, and T. A. McAllister. 2011. Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. Can. J. Anim. Sci. 91:133–146.

Alonso-Díaz, M. A., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Hoste, H., 2010. Tannins in tropical tree fodders fed to small ruminants: A friendly foe? Small. Ruminant Res. 89: 164-173.

Archimède, H., M. Eugène, C. M. Magdeleine, M. Boval, C. Martin, D. P. Morgavi, P. Lecomte, and M. Doreau. 2011. Comparison of methane production between C3 and C4 grasses and legumes. Anim. Feed Sci. Technol. 166:59–64.

Arndt, C., J. M. Powell, M. J. Aguerre, and M. A. Wattiaux. 2015. Performance, digestion, nitrogen balance, and emission of manure ammonia, enteric methane, and carbon dioxide in lactating cows fed diets with varying alfalfa silage-to-corn silage ratios. J. Dairy Sci. 98:418–430.

Baldwin, R. L., and M. J. Allison. 1983. Rumen metabolism. J. Anim. Sci. 57(Suppl. 2):461–477.

Baron, V. S., K. A. Beauchemin, P. Juskiw, and R. R. Doce. 2014. Managing short-season corn and small-grain silage in Western Canada. In Proc. of the 35th Western Nutrition Conference, 24 – 25 Sept. 2014, Edmonton, AB. p. 147–162.

Beauchemin, K. A., H. H. Janzen, S. M. Little, T. A. McAllister, and S. M. McGinn. 2010. Life cycle assessment of greenhouse gas emissions from beef production in western Canada: A case study. Agric. Syst. 103:371–379.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48:21–27.

Beauchemin, K. A., and S. M. McGinn. 2005. Methane emissions from feedlot cattle fed barley or corn diets. J. Anim. Sci. 83:653–661.

Beauchemin, K. A., and S. M. McGinn. 2006. Enteric methane emissions from growing beef cattle as affected by diet and level of intake. Can. J. Anim. Sci. 86:401–408. doi:10.4141/A06-021.

Beauchemin, K. A., and S. M. McGinn. 2008**.** Reducing methane in dairy and beef cattle operations: What is feasible? Prairie Soil Crop 1:17–21.

Beauchemin, K. A., S. M. McGinn, T. F. Martinez, and T. A. McAllister. 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. J. Anim. Sci. 85:1990–1996. doi:10.2527/jas.2006-686.

Becker, P. M., P. G. Wikselaar, M. C. R. Franssen, R. C. H. Vos, R. D. Hall, and J. Beekwilder. 2014. Evidence for a hydrogen-sink mechanism of (+) catechin-mediated emission reduction of the ruminant greenhouse gas methane. Metabolomics 10:179–189. doi:10.1007/s11306-013-0554-5.

Benchaar, C., F. Hassanat, R. Gervais, P. Y. Chouinard, H. V. Petit, and D. I. Massé. 2014. Methane production, digestion, ruminal fermentation, nitrogen balance, and milk production of cows fed corn silage- or barley silage-based diets. J. Dairy Sci. 97:961–974.

Berard, N. C., Y. Wang, K. M. Wittenberg, D. O. Krause, B. E. Coulman, T.A McAllister, and K. H. Ominski. 2011. Condensed tannin concentrations found in vegetative and mature forage legumes grown in western Canada. Can. J. Plant Sci. 91:669–675. doi:10.4141/cjps10153.

Bhat, T. K., B. Singh, and O. P. Sharma.1998. Microbial degradation of tannins – a current perspective. Biodegradation 9:343–357. doi:10.1023/A:100839750696.

Bhatta, R., M. Saravanan, L. Baruah, K. Sampath, and C. S. Prasad. 2013. Effect of plant secondary compounds on *in vitro* methane, ammonia production and ruminal protozoa population. J. Appl. Microbiol. 115:455–465. doi:10.1111/jam.12238.

Bhatta, R., Y. Uyeno, K. Tajima, A. Takenaka, Y. Yabumoto, I. Nonaka, O. Enishi, and M. Kurihara. 2009. Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. J. Dairy Sci. 92:5512–5522. doi:10.3168/jds.2008-1441.

Blaxter, K. L., and J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19:511–522.

Blümmel M., D. I. Givens, and A. R. Moss. 2005. Comparison of methane produced by straw fed sheep in open-circuit respiration with methane predicted by fermentation characteristics measured by an *in vitro* gas procedure. Anim. Feed Sci. Technol. 123–124:379–390

Broderick, G.A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370–1381.

Cammell, S. B., J. D. Sutton, D. E. Beever, D. J. Humphries, and R. H. Phipps. 2000. The effect of crop maturity on the nutritional value of maize silage for lactating dairy cows. Anim. Sci. 71: 381–390.

Capper, J. L. 2012. Is the grass always greener? Comparing the environmental impact of conventional, natural and grass-fed beef production systems. Animals. 2:127–143.

Capper, J. L., and D. E. Bauman. The role of productivity in improving the environmental sustainability of ruminant production systems. Annu. Rev. Anim. Biosci. 1:469–489

Carrasco, J. M. D., C. Cabral, L. M. Redondo, N. D. P. Viso, D. Colombatto, M. D. Farber, and M. E. F. Miyakawa. 2017. Impact of chestnut and quebracho tannins on rumen microbiota of bovines. BioMed. Res. Int. 2017:11. doi:10.1155/2017/961081.

Carulla, J. E., M. Kreuzer, A. Machmüller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Austr. J. Agric. Res. 56:961–970.

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

Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J. Galloway, M. Heimann, C. Jones, C. Le Quéré, R.B. Myneni, S. Piao and P. Thornton, 2013: Carbon and Other Biogeochemical Cycles. In: Climate Change 2013: The physical science basis contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and NY, USA.

Cole, N. A., and R. W. Todd. 2009. Nitrogen and phosphorus balance of beef cattle feedyards. In: E. Jordan, ed. Proceedings of the Texas Anim. Manure Manage. Iss. Conf., Sept. 29-30. Round Rock, TX. p. 17–24.

Cyriac, J., A. G. Rius, M. L. McGilliard, R. E. Pearson, B. J. Bequette, and M. D. Hanigan. 2008. Lactation performance of mid-lactation dairy cows fed ruminally degradable protein at concentrations lower than National Research Council recommendations. J. Dairy Sci. 91:4704–4713.

de Klein, C. A. M., and R. J. Eckard. 2008. Targeted technologies for nitrous oxide abatement from animal agriculture. Aust. J. Exp. Agric. 48:14–20.

Department for Environment, Food and Rural Affairs (DEFRA). 2010. Ruminant nutrition regimes to reduce methane and nitrogen emissions. Project AC0209 Report. DEFRA, Procurements and Contracts Division (Science R and D Team). http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=14952 (Accessed 11 November 2018).

Deppenmeier, U. 2002. The unique biochemistry of methanogenesis. Prog. Nucleic Acid Res. Mol. Biol. 71:223–283.

Deschamps A. M., G. Mohudeau, M. Conti, and J. M. Lebeault. 1980. Bacteria degrading tannic acid and related compounds. J. Ferment Technol. 58:93–97.

Dijkstra, J, E. Kebreab, A. Bannink, J. France, and S. López. 2005. Application of the gas production technique to feed evaluation systems for ruminants. Anim Feed Sci Technol. 123–124:561–578

Dijkstra, J., O. Oenema, J. W. van Groenigen, J. W. Spek, A.M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N_2O emissions. Animal 7 (Suppl. 2):292–302. doi:10.1017/S1751731113000578.

Eckard, R. J., C. Grainger, and C. A. M. de Klein. 2010. Options for the abatement of methane and nitrous oxide from ruminant production: A review. Livest. Sci. 130:47–56.

Eisler, M. C., M. R. F. Lee, J. F. Tarlton, G. B. Martin, J. Beddington, J. A. J. Dungait, H. Greathead, J. Liu, S. Mathew, and H. Miller. 2014. Agriculture: Steps to sustainable livestock. Nature. 507:32–34.

Ellis, J. L., E. Kebreab, N. E. Odongo, B. W. McBride, E. K. Okine, and J. France. 2007. Prediction of methane production from dairy and beef cattle. J. Dairy Sci. 90:3456–3467

Environment and Climate Change Canada (2018) National Inventory Report 1990-2016: Greenhouse gas sources and sinks in Canada. http://www.publications.gc.ca/site/eng/9.506002/publication.html (Accessed 12 September 2018)

Fanchone, A., P. Nozière, J. Portelli, B. Duriot, V. Largeau, and M. Doreau. 2013. Effects of nitrogen underfeeding and energy source on nitrogen ruminal metabolism, digestion and nitrogen partitioning in dairy cows. J. Anim. Sci. 91:895–906.

Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2013. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. J. Dairy Sci. 96:533–550.

Ferraretto, L. F., and R. D. Shaver. 2012. Meta-analysis: Impact of corn silage harvest practices on intake, digestion, and milk production by dairy cows. Prof. Anim. Sci. 28:141–149.

Ferris, C. P., F. J. Gordon, D. C. Patterson, M. G. Porter, and T. Yan. 1999. The effect of genetic merit and concentrate proportion in the diet on nutrient utilisation by lactating dairy cows. J. Agric. Sci. 132:483–490.

Field, J. A., S. Kortekaas, and G. Lettinga. 1989. The tannin theory of methanogenic toxicity. Biol. Wastes. 29:241–262.

Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. J. Dairy Sci. 80:1426–1437.

Follett, R. F., and J. L. Hatfield. 2001. Nitrogen in the environment: sources, problems, and management. In optimizing nitrogen management in food and energy production and environmental protection: Proceedings of the 2nd International Nitrogen Conference on Science and Policy. The Scientific World 1(S2):920–926.

Food and Agriculture Organization (FAO). 2006. Livestock's long shadow: environmental issues and options. Rome, Italy.

Food and Agricultural Organization (FAO). 2009. The state of food and agriculture: livestock in the balance. Rome, Italy.

Food and Agricultural Organization (FAO). 2011. Mapping supply and demand for animal-source foods to 2030. In Robinson T. S, Pozzi F, ed. Animal Production and Health Working Paper. N. 2, Rome, Italy.

Food and Agriculture Organization (FAO). 2015. Environmental performance of large ruminant supply chains: guidelines for assessment. Rome, Italy.

Gabruch, M., and R. Gietz. 2014. The potential of grain corn in Alberta. Alberta Agriculture and Forestry. Available http://www1.agric.gov.ab.ca/4Department/deptdocs.nsf/all/bus15031 (Accessed 4 November 2017).

Gerber, P. J., A. Mottet, B. Henderson, A. Mottet, C. Opio, A. Falcucci, and F. Teillard. 2013. Environmental impacts of beef production: Review of challenges and perspectives for durability. Meat Sci. 109:2–12.

Gerber, P. J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, and G. Tempio. 2013.Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. FAO, Rome, Italy.

Getachew, G., W. Pittroff, D. H. Putnam, A. Dandekar, S. Goyal, and E. J. DePeters. 2008. The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis. Anim. Feed Sci. Technol. 140:444–461. doi:10.1016/j.anifeedsci.2007.03.011.

Gladine, C., E. Rock, C. Morand, D. Bauchart, and D. Durand. 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. Br. J. Nutr. 98:691–701.

Goel, G., and H. S. Makkar. 2012. Methane mitigation from ruminants using tannins and saponins. Trop. Anim. Health Prod. 44:729–739.

Goopy J. P., C. Chang, and N. Tomkins. 2016. A Comparison of Methodologies for Measuring Methane Emissions from Ruminants. In: Rosenstock T., Rufino M., Butterbach-Bahl K.,

Wollenberg L., Richards M. eds. Methods for measuring greenhouse gas balances and evaluating mitigation options in smallholder agriculture. Springer, Cham.

Grainger, C., T. Clarke, M. J. Auldist, K. A. Beauchemin, S. M. McGinn, G. C. Waghorn, and R. J. Eckard. 2009. Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Can. J. Anim. Sci. 89:241–251.

Grainger, C., T. Clarke, S. M. McGinn, M. J. Auldist, K. A. Beauchemin, M. C. Hannah, G. C. Waghorn, H. Clark, and R. J. Eckard. 2007. Methane emissions from dairy cows measured using the sulfurhexafluoride (SF_6) tracer and chamber techniques. J. Dairy Sci. 90:2755–2766

Hagerman, A. E. 2011. Tannin Handbook, Department of Chemistry and Biochemistry, Miami University, USA. http://www.users.muohio.edu/hagermae (Accessed 17 October 2017).

Hammond, K. J., S. O. Hoskin, J. L. Burke, G. C. Waghorn, J. P. Koolaard, and S. Muetzel. 2011. Effects of feeding fresh white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*) on enteric methane emissions from sheep. Anim. Feed Sci. Technol. 166–167:398–404.

Hassanat, F., R. Gervais, C. Julien, D. I. Masse, A. Lettat, P. Y. Chouinard, H. V. Petit, and C. Benchaar. 2013. Replacing alfalfa silage with corn silage in dairy cow diets: Effects on enteric methane production, ruminal fermentation, digestion, nitrogen balance, and milk production. J. Dairy Sci. 96:4553–4567.

Hatew, B., A. Bannink, H. Van Laar, L. H. De Jonge, and J. Dijkstra. 2016. Increasing harvest maturity of whole-plant corn silage reduces methane emission of lactating dairy cows. J. Dairy Sci. 99:354−368

Hegarty, R. S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. Aust. J. Agric. Res. 50:1321–1327.

Hegarty, R. S., and R. Gerdes. 1999. Hydrogen production and transfer in the rumen. Rec. Adv. Anim. Nutr. Austr. 12:37–44.

Henry, B. K., R. J. Eckard, and K. A. Beauchemin. 2018. Review: Adaptation of ruminant livestock production systems to climate changes. Animal. 12(Suppl2):S445-S456.

Holter, J. B., and A. J. Young. 1992. Methane prediction in dry and lactating Holstein cows. J. Dairy Sci. 75:165–2175.

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

Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico. 2013. Special topics-Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069.

Intergovernmental Panel on Climate Change (IPCC). 2006. Guidelines for national greenhouse gas inventories. Prepared by the National Greenhouse Gas Inventories Programme, IGES, Hayama, Kanagawa, Japan.

Intergovernmental Panel on Climate Change (IPCC). 2007. Changes in atmospheric constituents and in radiative forcing. in climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change.

Intergovernmental Panel on Climate Change (IPCC). 2013: Summary for policymakers. In: Climate Change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and NY, USA.

Ishler, V., and G. Varga. 2001. Carbohydrate nutrition for lactating dairy cattle. Dept. Dairy Anim. Sci. Pennsylvania State University. pp. 1–29.

Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160:1–22.

Jayanegara A., F. Leiber, and M. Kreuzer. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments. J. Anim. Physiol. Anim. Nutr. 96:365–375.

Jayanegara, A., G. Goel, H. P. S. Makkar, and K. Becker. 2015: Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol. 209:60–68.

Jerónimo, E., C. Pinheiro, E. Lamy, M. T. Dentinho, E. Sales-Baptista, O. Lopes, and F. C. Silva. 2016. Tannins in ruminant nutrition: Impact on animal performance and quality of edible products. In Tannins: Biochemistry, Food Sources and Nutritional Properties; Combs, C.A., Ed.; Nova Science Publisher Inc.: Hauppauge, NY, USA. pp. 121–168

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

Johnson, K., M. Huyler, H. Westberg, B. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. Environ. Sci. Technol. 28:359–362.

Kasuya, H., and J. Takahashi. 2010. Methane emissions from dry cows fed grass or legume silage. Asian-Australas. J. Anim. 23:563−566.

Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231–3261.

Krumholz, L. R., and M. P. Bryant.1986. *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. Arch. Microbiol. 144:8–14.

Kumar, R., and M. Singh. 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food Chem. 32:447-453.

Lamy, E., G. da Costa, R. Santos, F. Capela e Silva, J. Potes, A. Pereira, A. V. Coelho, and E. Sales Baptista. 2011. Effect of condensed tannin ingestion in sheep and goat parotid saliva proteome. J. Anim. Physiol. Anim. Nutr.95:304–312.

Lee, C., A. N. Hristov, C. J. Dell, G. W. Feyereisen, J. Kaye, and D. Beegle. 2012. Effect of dietary protein concentration on ammonia and greenhouse gas emissions from dairy manure. J. Dairy Sci. 95:1930–1941.

Leng, R. A. 1993. Quantitative ruminant nutrition—A green science. Aust. J. Agric. Res. 44:363–380.

Leng, R. A. 2018. Unravelling methanogenesis in ruminants, horses and kangaroos: the links between gut anatomy, microbial biofilms and host immunity. Anim. Prod. Sci. **58**:1175–1191.

Lesschaeve, I., and A. C. Noble. 2005. Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. Am J. Clin. Nutr. 81:330S-335S.

Li, L., J. Cyriac, K. F. Knowlton, L. C. Marr, S. W. Gay, M. D. Hanigan, and J. Arogo Ogejo. 2009. Effects of reducing dietary nitrogen on ammonia emissions from manure on the floor of a naturally ventilated free stall dairy barn at low (0-20ºC) temperatures. J. Environ. Qual. 38:2172 _2181

Makkar, H. P. S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Res. 49:241-256.

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

McCartney, C. A., I. D. Bull, and R. J. Dewhurst. 2012. Archaeol concentration in total rumen contents of cows offered diets based on ryegrass or white clover. In: Proc. of the Irish Agric. Res. Forum. Tullamore, Ireland. p. 99.

McGeough, E. J., P. O'Kiely, P. A. Foley, K. J. Hart, T. M. Boland, and D. A. Kenny. 2010. Methane emissions, feed intake, and performance of finishing beef cattle offered maize silages harvested at 4 different stages of maturity. J. Anim. Sci. 88:1479–1491.

McGinn, S. M., K. A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82:3346–3356.

McGinn, S. M, K. A. Beauchemin, T. K. Flesch, and T. Coates. 2009. Performance of a dispersion model to estimate methane loss from cattle in pens. J. Environ. Qual. 38:1796–1802.

Menke, K. H., L. Raab, A. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. 93:217–222.

Min, B. R., T. N. Barry, G. T. Attwood, and W. C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperature forages. A review. Anim. Feed Sci. Technol. 106:3-19.

Moe, P. W., and H. F. Tyrrell. 1979. Methane production in dairy cows. J. Dairy Sci. 62:1583–1586.

Morgavi, D. P., E. Forano, C. Martin, and C. J. Newbold. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal. 4:1024–1036.

Murdiati, T. B., C. S. McSweeney, and J. B., Lowry. 1992. Metabolism in sheep of gallic acid, tannic acid, and hydrolysable tannin from *Terminalia oblongata*. Aust. J. Agric. Res. 43:1307–1319.

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

National Research Council (NRC). 2001. Nutrient requirements of dairy cattle. 5th rev. ed. National Academy Press. Washington, DC.

Naumann, H. D., L. O. Tedeschi, W. E. Zeller, and N. F. Huntley. 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. R. Bras. Zootec. 46: 929–949.

Patra, A.K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91:24–37.

Pelletier, N., R. Pirog, and R. Rasmussen. 2010. Comparative life cycle environmental impacts of three beef production strategies in the Upper Midwestern United States. Agric. Sys. 103: 380–389.

Pinares-Patino, C. S., G. C. Waghorn, R. S. Hegarty, and S. O. Hoskin. 2009. Effects of intensification of pastoral farming on greenhouse gas emissions in New Zealand. N. Z. Vet. J. 57:252–261.

Reed, J. D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73:1516–1528.

Reynolds, C. K., D. J. Humphries, P. Kirton, M. Kindermann, S. Duval, and W. Steinberg. 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. J. Dairy Sci. 97:3777–3789.

Sauvant, D., and S. Giger-Reverdin. 2009. Modélisation des interactions digestives et de la production de méthane chez les ruminants. (In French.) INRA Prod. Anim. 22:375–384.

Satter, L. D., T. J. Klopfenstein, and G. E. Erickson. 2002. The role of nutrition in reducing nutrient output from ruminants. J. Anim. Sci. 80:143–156.

Sere, C., and S. Steinfeld. 1996. World livestock production systems. Current status, issues and trends. FAO. Rome, Italy.

Staerfl, S. M., J. O. Zeitz, M. Kreuzer, and C. R. Soliva. 2012. Methane conversion rate of bulls fattened on grass or maize silage as compared with the IPCC default values, and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca and lupine. Agric. Ecosyst. Environ. 148:111–120.

Statistics Canada Agriculture Census, 2011. Highlights and analyses. http://www.statcan.gc.ca/pub/95-640-x/2011001/p1/prov/prov-48-eng.htm (Accessed 3 August 2017).

Steinfeld, H., C. de Haan, and H. Blackburn. 1996. Balancing Livestock and the Environment: Summary of the Results of a Multi-Donor Study. Mimeo. The World Bank, Washington, DC.

Thauer, R. 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson. Microbiology 144:2377–2406.

Torrent, J., and D. E. Johnson, D.E. 1994. Methane production in the large intestine of sheep. In: Energy metabolism of farm animals. Aquilera, J. F ed. CSIC Publishing Service. Granada, Spain. pp. 391–394.

United Nations (UN). 2017. Department of Economic and Social Affairs. Population Division. World Population Prospects, 2017. Available from: http://esa.un.org/unpd/wpp/Graphs/Probabilistic/POP/TOT/. (Accessed 07 September, 2018).

Ungerfeld, E. M. 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. Front. Microbiol. 6:1–17.

van Dorland, H. A., H. R. Wettstein, H. Leuenberger, and M. Kreuzer. 2007. Effect of supplementation of fresh and ensiled clovers to ryegrass on nitrogen loss and methane emission of dairy cows. Livest. Sci. 111:57–69.

van Lingen, H. J., C. M. Plugge, J. G. Fadel, E. Kebreab, A. Bannink, and J. Dijkstra. 2016. Thermodynamic driving force of hydrogen on rumen microbial metabolism: A theoretical investigation. PLoS One 11:e0161362.

Waghorn, G. C. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production – progress and challenges. Anim. Feed Sci. Technol. 147:116–139.

Waghorn, G. C., and D. A. Clark. 2006. Greenhouse gas mitigation opportunities with immediate application to pastoral grazing for ruminants. Int. Congr. Ser. 1293:107–110.

Waghorn, G. C., M. H. Tavendale, and D. R. Woodfield. 2002. Methanogenesis from forages fed to sheep. Proc. N. Z. Grassland Assoc. 64:167–171.

Waghorn, G. C., M. J. Ulyatt, A. John, and M. T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on lotus. Br. J. Nutr. 57:115–126.

Wang, M., X. Z. Sun, P. H. Janssen, S. X. Tang, and Z. L. Tan. 2014. Responses of methane production and fermentation pathways to the increased dissolved hydrogen concentration generated by eight substrates in in vitro ruminal cultures. Anim. Feed Sci. Technol. 194:1–11.

Wei, C., K. Yang, G. Zhao, S. Lin, and Z. Xu. 2016. Effect of dietary supplementation of gallic acid on nitrogen balance, nitrogen excretion pattern and urinary nitrogenous constituents in beef cattle. Arch. Anim. Nutr. 70:416–423.

Williams, S. R. O., P. J. Moate, M. C. Hannah, B. E. Ribaux, W. J. Wales, R. J, Eckard. 2011. Background matters with the SF 6 tracer method for estimating enteric methane emissions from dairy cows: a critical evaluation of the SF 6 procedure. Anim. Feed Sci. Technol. 170:265–276.

Woodward, S. L., G. C. Waghorn, and P. G. Laboyrie. 2004. Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduce methane emissions from dairy cows. Proc. of the New Zealand Soc. Anim. Prod. 64:160-4.

Yan, T., R. E. Agnew, F. J. Gordon, and M. G. Porter. 2000. Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. Livest. Prod. Sci. 64:253–263.

Zahiroddini, H., J. Baah, W. Absalom, and T. A. McAllister. 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Anim. Feed Sci. Technol. 117: 317–330.

Zom, R. L. G., A. Bannink, and R. M. A. Goselink. 2012. Effect of increased maturity of silage maize at harvest on conservation dairy cow performance and methane emission. Livestock Research Wageningen, Wageningen, the Netherlands. Report 578.

CHAPTER 2 - *In vitro* degradation and methane production of short-season corn hybrids harvested before or after a light frost[1]

2.1. Introduction

A life cycle assessment of beef production in western Canada showed that enteric CH_4 is the largest contributing source of greenhouse gases, accounting for 63% of total emissions (Beauchemin et al., 2010). Feeding grain to ruminants may reduce CH_4 emission (Van Soest 1994; Janssen 2010), but it limits their ability to digest cellulosic materials. Furthermore, forage-based diets can be less expensive than grain-based diets and forages provide numerous environmental benefits including storage of soil carbon (Guyader at al., 2016).

Corn silage (CS), a starch-containing forage, can be a suitable alternative to grass and legume forages in ruminant diets in areas where they are adapted. As CS contains relatively high starch concentration (182 to 350 g/kg DM, Baron et al., 2014), it may result in lower CH_4 emissions compared with diets based on grass-legume forages (Beauchemin and McGinn, 2008). However, in past years, corn was not suited to a large area of western Canada due to cold temperatures because optimum temperature for corn growth is 31ºC (Yan and Hunt, 1999). Consequently, production of corn in short-season regions historically resulted in CS with low DM and grain concentrations (Daynard, 1978; Narasimhalu et al., 1986; Baron et al., 1987). In recent years development of earlier maturing corn hybrids with ≤ 2600 CHU has resulted in corn maturing in fewer days, more optimum DM concentration for silage production, and greater grain and starch concentrations than in the past (Gabruch and Gietz, 2014; Guyader at al., 2018).

[1]A version of this chapter is in press. Aboagye, I. A., V. S. Baron, M. Oba, J. Guyader, and K. A. Beauchemin.2019. *In vitro* degradation and methane production of short-season corn hybrids harvested before or after a light frost. Canadian Journal of Animal Science

Corn heat units are used in Canada to track accumulated heat required from planting to grain maturity (Brown and Bootsma, 1993). The adaptation zones for corn production are delineated by mapping CHU received from spring to fall frost, while corn hybrids are rated in number of CHU needed from planting to reach grain maturity (Dwyer et al., 1999). However, when grown for silage, year to year variation in CHU received within and among adaptation zones, combined with the objective of maximizing whole plant DM yield by using corn hybrids with greater CHU rating than recommended, results in variation in grain and starch concentrations (Guyader et al., 2018).

Advancing maturity of corn during grain filling within an adaptation zone usually results in greater DM and starch concentrations and reduced neutral detergent fiber (NDF) concentration at harvest (Darby and Lauer, 2002; Ferraretto and Shaver, 2012). However, for short-season areas, frost can occur before ideal plant maturity for ensilage (320-380 g/kg DM; Narasimhalu et al., 1986); and this can negatively affect starch and kernel moisture concentrations. Therefore, growers sometimes leave the whole plant standing until after frost to increase the drying rate so that ensiling occurs at an optimum DM concentration (Baron et al., 2008). However, drying can occur quickly after frost due to damage to cellular structures (Lee and Herbek, 2004) and harvesting can be delayed due to inclement weather. Consequently, the DM concentration may exceed the optimum range and this may affect the chemical composition of the whole-plant, rumen degradability (St. Pierre et al., 1983; Narasimhalu et al., 1986), and subsequently enteric CH_4 production when fed to animals.

Differences in chemical composition as a result of relative maturity at harvest, adaptation zone (location), plant genetics and frost prior to harvest may cause variability in enteric CH_4 production in ruminants. In beef (McGeough et al., 2010) or dairy cattle (Hatew et al., 2016),

increasing DM concentration of whole plant corn at harvest (from 250 to 400 g/kg fresh forage) tended to linearly reduce or reduced CH_4, respectively. However, Cammell et al. (2000) and Zom et al. (2012) did not report a linear reduction in enteric CH_4 emissions in dairy cattle fed CS diets with increasing DM concentration at harvest (230 to 420 g/kg fresh weight).

There is insufficient information describing the variability in nutrient concentration, degradability and CH_4 production of short-season whole plant corn hybrids (**WPCH**), yet these hybrids are growing in popularity for milk and beef production in parts of Canada. Therefore, the first objective of this study was to determine the variability in nutrient concentrations, degradability of DM and NDF, and CH_4 production of short-season WPCH grown and adapted in two locations of AB and harvested before or after frost. The second objective was to determine the relationship between CH_4 production and the nutrient concentrations and degradability variables. We hypothesized that relative maturity of corn hybrids as determined by their CHU rating within a location would influence relative grain maturity, starch concentration and NDF degradability of WPCH, and therefore, CH_4 emissions among hybrids would vary. We further hypothesized that delaying harvest until after frost would increase DM and starch concentrations and reduce CH_4 emissions compared with harvesting before frost.

2.2. Materials and Methods

Short-Season Whole Plant Corn Hybrids

Short-season corn hybrids were grown (two-row corn planter; John Deere, Moline, IL, US) in 2 locations [Vauxhall (AB) and Lacombe (AB)], with 2 field plots per site (replications), in western Canada and harvested before or after frost in two different years. The agronomic details are reported by Guyader et al. (2018). Corn silage hybrids grown in central or southern AB, two areas characterized by having different CHU ratings, during 2013 and 2015 growing

seasons were examined. Seeding dates, harvesting dates, and CHU received at the two locations are shown in Table 2.1. The CHU received between seeding and harvesting were calculated for each year and location as the sum of daily CHU using the method described by Huggins-Rawlins (2015; after seeding, starting the day after three consecutive days with mean daily temperature ≥ 12.8°C, until harvest).

Different hybrids were grown at each location (except P7632HR), with hybrids selected based on their CHU rating to represent the range of CHU typically received in that location. Hybrids grown in central AB were rated 2000 to 2200 CHU, while those grown in southern AB ranged from 2200 to 2600 CHU. The four CS hybrids grown at Lacombe (central AB hybrids; CHU rating) were: P39F44 (2000), P39M26 (2050), PS2262RR (2150), and P7632HR (2200); those grown at Vauxhall (southern AB hybrids; CHU rating) were: P7632HR (2200), P8210HR (2400), P8673AM (2550), and P8622AM (2600). Hybrids were from Pioneer Hi-Bred (Johnston, IA, USA) except PS2262RR, which was obtained from Pickseed (Lindsay, ON).

At both locations and in each year, the first harvest (Table 2.1) occurred before a forecasted frost (i.e. temperature dropping below -2°C overnight), while the second harvest was after frost (i.e., on average, 10 d later and after an air temperature of -1.5°C). The minimum, maximum and mean daily temperatures from seeding until harvesting were recorded at the closest (< 25 km) weather station.

Chemical Analyses

The corn hybrids were harvested using a small research silage chopper (two-row harvester; John Deere, Moline, IL, US; Model 8320 in Vauxhall and Model 3940 in Lacombe) and a 5 kg subsample of whole plant material was obtained. The samples were dried at 55°C for at least 72 h and ground through a 4-mm screen (standard model 4 Wiley Mill; Arthur Thomas

Co., Philadelphia, PA, USA). The ground sample was split, and a representative 250 g sample was reground through a 1-mm screen (standard model 4 Wiley Mill) and retained for chemical and *in vitro* analysis. Chemical analysis was conducted in duplicate for DM, ash, starch, and NDF including ash. Analytical DM and ash were determined by drying samples at 135ºC for 2 h and combusting samples in a muffle furnace at 550°C for 5 h respectively, followed by hot weighing (method 930.15; Association of Official Analytical Chemists 2005). Neutral detergent fiber was determined as described by Van Soest et al. (1991) using the Ankom 200® system (Ankom Technology Corporation, Fairport, NY, USA). Heat-stable α-amylase and sodium sulfite were used in the NDF assay. The 1-mm ground samples were reground using a ball grinder (Mixer Mill MM2000; Retsch, Haan, Germany) before determination of starch concentration. Starch concentration was determined by enzymatic hydrolysis of α-linked glucose polymers as described by Chung et al. (2011).

In Vitro Gas Production And Fermentation End Products

Animal handling and care procedures in this study were approved by the Lethbridge Research and Development Centre Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care (2009). An *in vitro* experiment was conducted (2 runs conducted sequentially) to measure gas production (total gas and CH_4) and rumen fermentation characteristics from the various WPCH samples. Dry matter degradability (**DMD**) after 48 h incubation was also measured to express CH_4 on the basis of DM degraded. The study was designed as a completely randomized design. The ground WPCH samples from each field plot within each run were replicated three times over a 48-h period of incubation and averaged.

The ground samples were weighed (0.7 ± 0.01 g) into acetone-washed ANKOM F57 filter bags (50 μm pore size; Ankom Technology Corp., Macedon, NY, USA), heat-sealed and

placed individually into a 125-mL capacity serum vial. Rumen inoculum was collected before feeding on the morning of the incubations. Rumen contents were collected at different locations within the rumen of 3 cannulated cattle (mean body weight ± SD; 867 ± 39.3 kg) fed a diet containing 77% corn silage, 18% corn dry distillers grain and 5% vitamin-mineral supplement (DM basis). Animals were adapted to the diet for about two weeks beforehand. Whole rumen contents pooled across animals and squeezed through two layers of cheesecloth and immediately transferred to the laboratory in an insulated, airtight container and kept at 39°C in a water bath. The pH of the rumen fluid (mean ± SD, 6.35 ± 0.06) was measured using a pH meter (Orion Model 260A; Fisher Scientific, Toronto, ON, Canada).

Anaerobic buffer medium (pH 6.97) prepared as described by Goering and Van Soest (1970) was added to the vials together with the rumen fluid in a ratio 3:1 (buffer medium: rumen fluid). Each vial was flushed with CO_2 before the addition of 60 mL of the anaerobic buffer medium and 20 mL of rumen fluid. The bottles were sealed with a 14 mm butyl rubber stopper plus aluminum crimp cap, and placed on a rotary shaker platform at 120 rpm in an incubator and kept for 48 h at 39ºC. Blanks containing buffer medium, rumen fluid, and empty filter bag were also incubated in three replications. Headspace gas production (**GP**) was measured at 3, 6, 12, 18, 24, and 48 h of incubation by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer (model PX4200-015GI; Omega Engineering, Inc., Laval, Que., Canada) connected to a visual display unit (Data Track, Christchurch, UK). Pressure values, corrected for the amount of substrate and from blanks were to generate gas volume (mL) estimates using the quadratic equation (0.18 + [3.697 × gas pressure (psi)] + [0.0824 × gas pressure^2 (psi)]) of Mauricio et al. (1999).

After each GP measurement, about 15 mL of gas sample from each vial was taken using 20 mL graduated plastic syringes connected to the three-way stopcocks. The needle was left in the seal to allow for gas release following pressure reading or/and gas sampling. Each sample was injected into an evacuated 6.8 mL exetainer (Labco Ltd., High Wycombe, England) for gas composition analysis. The CH_4 concentration was analyzed using a gas chromatograph (Varian 4900; Agilent Technologies Canada Inc., Mississauga, Ontario) equipped with a 10-m PPU column and thermal conductivity detector. Helium was used as carrier gas. The temperature of column and injector was 40°C and 70°C, respectively. Run was isothermal for 80 s and injection time was 40 min.

After 48 h of incubation, the bags were removed from the vials and the pH of the liquid within the vials was measured. The bags were washed with cold water until the excess water ran clear, dried in the oven at 55°C for 24 h and residual DM weighed for DMD calculation. The amount of DM degraded was used to express the quantity of CH_4 produced and the blanks were used to correct for gas released.

After 48 h of incubation, 1 mL of rumen fluid mixed with the medium in the vials was acidified with 0.2 mL metaphosphoric acid to determine the VFA concentrations. The VFA were analyzed using gas-liquid chromatography (model 6890; Agilent, Wilmington, DE) with a polar capillary column (30 m × 0.32 mm × 1 μm; ZB-FFAP; Phenomenex Inc., Torrance, CA), a flame ionization detector and helium as carrier gas. Crotonic acid was used as an internal standard. The oven temperature was started at 150°C for 1 min and increased to 195°C for 3 min (5°C min^{-1}). The injector and detector temperatures were set at 225°C and 250°C, respectively. Total VFA produced from the WPCH were corrected by subtracting the VFA production measured in the blanks containing buffer medium, rumen fluid and empty filter bag after 48 h incubation.

Dry matter and NDF degradation

Degradability of DM and NDF (**NDFD**) were determined in two different runs separately from gas production to prevent the possibility of gas readings negatively affecting degradability. Ground samples were weighed (0.5 ± 0.01) in triplicate into acetone-washed ANKOM F57 filter bags (50 μm pore size; Ankom Technology Corp., Macedon, NY, USA), heat-sealed and placed in jars of the DAISY^{II} Incubator (Ankom Technology Corp.). Blank bags were added to each jar. Anaerobic buffer (1600 mL) and rumen fluid (400 mL) as described above were added in a ratio of 4:1 to the jars. After 48 h of incubation the bags were removed from the jars, washed with cold water until the excess water ran clear, dried in the oven at 55°C for 24 h and weighed to determine DMD from DM lost from the bags. The bag residues were further analyzed for NDF concentration as described above and used to determine NDFD.

Calculation and statistical analyses

Dry matter degradability and NDFD were calculated as the difference between chemical composition of the initial substrate incubated and that of the bag residues after 48 h incubation. The following equations were used to calculate CH_4 production:

$CH_4\,(mL) = \sum ((\text{gas produced}_t \times [CH_4]_t) - (\text{gas produced blank}_t \times [CH_4\,\text{blank}]_t))$

where t = 3, 6, 12, 24, or 48 h incubation

$CH_4\,(mg\ /g\ \text{degraded DM}) = ((((mL\ CH_4\ /1000)/22.4) \times 16)/\ g\ \text{degraded DM}) \times 1000$

The R version 3.2.3 (2015) and the 'nlme' package by Pinheiro et al. (2016) were used to analyze the data, where autoregressive-1 was used to correct for lack of independence in the residual and varPower to correct homogeneity of variance. For the *in vitro* studies, the three replicates of each WPCH within run and the two runs were averaged for each hybrid within field plot. All variables were analyzed by location using a model that included hybrid, harvest and

their interaction as fixed effects with year and plot (replicate) as random effects. Means were declared significantly different when $P < 0.05$ while tendencies were indicated at $0.05 \leq P < 0.10$. The data were combined over locations to appraise the general relationships between CH_4 production and chemical composition (starch and NDF) and degradability (DMD and NDFD) or fermentation end products (gas and VFA) by multiple regressions using the 'lm' function. The 'step' function was used to identify the best model with the lowest Akaike's information criterion and the 'chart. Correlation' function was used to identify the correlation between variables measured. Multicollinearity between independent variables was assessed using the 'vif' function and tolerance level was declared at VIF < 10. The multiple regression equations, predictors and correlation coefficients were declared significant at $P < 0.05$.

2.3. Results

The CHU received over the growing seasons of 2013 and 2015 for central and southern AB are shown in Table 2.1. Harvesting after frost increased the CHU received by about 52 to 55 CHU in both locations in 2013, but in 2015 harvesting after frost increased CHU by 127 in southern AB, with only a minimal increase of 5 CHU in central AB. The CHU received as a percentage of CHU rating of the individual hybrids (mean ± standard deviation) in 2013 was 102 ± 4.3% for central AB and 102 ± 7.3% in southern AB. However, in 2015, the CHU rating of the hybrids exceeded the CHU received in both locations, with one exception (P7632HR, Southern AB, after frost). On average in 2015, hybrids in central AB received only 88 ± 3.4% of their rating, while hybrids in southern AB received only 93 ± 7.0%. Thus, in central AB, all hybrids did not receive their recommended accumulation of CHU to meet grain or silage maturity requirements prior to the first harvest.

Table 2.1. Seeding and harvesting dates of the whole plant corn hybrids and the corn heat units (CHU) received in central or southern Alberta in two years and harvested before or after light frost.

Location[1]	2013		2015	
	Before frost	After frost[2]	Before frost	After frost
Central AB				
Seeded date	10 May 2013	10 May 2013	05 May 2015	05 May 2015
Harvested date	18 Sept. 2013	01 Oct. 2013	15 Sept. 2015	23 Sept. 2015
CHU received[3]	2113	2168	1837	1842
Southern AB				
Seeded date	13 May 2013	13 May 2013	07 May 2015	07 May 2015
Harvested date	24 Sept. 2013	01 Oct. 2013	18 Sept. 2015	29 Sept. 2015
CHU received	2458	2510	2182	2309

Note: Accumulated CHU in each column calculated starting from the day following three consecutive days after seeding with mean daily temperature ≥12.8°C until harvest.
[1]Corn hybrids were seeded and harvested in central and southern AB.
[2]Occurrence of frost at minimum temperature of -0.2 to 2.2°C.
[3]Corn heat unit calculated each day using the equation: (1.8(Tmin - 4.4) + 3.33(Tmax - 10) – 0.084(Tmax- 10)2) / 2; where Tmin = daily minimum temperature (ºC); Tmax = daily maximum temperature (ºC).

Average DM concentration of the samples ranged from 253 to 372 g/kg for central AB and from 309 to 466 g/kg for southern AB with effect of hybrid at both locations ($P \leq 0.02$; Table 2.2). At both locations, DM concentration was affected by harvest ($P \leq 0.01$). In central AB, DM concentration after light frost was 75 g/kg greater than before frost, and in southern AB, DM concentration after frost was 58 g/kg greater than before frost.

Starch concentration of central AB hybrids ranged from 155 to 293 g/kg DM with differences among hybrids ($P < 0.001$), while those in southern AB ranged from 228 to 329 g/kg DM with a tendency for differences among hybrids ($P = 0.09$), when averaged over the two years (Table 2.2). Harvesting after frost increased ($P < 0.01$) starch concentration by 35 g/kg DM in central AB but in southern AB the effect of harvest tended to differ among hybrids (hybrid × harvest interaction; $P = 0.06$).

The NDF concentration of central hybrids ranged from 519 to 574 g/kg DM with a tendency for differences among hybrids ($P = 0.07$) while southern AB hybrids ranged from 512

to 564 g/kg DM with no difference among hybrids ($P = 0.94$; Table 2.2). There was no effect of harvest on the NDF concentration in either location ($P \geq 0.21$).

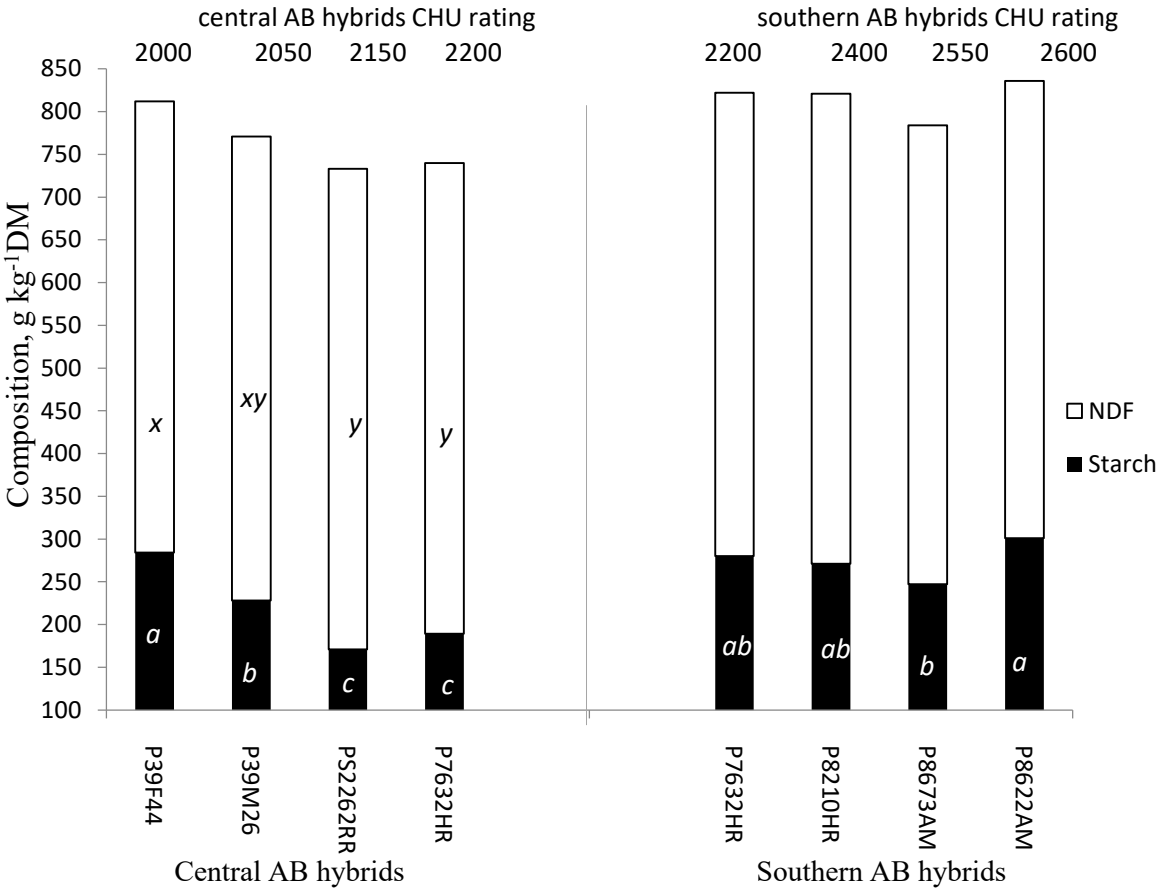
The hybrid grown in central AB with the lowest starch concentration and greatest NDF concentration irrespective of harvest time was P7632HR, and it had the greatest CHU rating (2200) for that location (Table 2.2; Figure 2.1). However, in southern AB the same hybrid (least CHU rating for the location) irrespective of harvest time did not differ in starch and NDF concentration compared with hybrids with greater CHU ratings. Hybrid P7632HR was the only one grown in both locations because it was recommended within the CHU mapping zone of both locations (maximum for central AB and minimum for southern AB).

Table 2.2. Whole plant dry matter (DM), starch and neutral detergent fiber (NDF) concentrations of short-season whole plant corn hybrids harvested before or after light frost.[1]

Item	Whole plant DM (g/kg)		Starch (g/kg DM)		NDF (g/kg DM)	
	Before frost	After frost	Before frost	After frost	Before frost	After frost
Central AB						
P39F44	301	372	274	293	519	536
P39M26	273	352	212	244	543	543
PS2262RR	265	340	155	223	551	574
P7632HR	253	326	160	181	552	550
Mean	273*b*	348*a*	200*b*	235*a*	541	551
SEM[2]						
Hybrid	27.1		13.0		12.5	
Harvest	26.1		9.2		10.7	
Hybrid × Harvest	28.8		18.5		15.5	
P-value						
Hybrid	0.02		<0.001		0.07	
Harvest	<0.001		0.01		0.30	
Hybrid × Harvest	0.99		0.54		0.71	
Southern AB						
P7632HR	407	466	263*xy*	296*xy*	564	519
P8210HR	380	422	298*x*	244*y*	541	559
P8673AM	309	373	228*y*	266*y*	549	524
P8622AM	340	406	274*xy*	329*x*	558	512
Mean	359*b*	417*a*	266	284	553	529
SEM						
Hybrid	35.6		31.3		18.8	
Harvest	33.4		29.6		13.3	
Hybrid × Harvest	39.6		34.4		26.6	
P-value						
Hybrid	<0.01		0.09		0.94	
Harvest	<0.01		0.22		0.21	
Hybrid × Harvest	0.95		0.06		0.61	

Note: Different lowercase letters following means within a variable for hybrid × harvest interaction or harvest effect differ at $P < 0.05$ (a-b) or have a tendency to differ at $0.05 < P < 0.1$ (x-y).
[1]Hybrids grown at two locations in Alberta (AB) were rated within 2000 to 2200 and 2200 to 2600 CHU for central and southern AB, respectively; and harvested before or after light frost with n = 4 for each hybrid within harvest.
[2]Standard error of means.

Figure 2.1. Starch and neutral detergent fiber (NDF) concentrations of short-season whole plant corn hybrids grown and harvested in central or southern AB (Averaged across harvest).[1]



[1]Means without a common letter differ at $P < 0.05$ (*a*-*b*) or have a tendency to differ at $0.05 < P < 0.1$ (*x*-*y*). The central AB hybrids are (CHU rating): P39F44 (2000), P39M26 (2050), PS2262RR (2150), and P7632HR (2200); southern AB hybrids are (CHU rating): P7632HR (2200), P8210HR (2400), P8673AM (2550), and P8622AM (2600). Hybrids were from Pioneer Hi-Bred (Johnston, IA, USA) except PS2262RR, which was obtained from Pickseed (Lindsay, ON).

The DMD did not vary among hybrids or harvest times for central AB (671 ± 15.7 g/kg DM; $P \geq 056$; Table 2.3). In southern AB, the DMD did not differ among hybrids ($P = 0.82$), but harvesting after frost tended to increase ($P = 0.06$) DMD by 24 g/kg DM. In central AB, the NDFD did not vary among hybrids or between harvest times (568 ± 7.5 g/kg NDF $P \geq 0.15$). However, in southern AB, NDFD increased ($P < 0.01$) by 39 g/kg NDF after light frost, but there was no effect of hybrid (545 ± 21.1 g/kg NDF; $P = 0.16$).

Total gas produced after 48 h incubation did not vary among hybrids in either location ($P \geq 0.73$; Table 2.3). However, in central AB total gas produced decreased by 15 mL/g DM incubated after slight frost ($P < 0.01$). Methane produced after 48 h incubation was affected by hybrid and harvest in central AB ($P \leq 0.01$), but in southern AB, CH_4 production was not affected by hybrid or harvest ($P \geq 0.10$). Harvesting after frost in central AB decreased CH_4 production by 11.5%.

Table 2.3. Dry matter, neutral detergent fiber degradabilities, accumulated gas and methane produced by short-season whole plant corn hybrids harvested before or after light frost.[1]

Item	DMD[2] (g/kg DM)		NDFD[2] (g/kg NDF)		Gas produced[3] (mL/g DM incubated)		Methane produced[3] (mg/g DM degraded)	
	Before frost	After frost	Before frost	After frost	Before frost	After frost	Before frost	After frost
Central AB								
P39F44	673	680	578	579	204	197	22	22
P39M26	672	667	565	555	212	185	25	22
PS2262RR	659	692	542	587	207	200	28	24
P7632HR	670	657	562	576	214	195	28	24
Mean	668	674	562	574	209*a*	194*b*	26*a*	23*b*
SEM[4]								
Hybrid	17.5		8.7		9.9		1.4	
Harvest	13.9		6.2		9.5		1.4	
Hybrid × Harvest	23.1		12.4		10.8		1.6	
P-value								
Hybrid	0.77		0.48		0.73		<0.001	
Harvest	0.56		0.15		<0.01		<0.01	
Hybrid × Harvest	0.38		0.18		0.30		0.21	
Southern AB								
P7632HR	651	704	521	585	193	200	22	22
P8210HR	675	659	557	558	202	189	23	21
P8673AM	661	689	515	567	200	203	25	24
P8622AM	650	678	506	544	192	196	22	23
Mean	659*y*	683*x*	525*b*	564*a*	197	197	23	23
SEM								
Hybrid	27.3		21.1		9.0		1.1	
Harvest	26.0		19.7		8.2		1.0	
Hybrid × Harvest	29.7		23.6		11.8		1.3	
P-value								

Hybrid	0.82	0.16	0.77	0.10
Harvest	0.06	<0.01	0.96	0.55
Hybrid × Harvest	0.25	0.24	0.59	0.62

Note: Different lowercase letters following means for the effect of harvest differ at $P \leq 0.05$ (*a*-*b*) or have a tendency to differ at $0.05 < P < 0.1$ (*x*-*y*).

[1]Hybrids grown at two locations in Alberta (AB) were rated within 2000 to 2200 and 2200 to 2600 CHU for central and southern AB, respectively; and harvested before or after light frost with n = 4 for each hybrid within harvest

[2]DMD = *in vitro* dry matter degradability; NDFD = *in vitro* neutral detergent fiber degradability.

[3]Accumulated gas and methane after 48 h incubation.

[4]Standard error of means.

The effect of harvest on total VFA did not differ among hybrids in either location ($P \geq 0.58$; Table 2.4). However, harvest times tended to affect VFA in both locations, such that in central AB harvesting before frost tended to produce more VFA than after frost harvest ($P = 0.059$) while in southern AB harvesting after frost tended to produce more VFA than before frost ($P = 0.08$). In central AB, harvesting after frost decreased acetate ($P < 0.001$) and increased propionate ($P < 0.001$) proportions, with differences ($P \leq 0.001$) among hybrids. However, in southern AB, there was no effect of hybrid or harvest times on VFA proportions ($P \geq 0.12$). Differences in acetate:propionate ratio due to hybrid and harvest at both locations reflected the differences in acetate and propionate proportions.

When examined over both locations, CH_4 production expressed in mg/g DM degraded did not correlate with NDF concentration, NDFD, gas produced after 48 h, DMD, or butyrate proportion (r = 0.094, 0.16, 0.22, 0.23 and -0.23, respectively; $P > 0.05$). However, CH_4 production was positively correlated with acetate proportion and inversely correlated with total VFA, starch concentration, and molar proportion of propionate (r = 0.57, -0.26, -0.31, and -0.72, respectively; $P < 0.05$). Over both locations, multiple regression analysis showed that variation in CH_4 production was mostly explained by variation in DMD, cumulative gas produced after 48 h, total VFA, and proportions of propionate and butyrate ($CH_4 = 36.89 + 0.21$ DMD + 0.08 GP.48h – 0.37 total VFA – 0.65 propionate – 0.49 butyrate; $r^2 = 0.79$; $P < 0.001$). Chemical composition (starch and NDF concentrations) of the hybrids alone failed to explain variation in CH_4 production ($CH_4 = 29.14 – 0.14$ starch – 0.04 NDF; $r^2 = 0.07$; $P = 0.045$). However, with nutrient degradability added to chemical composition, starch concentration and DMD explained 33% of the variation in CH_4 production ($CH_4 = 2.44 – 0.29$ starch + 0.42 DMD; $r^2 = 0.33$ $P < 0.001$).

Table 2.4. *In vitro* total volatile fatty acid (VFA) concentration and individual VFA proportions of short-season whole plant corn hybrids harvested before or after frost.[1]

Item	Total VFA[2] (m*M*)		VFA proportions (mol/100 mol)						A:P	
			Acetate (A)		Propionate (P)		Butyrate			
	Before frost	After frost	Before frost	After frost	Before frost	After frost	Before frost	After frost	Before frost	After frost
Central AB										
P39F44	38.5	37.1	47.2	46.0	30.3	32.3	19.5	19.0	1.6	1.5
P39M26	39.1	34.9	49.6	46.3	28.0	31.8	19.3	19.1	1.8	1.5
PS2262RR	35.3	37.0	53.5	47.7	26.4	30.1	17.3	19.5	2.0	1.7
P7632HR	38.1	34.8	51.7	48.9	26.7	29.3	18.5	19.1	2.0	1.7
Mean	37.8*x*	36.0*y*	50.5*a*	47.2*b*	27.8*b*	30.8*a*	18.7	19.2	1.9*a*	1.6*b*
SEM[3]										
Hybrid	2.21		1.65		0.92		1.66		0.08	
Harvest	2.12		1.54		0.83		1.57		0.08	
Hybrid × Harvest	2.39		1.84		1.08		1.82		0.10	
P-value										
Hybrid	0.58		<0.01		0.001		0.27		<0.001	
Harvest	0.059		<0.001		<0.001		0.41		<0.001	
Hybrid × Harvest	0.14		0.28		0.59		0.69		0.23	
Southern AB										
P7632HR	34.2	39.8	45.6	45.6	29.8	30.7	21.9	20.6	1.57	1.54
P8210HR	36.1	36.0	44.5	46.3	30.8	30.6	21.7	20.3	1.49	1.56
P8673AM	35.9	36.0	47.9	46.5	29.0	28.7	20.4	22.1	1.70	1.65
P8622AM	34.5	37.6	46.5	44.7	29.4	30.4	21.6	21.8	1.62	1.52
Mean	35.1*y*	37.4*x*	46.1	45.8	29.8	30.1	21.4	21.2	1.59	1.57
SEM										
Hybrid	1.48		2.31		0.66		1.66		0.11	
Harvest	1.20		2.18		0.54		1.62		0.10	
Hybrid × Harvest	1.92		2.54		0.85		1.74		0.12	
P-value										
Hybrid	0.92		0.61		0.12		0.82		0.30	
Harvest	0.08		0.75		0.49		0.78		0.66	
Hybrid × Harvest	0.33		0.64		0.71		0.16		0.76	

Note: Different lowercase letters following means for the effect of harvest differ at $P < 0.05$ (*a-b*) or have a tendency to differ at $0.05 < P < 0.1$ (*x-y*).

[1]Hybrids grown at two locations in Alberta (AB) were rated within 2000 to 2200 and 2200 to 2600 CHU for central and southern AB, respectively; and harvested before or after light frost with n = 4 for each hybrid within harvest .
[2]Total volatile fatty acid corrected from blank (containing buffer medium, rumen fluid, and empty filter bag) after 48 h incubation period.
[3]Standard error of means.

2.4. Discussion

In both growing seasons the average CHU received in central AB (mean ± SD, 1990 ± 174.9) was lower than that received in southern AB (2365 ± 148.4), thus the two locations provided a range in environmental conditions for corn growth as planned. Long-term CHU rating is 1800 to 2000 for central AB (Lacombe) and 2200 to 2400 for southern AB (Vauxhall; Thygesen, 2003). Due to the different CHU ratings of the two locations, the hybrids grown at each location differed and hybrid and harvest effects were examined within location. As very few hybrids are adapted to and recommended for both locations, it was not possible or relevant to grow the same hybrids in both locations (with the exception of P7632HR with 2200 CHU rating). The two years of study were representative of the long term averages for these locations. Corn heat units are used in Canada to rate corn hybrids based on the accumulated heat required from planting to grain maturity (Brown and Bootsma, 1993).

In central AB harvest time had a significant effect on whole plant DM. Harvesting after frost can increase the drying rate of WPCH due to rupturing of cells (Lee and Herbek, 2004), so growers sometimes leave the whole plant standing until after frost to optimize DM concentration for ensiling (Narasimhalu et al., 1986; Baron et al., 2008). Frost damage depends on its intensity, which is influenced by temperature, duration of temperature less than 0°C and corn growth stage at the time of frost (White et al., 1976; Lee and Herbek, 2004). When frost is light as was the case of central AB in this study (-0.2 to -2.0°C), and cells in the corn stalks and ear-shanks are still alive, it may be possible for kernels to fill for a short period. The frost reported herein, falls in the range reported by White et al. (1976; -0.0 to -2.0°C) and less intense than the frost reported by Narasimhalu et al. (1986; -2.0 to -3.0 °C). Delaying harvest until after a frost also increased the CHU received, but the increase was relatively small and inconsistent between years

for central AB. On average, harvesting after frost in central AB allowed the plants to receive an 1.5% increase of CHU. Nevertheless, an increase in starch and DM concentration (mean increase, 35 g/kg DM and 75 g/kg; respectively) occurred in central AB by delaying harvest. Although, grain yield was not reported in the present study, White et al. (1976) reported an increase in grain yield (proxy for starch concentration) and whole plant DM concentration after frost.

As starch concentration increases, there is usually a proportional decline in NDF concentration due to increased grain yield and dilution of NDF by starch (Allen et al., 2003). Despite changes in starch concentration there was no change in NDF concentration with harvest time in central hybrids. The relatively small additional accumulation of CHU by harvesting after frost in central AB, may have caused the lack of effect of late harvest on NDF concentration, indicating that while kernel filling (starch concentration) was affected by CHU received, stover fiber (main source of NDF concentration) was not affected. The light frost was likely not sufficient to kill the whole plant or the stalk but may have frozen some leaf tissues causing the leaves to fall from the plant, reducing photosynthesis, and consequently reducing sucrose translocation from leaves to the kernels. As the air temperature warms after frost, the plants may translocate more reserved stalk carbohydrate reserves to increase grain yield (White et al., 1976; Slewinski, 2012), which may in turn increase starch concentration.

In southern AB, harvesting after frost the CHU received after frost only increased whole-plant DM and did not affect kernel development and therefore the lack of change in NDF concentration in southern hybrids due to late harvest was as expected. The frost in southern AB (-0.8 to -2.2°C) and the accumulated CHU (3.9% increase) were relatively greater than for central AB. When the air temperature falls below -2.0°C, it is possible that translocation of

sucrose from the stalk may not occur and this may terminate grain filling, which may partly explain the lack of effect of the late harvest on starch concentration for southern AB hybrids. Also, in southern AB, it appears the effects of harvesting after frost were dependent on the difference in maturities among the hybrids at the time of frost (i.e., some hybrids were closer to the end of grain filling). Therefore, for a relatively warmer area where the accumulated CHU acquired during the growing season is adequate for plants to mature before frost, delaying harvest until after frost is not a best practice as DM yield may decrease due to loss of leaves and the whole-plant may become drier than the recommended DM concentration (320-380 g/kg) for silage making.

Due to the relatively short growing season for corn hybrids in AB, the average starch concentration (247 g/kg DM) was less than reported by commercial laboratories for hybrids grown in the U.S. [mean ± SD, 326 ± 69.5 g/kg DM, 169,620 samples; NASEM, 2016]. However, the range of starch concentrations in the present study (155 to 329 g/kg DM) was within the range reported by NASEM (2016). Also the NDF concentrations of the WPCH in the study (mean: 544 g/kg DM; range: 512 to 574 g/kg DM) were considerably greater than those reported by NASEM (2016; mean ± SD: 430 ± 54.9 g/kg DM, 193,210 samples). It is important to note that samples used in this study were not ensiled, and therefore pre-ensiling nutritional quality is presented. However, starch and NDF concentrations are not likely to change significantly during the ensiling process. Although factors such as water availability, soil fertility, and photoperiod can influence the development of crops, corn is a warm-season crop, and so, it is predominantly sensitive to temperature for growth and development. Starch concentration of WPCH within an adaptation zone is largely determined by CHU received from planting to grain maturity, relative to the number of CHU rating of the hybrid, which would

account for the lower starch concentrations observed in the present study compared with the U.S. database. In areas where corn is well adapted, grain-filling occurs during a prolonged frost-free period that is conducive to high grain yield and starch concentration.

The study demonstrates the importance of matching the hybrid rating to the CHU zone to maximize starch concentration while producing silage with an optimum DM concentration especially for cooler areas. For example, in central AB hybrid P7632HR had 113 g/kg DM less starch concentration compared with the hybrid with the lowest CHU rating of 2000 (P39F44) in central AB (Figure 2.1). The CHU received in central AB during the study was insufficient for hybrid P7632HR to reach a desirable stage of maturity by harvest time. The average DM concentration of this hybrid at harvest was only 290 g/kg compared with 337 g/kg of hybrid P39F44 in central AB, whereas the optimum DM for ensilaging WPCH is 320 to 380 g/kg (Allen et al., 2003). Thus, hybrid P39F44 with a low CHU rating of 2000 was ideal for ensilaging indicating the potential of appropriate hybrids to reach a desirable stage of maturity in the short growing season of central AB. The current study agrees with Baron et al. (2008), where a short-season WPCH (2000 CHU rating) grown in central AB increased in starch concentration as actual CHU received increased from 1950 to 2200 at harvest. Grain maturity occurs after yield maximizes; therefore, hybrids selected for CS often have CHU rating of 100-200 CHU more than required for grain (Daynard, 1978). For instance, a corn hybrid with 2600 CHU rating grown for silage would typically be grown in a 2400 CHU zone (i.e., 92% CHU received as a percentage of CHU rating). However, weather conditions during the growing season are variable from year to year and as the CHU zone becomes shorter and cooler, nutrient concentration can be compromised (Baron et al., 2008). Thus, to maximize starch concentration of CS in very short

season areaa, it is recommended that the CHU rating of hybrids when grown for silage not exceed the long-term rating of the adaptation zone in very short-season areas.

In ground samples, starch is almost completely degraded (Martin et al., 2008). Thus, degradability of starch and hence DMD in the current study may be overestimated *in vitro* because grinding alleviates potential negative effects of kernel vitreousness on starch degradability (Allen, 2012). Starch concentration, NDF concentration and NDFD are the major contributors to DMD. The study showed that despite variations in starch concentration, NDF concentration and NDFD among the WPCH samples, there were surprisingly minimal effects on DMD. White et al. (1976) reported a decline in DMD of WPCH after frost but did not report the same trend in a different growing year in Prince Edward Island. Also, in Ontario, Daynard and Hunter (1975) observed no change in whole-plant DMD over a range of 240 to 440 g/kg whole-plant DM prior to frost. The DMD is an important trait for WPCH selection because it affects digestible energy intake of ruminants. However, the relative importance of the determinants of DMD, specifically high starch concentration versus high NDFD for cattle is not clear, and requires further study.

The present study showed harvesting after frost resulted in no change in NDFD for central AB hybrids. The WPCH grown in central AB were harvested at a less mature stage than those grown in southern AB as indicated by the numerically lower whole plant DM and starch concentrations. Lack of change in NDFD for the central AB hybrids is consistent with the findings of Darby and Lauer (2002), who reported that cell wall digestibility remained fairly constant throughout early to mid-stages of growth of WPCH, decreasing only in late maturity.

In southern AB, greater NDFD after frost was not anticipated. This result may partly be due to the maturity of these hybrids being close to the end of grain-filling coupled with the loss

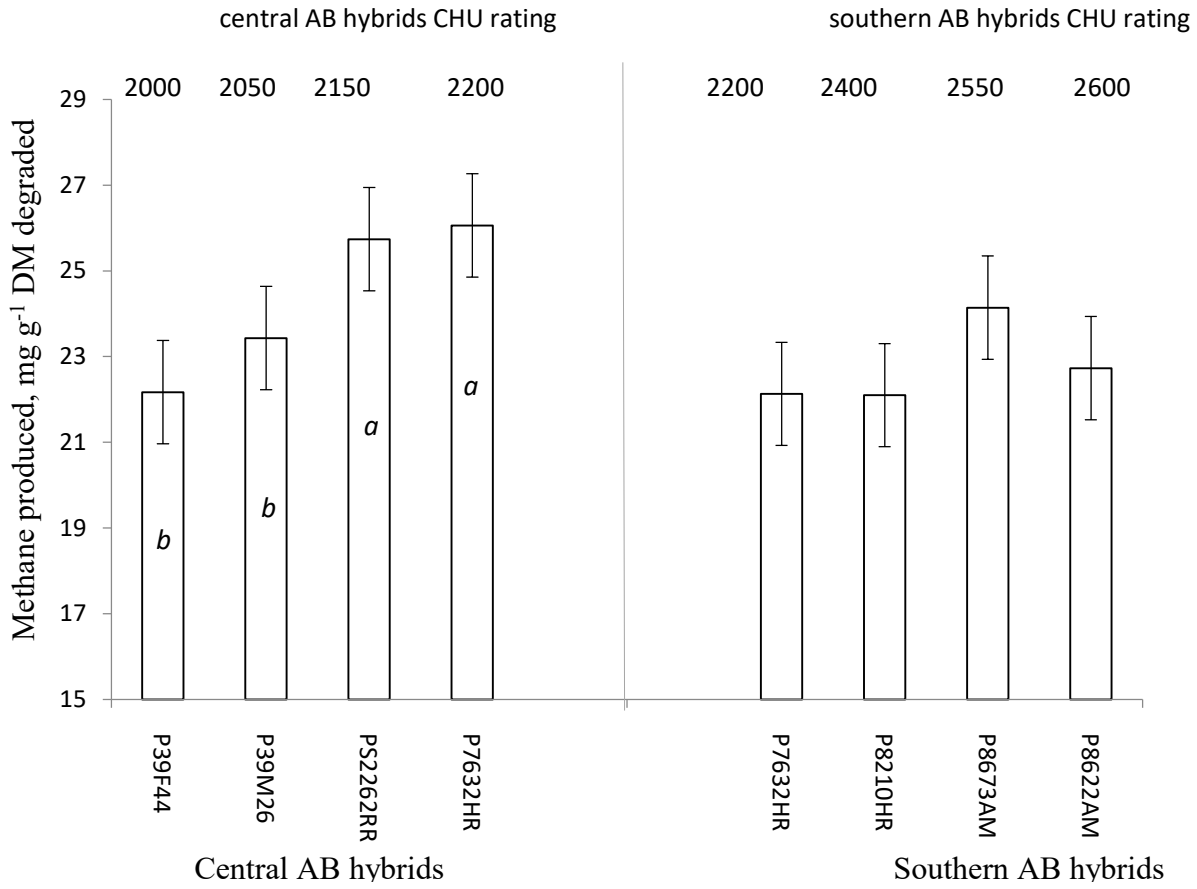
of mature leaves (mainly fiber). In support of our findings for NDFD, Ferraretto and Shaver (2012) summarized data from 27 dairy cow experiments and reported a tendency for increased total tract NDF digestibility with an increase in DM concentration (35 to 40%) of CS harvested. In the present study, increased NDFD of hybrids grown in the relatively warmer area after frost, in comparison with the lack of change in NDFD in hybrids grown in the cooler area, indicates that harvesting after frost may affect NDFD differently, depending on location.

The lack of hybrid effects on total VFA concentrations at each location is consistent with the lack of difference in DMD among hybrids. Diets with higher starch concentration favour propionate production at the expense of acetate (Ellis et al., 2008), which accounts for the decreased acetate proportion, increased propionate proportion, and decreased acetate:propionate ratio observed for central hybrids harvested after frost. It should be mentioned that the total VFA concentrations reported are seemingly low compared with many other studies (e.g., Fernandez et al., 2004; Hatew et al., 2016). This discrepancy occurred because the concentrations were corrected for the background concentrations contributed by the rumen fluid inoculum, such that changes in VFA represented the effect of substrate only.

An important finding of our study is that there were differences in CH_4 production among WPCH in central AB indicating that it might be possible to grow corn hybrids in cooler growing zones that result in less CH_4 produced by cattle. For example, the earliest hybrids P39F44 and P39M26 harvested in central AB produced less CH_4 compared with later hybrids (Figure 2.2). In contrast, hybrids grown in southern AB, which represents a warmer adaptation zone, did not differ in CH_4 production. Increased starch concentration is associated with decreased enteric CH_4 production while increased NDF concentration and degradation increases CH_4 production (Moe and Tyrrell, 1979). As starch concentration increased, CH_4 decreased for central AB hybrids

harvested after frost; however, the increase in NDFD in southern AB hybrids after frost did not increase CH_4 production for southern AB hybrids harvested after frost. The results indicate that CH_4 production did not correspond directly to changes in chemical composition and degradabilities. When examined over all WPCH samples, chemical composition was not the major factor associated with CH_4 production. As starch increases in a diet, there is a shift by rumen microbes to convert pyruvate to propionate using the acrylate pathway and this reduces the acetate:propionate ratio of the rumen (Van Soest, 1994). The formation of propionate uses reducing equivalents and therefore, stoichiometrically lowers H_2 available for CH_4 production (Janssen, 2010; Patra et al., 2017). This effect accounts for the observed high negative correlation between propionate concentration and CH_4 production. As CH_4 is an energy loss to the animal, reducing its formation in the rumen may potentially improve animal performance (Mathison et al., 1998).

Figure 2.2. *In vitro* methane production for short-season whole plant corn hybrids grown and harvested in central or southern AB (Averaged across harvest). [1]



[1]Means without a common letter differ at $P < 0.05$ (*a*-*b*). The central AB hybrids are (CHU rating): P39F44 (2000), P39M26 (2050), PS2262RR (2150), and P7632HR (2200); southern AB hybrids (CHU rating) are: P7632HR (2200), P8210HR (2400), P8673AM (2550), and P8622AM (2600). Hybrids were from Pioneer Hi-Bred (Johnston, IA, USA) except PS2262RR, which was obtained from Pickseed (Lindsay, ON).

Multiple regression analysis showed that variations in enteric CH_4 production of short-season WPCH (mg/g degraded) could be best predicted by combined effects of DMD, cumulative gas produced after 48 h, total VFA, propionate and butyrate proportion. However, chemical composition alone (i.e., starch and NDF concentrations) poorly predicted CH_4 production. Without measuring gas production and VFA, starch concentration and DMD combined predicted 33% of the variation in CH_4 production. A unit increase in starch

concentration decreased *in vitro* CH_4 production by 0.29 percentage units while a unit increase in DMD increased it by 0.42 percentage units.

In conclusion, for short-season growing areas, attainment of whole-plant DM concentration within the optimum range (320 to 380 g/kg) for ensilage can be challenging. Therefore, it is common practice to delay corn harvest to after frost to increase DM concentration. However, this practice may have differing effects on nutritional quality depending upon location and hybrid. In the central, cooler area of AB, hybrids were less mature due to the lower accumulated CHU received and thus, delaying harvest until after a mild frost increased DM concentration, improved quality and reduced CH_4 production. Therefore, delayed harvest in cooler areas has the potential to improve animal performance, although harvesting at an optimum DM concentration after frost can be challenging due to rapid drying and inclement weather. Because of greater accumulated CHU in the warmer, southern region of AB, the WPCH tended to reach maturity before frost and therefore, harvesting after frost increased the DM concentration of some hybrids above optimum for ensiling without any major improvements in quality or decrease in CH_4 production.

Methane production from short-season WPCH hybrids is variable, depending upon the location and hybrid. This effect was attributed to differences in chemical composition, rumen fermentation, and digestibility. Further *in vivo* research is needed to document the relationships reported in the present *in vitro* study that identified factors that may reduce CH_4 emissions of ruminants fed short-season corn hybrids.

2.5. Literature Cited

Allen, M. S. 2012. Adjusting concentration and ruminal digestibility of starch through lactation. In Proc. of the Four-State Dairy Nutr. Managt. Conf., Madison, WI. p. 24–30.

Allen, M. S., J. G. Coors, and G. W. Roth. 2003. Corn silage. In Silage Science and Technology, D. Buxton, R. Muck, and J. Harrison, eds. ASA, CSSA, and SSA, Madison, WI.

Association of Official Analytical Chemists. 2005. Official methods of analysis, 18th ed. AOAC Int., Gaithersburg, MD.

Baron, V. S., K. A. Beauchemin, P. Juskiw, and R. R. Doce. 2014. Managing short-season corn and small-grain silage in Western Canada. In Proc. of the 35th Western Nutrition Conference, 24 – 25 Sept. 2014, Edmonton, AB. p. 147–162.

Baron, V. S., T. B. Daynard, G. Dupuis, N. A. Fairey, D. J. Major, J. F. Muldoon, and R. P. White. 1987. Evaluation of early-maturing European and Canadian corn hybrids for grain and forage production in Canada. Maydica. XXXII: 33–48.

Baron, V. S., M. Oba, and A. Aasen. 2008. Challenges for growing corn silage suitable for the dairy industry in a northern climate. WCDS Advances Dairy Technol. 20:181–192.

Beauchemin, K. A., H. H. Janzen, S. M. Little, T. A. McAllister, and S. M. McGinn. 2010. Lifecycle assessment of greenhouse gas emissions from beef production in western Canada: A case study. Agric. Syst. 103:371–379.

Beauchemin, K. A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008**.** Nutritional management for enteric methane abatement: a review. Australian J. Exp. Agriculture 48:21–27.

Beauchemin, K. A., and S. M. McGinn. 2008**.** Reducing methane in dairy and beef cattle operations: What is feasible? Prairie Soil Crop 1:17–21.

Brown, D.M. and Bootsma, A. 1993. Crop heat units for corn and other warm-season crops in Ontario. Ontario Ministry of Agriculture and Food Factsheet No. 93–119, Agdex 111/31. p.

4. [Online] Available: http://www.omafra.gov.on.ca/english/crops/facts/93-119.htm (Acessed 16 September 2016).

Cammell, S. B., J. D. Sutton, D. E. Beever, D. J. Humphries, and R. H. Phipps. 2000. The effect of crop maturity on the nutritional value of maize silage for lactating dairy cows. Anim. Sci. 71: 381–390.

Canadian Council on Animal Care (CCAC). 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. Ottawa, ON, Canada.

Chung, Y.-H., N. D. Walker, S. M. McGinn, and K. A. Beauchemin. 2011**.** Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. J. Dairy Sci. 94:2431–2439.

Darby, H. M., and J. G. Lauer. 2002. Harvest date and hybrid influence on corn forage yield, quality, and preservation. Agron. J. 94:559–566.

Daynard, T. B. 1978. Practices affecting quality and preservation of whole plant corn silage. Can. J. Plant Sci 58:651–659.

Daynard, T. B., and R. B. Hunter. 1974. Relationships among whole-plant moisture, grain moisture, dry matter yield and quality of whole-plant corn silage. Can. J. Plant Sci. 55:77–84.

Dwyer, L. M., D. W. Stewart, L. Carrigan, B. L. Ma, P. Neave, and D. Balchin. 1999. Guidelines for comparisons among different maize maturity rating systems. Agron. J. 91: 946–949.

Ellis, J. L., J. Dijkstra, E. Kebreab, A. Bannink, N. E. Odongo, B. W. McBride, and J. France. 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agr. Sci. 146:213–233.

Fernandez, I., P. Noziere, and B. Michalet-Doreau. 2004. Site and extent of starch digestion of whole-plant maize silages differing in maturity stage and chop length, in dairy cows. Livest. Prod. Sci. 89:147–157.

Ferraretto, L. F., and R. D. Shaver. 2012. Meta-analysis: Impact of corn silage harvest practices on intake, digestion, and milk production by dairy cows. Prof. Anim. Sci. 28:141–149.

Gabruch, M., and R. Gietz. 2014. The potential of grain corn in Alberta. Alberta Agriculture and Forestry. Available http://www1.agric.gov.ab.ca/4Department/deptdocs.nsf/all/bus15031 (Accessed 04 November 2017).

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis. Agric. Handbook No. 379. Agricultural Research Service, USDA, Washington, DC.

Guyader, J., V. S. Baron, and K. A. Beauchemin. 2018. Corn forage yield and quality for silage in short growing season areas of the Canadian prairies. Agronomy 8:1-25.

Guyader, J., H. H. Janzen, R. Kroebel, and K.A. Beauchemin. 2016. Invited Review: Forage utilization to improve environmental sustainability of ruminant production. J. Anim. Sci. 94:3147–3158.

Hatew, B., A. Bannink, H. Van Laar, L. H. De Jonge, and J. Dijkstra. 2016. Increasing harvest maturity of whole-plant corn silage reduces methane emission of lactating dairy cows. J. Dairy Sci. 99:354−368.

Huggins-Rawlins, N. 2015. Agroclimatic atlas of Alberta. Alberta Agriculture and Forestry. Available http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sag6301 (Accessed 01 November 2016)

Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160:1–22.

Lee, C., and J. Herbek. 2004. Late-season frost damage to corn grown for silage: AGR-183. University of Kentucky. Available: https://www.uky.edu/Ag/Forage/frost%20damage%20to%20corn%20silage.pdf Accessed (Accessed 28 August 2017)

Martin, N. P., D. R. Mertens, M. B. Hall, and J. G. Lauer. 2008. Fiber digestibility and starch content of corn silage. In Proc. of the Idaho Alfalfa and Forage Conference. p. 26–27

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

Mauricio, R. M., F. L. Mould, M. S. Dhanoa, E. Owen, K. S. Channa, K.S. and M. Theodorou. 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. Anim. Feed Sci. Technol. 79:321–330.

McGeough, E. J., P. O'Kiely, P. A. Foley, K. J. Hart, T. M. Boland, and D. A. Kenny. 2010. Methane emissions, feed intake, and performance of finishing beef cattle offered maize silages harvested at 4 different stages of maturity. J. Anim. Sci. 88:1479–1491.

Narasimhalu, P, R. P. White, and K. B. MaCrae. 1986. The effect of harvesting before and after frost on corn silage composition and its intake and digestibility in sheep. Can. J. Plant Sci. 66:579–586.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient Requirements of Beef Cattle. 8th rev. ed. The National Academies Press, Washington, DC.

Owens, F. 2008. Corn silage – Facts, fantasies and future. Proc. 19th Florida Ruminant Nutrition Symposium, Gainsville, Florida, US. Available: http://vffarms.com/Images/Resources/ProductionChoices/Pioneer_silage_data.pdf. (Accessed 12 May 2017).

Patra, A., T. Park, M. Kim, and Z. Yu. 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. J. Anim. Sci. Biotechnol. 8:13.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-126, <URL: http://CRAN.R-project.org/package=nlme>.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Slewinski, T. L. 2012. Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. J. Exp. Bot. 63:4647–4670.

St. Pierre, N. R., R. Bouchard, G. St. Laurent, C. Vinet, and G. L. Roy. 1983. Effects of stage of maturity and frost on nutritive value of com silage for lactating dairy cows. J. Dairy Sci. 66:1466–1473.

Thygesen, L. 2003. Annual total corn heat unit from 1971 to 2000. Alberta and Agriculture Forestry. 2003. Available:
https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sag6442?opendocument (Accessed 25 January 2019)

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd rev. ed. Cornell University Press, Ithaca, NY.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

White, R. P., K. A. Winter, and H. T. Kunelius. 1976. Yield and quality of silage corn as affected by frost and harvest date. Can. J. Plant Sci. 56:481–486.

Yan, W., and L. A. Hunt. 1999. An equation for modeling the temperature response of plants using only the cardinal temperatures. Ann. Bot. 84:607–614.

Zom, R. L. G., A. Bannink, and R. M. A. Goselink. 2012. Effect of increased maturity of silage maize at harvest on conservation dairy cow performance and methane emission. Livestock Research Wageningen, Wageningen, the Netherlands. Report 578.

CHAPTER 3 - Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high-forage diet[2]

3.1. Introduction

There is inefficiency in dietary N utilization by cattle that are fed forage diets high in soluble protein, because greater N intake increases N excretion in feces and urine (Dijkstra et al.,2013). In addition, growing concerns of elevated CH_4 emissions from ruminant production have prompted efforts to develop mitigation strategies. Beef cattle fed high forage diets lose about 6 to 12% of their energy intake into the atmosphere as enteric CH_4 (Johnson and Johnson, 1995) representing a potential source of inefficiency. Tannins are plant secondary compounds with the ability to form complexes with proteins and carbohydrates that can potentially improve N use and decrease CH_4 production (Patra and Saxena, 2011). However, the effects of these polyphenolic polymers may depend on the type and quantity consumed.

Tannins can be categorized as CT or HT based on their structure and reactivity. Condensed tannins have high binding capacity for dietary protein and can decrease degradability of protein in the rumen with negative effects on microbial protein synthesis but increase rumen bypass protein (MacAdam and Villalba, 2015). Thus, the net effect of MP flow to the small intestine is not necessarily reduced (Wang et al., 1996). For instance feeding CT containing forages such as birdsfoot trefoil as a sole diet improved weight gain of sheep due to an increase in amino acid absorption from the small intestine (Wang et al., 1996; Waghorn, 2008). However, ruminal undegraded proteins cannot completely elucidate the improvement in animal performance, other factors such as the origin, concentration, molecular structure, and dosage,

[2]A version of this chapter is published. Aboagye, I. A., M. Oba, A. R. Castillo, K. M. Koenig, A. D. Iwaasa, and K. A. Beauchemin. 2018. Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high-forage diet. J. Anim. Sci. 96:5276–5286. doi: 10.1093/jas/sky352.

could throw more light on tannins inclusion in animal diets. Condensed tannins can also decrease enteric CH_4 production (Carulla et al., 2005; Grainger et al., 2009), but the binding capacity of CT may also decrease fiber digestibility (Reed, 1995). Hydrolyzable tannins have smaller molecular weight (500-3000 Da) than CT (1900-28000 Da). Compared with CT, HT have a weaker affinity for proteins and thus are more easily absorbed from the digestive tract increasing potential toxicity to the animal (McLeod, 1974). For this reason previous research on use of tannin in ruminant diets focused on CT rather than HT. However, an *in vitro* study indicated that there was no difference between tannin sources in preventing protein degradation (Getachew et al., 2008). Furthermore, supplementing HT at 3% of DM showed no evidence of toxicity to sheep, yet CH_4 emissions were decreased by 24% compared with control (Liu et al., 2011). Hydrolyzable tannins can permeate rumen protozoa and exert toxic effects, thereby disrupting their association with methanogens (Patra and Saxena, 2011).

Feeding HT or CT at a dose of 0.6% dietary DM to feedlot Holstein steers had no effect on growth or DMI, but a combination of the 2 sources (0.3% each) increased average daily gain ADG and DMI compared with the control treatment (Rivera-Méndez et al., 2016). The relatively low dose of tannin used in the study of Rivera-Mendez et al. (2016) may have avoided potential toxicity of HT to steers. Aguerre et al. (2015) supplemented a combination of HT and CT at 0, 0.45 and 1.8% of dietary DM in the diet of lactating dairy cattle and reported a linear increase in DMI with no adverse effect on milk production but a decrease in milk urea N and urine N. Coupled with the abilities of both CT and HT to form complexes with protein and subsequently decrease protein digestion and N excretion, each has an individual ability, which when combined and fed as a supplement, may be advantageous to beef cattle and the environment. Condensed tannin has the ability to reduce fiber digestion (Reed et al., 1999), which may decrease H_2

available for methanogens and HT is more toxic to methanogenic activity (Jayanegara et al., 2015); thus supplementing the diet of beef cattle with the combination of CT and HT may decrease CH_4 emission and improve N use. However, there is limited information available on their combined effects and optimum dose. Therefore, we hypothesized that a combination of HT and CT compared with the control at an optimum dose may lower rumen NH_3 concentration (an indicator for N excretion), decrease CH_4 emissions and improve animal performance. The objective of the study was to determine the effects of HT alone and in combination with CT at low and high doses on growth performance, N use, ruminal fermentation, protozoa populations and CH_4 emissions in growing beef steers.

3.2. Materials and Methods

The experimental protocol (ACC 1630) was reviewed and approved by the Animal Care Committee of the LeRDC. The animals were cared for and the experiment was conducted according to the guidelines of the Canadian Council on Animal Care (2009).

Animals, Diet and Experimental Design

A total of 75 weaned crossbred steers with an initial body weight (**BW**) of 292 ± 4.1 kg (mean ± SEM) were grouped by BW (3 groups; 25 steers per group) and randomly assigned to one of 5 dietary treatments (15 animals per treatment) within group. Prior to the study, the steers were processed using standard management procedures for ear tagging and vaccination. Over a 2-week period, they were gradually introduced to the control diet and adapted to their individual pens (2.5 m × 3 m) bedded with wood shavings. Each pen had a separate feeder, and animals in adjacent pens shared a water bowl. The steers were fed a basal diet of alfalfa and barley silages (50:50; DM basis) and a supplement containing minerals and vitamins to meet or exceed the nutrients requirement of growing beef cattle (NASEM, 2016; Table 3.1). The basal diet was

supplemented with HT extract (CN; 74% tannin) or a combination (50:50) of HT and CT extract (Q; 91% tannin) in powdered forms (SilvaTeam, León, Gto., Mexico) at different levels of dietary DM (Table 3.1). The treatments for determining animal performance were: control (no tannin), 0.25% CN, 1.5% CN, combination of CN and Q at 0.125% each (0.25% **CNQ**), and CN and Q at 0.75% each (1.5% CNQ) of dietary DM. The lower (0.25%) and the higher (1.5%) levels of tannins applied were chosen for the present study because at 3 levels of Q extract applied at 0.2%, 0.4%, and 0.6% to the diet of Holstein steers there was no difference in DMI and gain efficiency and subsequently at the relatively highest level (0.6%) of CNQ did not improve ADG compared with CN or Q applied alone (Rivera-Mendez et al., 2016).

Following the performance study, 45 steers from 3 of the treatment groups were used for CH_4 measurements. Treatments for the CH_4 measurement were control, 1.5% CN, and 1.5% CNQ of dietary DM. The lower levels of tannins were not considered because an effect on CH_4 emissions was not expected at lower application rates (Jayanegara et al., 2012). Individual feed ingredients including tannins were mixed as a TMR using a Calan Data Ranger (American Calan, Northwood, NH) and offered once a day (0930 h) to the steers for ad libitum (5% ort) intake.

Sampling Procedures

Steers were weighed before feeding on 2 consecutive days at the beginning (8 and 9 Nov. 2016) and end (31 Jan. and 01 Feb. 2017) of the performance study. In addition animals were weighed before feeding every 3 wk, so that there were four experimental periods (period 1, wk 1 to 3; period 2, wk 4 to 6; period 3, wk 7 to 9; and period 4, wk 10 to 12). The amount of TMR offered was recorded daily and sampled weekly; orts were collected daily, weighed, composited, and subsampled weekly. The DM of the weekly sample of TMR was determined and used to

calculate weekly DMI. The silages, supplements and tannins were sampled weekly to monitor DM and adjust diet composition when necessary. A subsample of a weekly composite of the silages and supplements in each period was used for chemical analysis. Average daily gain and feed efficiency (ADG/DMI; gain:feed; G:F) were calculated by period and averaged over the entire study.

Table 3.1. Feed ingredients of dietary treatments and chemical composition of the basal diet.

	Dietary Treatment[1]				
	Control	CN		CNQ	
Item		0.25%	1.5%	0.25%	1.5%
Ingredients, % DM					
Alfalfa silage[2]	47.5	47.38	46.75	47.38	46.75
Barley silage[3]	47.5	47.38	46.75	47.38	46.75
Supplement[4]	5.00	5.00	5.00	5.00	5.00
Barley ground	4.90	4.90	4.90	4.90	4.90
Salt (sodium chloride)	0.05	0.05	0.05	0.05	0.05
Vitamin and mineral premix[5]	0.05	0.05	0.05	0.05	0.05
CN extract[6]	—	0.25	1.50	0.125	0.75
Q extract[6]	—	—	—	0.125	0.75
Chemical composition, % DM					
DM (as is)	35.8				
CP	17.1				
NDF	43.8				
Starch	12.7				
NEg (Mcal/kg DM)[7]	0.82				

[1]CN, Chestnut; CNQ, chestnut and quebracho mix.
[2]Contained 31.9% ± 1.48% DM and 79.6% ± 1.55% OM, 21.7% ± 0.74% CP, 43.7% ± 1.35 % NDF and 35.0% ± 1.24% ADF on a DM basis using pooled samples for each period and from the gas measurement phases (mean ± SD; n = 7).
[3]Contained 38.0% ± 0.70% DM and 91.9% ± 0.53% OM, 13.1% ± 0.51% CP, 46.4% ± 1.45% NDF, 25.5% ± 1.07% ADF and 18.7% ± 4.44% starch on a DM basis using pooled samples for each period and from the gas measurement phases (mean ± SD; n = 7).
[4]Contained 89.8% ± 0.23% DM and 94.7% ± 0.81% OM, 10.8% ± 0.32% CP, 19.7% ± 3.39% NDF, 6.13% ± 1.19% ADF and 60.1% ± 3.83% starch on a DM basis using pooled samples for each period and from the gas measurement phases (mean ± SD; n = 7); provided as mash.
[5]Contained 35.01% $CaCO_3$, 10.37% $CuSO_4$, 28.23% $ZnSO_4$, 0.15% ethylenediamine dihydriodide (80% concentration), 5.01% Se 1% (10,000 mg Se/kg), 0.1% $CoSO_4$, 14.54% $MnSO_4$, 1.71% vitamin A (500,000,000 IU/kg), 0.17% vitamin D (500,000,000 IU/kg), and 4.7% vitamin E (500,000 IU/kg).
[6]Chestnut extract contained 74% tannin and quebracho extract contained 91% tannin in powdered forms (SilvaTeam, León, Gto., Mexico); thus, the 0.25% CN, 1.5% CN, 0.25% CNQ, and 1.5% CNQ treatments contained 0.19, 1.11, 0.20 (0.09% HT and 0.11% CT), and 1.24% (0.56% HT and 0.68% CT) tannin, respectively.
[7]Estimated using the NASEM (2016), Empirical Level of Solution, with the measured nutrient compositions of the alfalfa silage, barley silage and supplement with animal, management, and environmental inputs.

Blood samples were collected from all 15 animals per treatment at the beginning of the study and at the end of every period at the time of weighing animals. Blood samples were taken from the jugular vein into sterile evacuated tubes containing an anticoagulant (10 mL, lithium heparin, Vacutainer, Becton Dickinson, Oakville, ON). The blood was centrifuged at 3000 × g and 4°C for 20 min to obtain plasma and stored at -20°C until analysis of plasma urea N (**PUN**) concentration as an indicator of protein status of the animal (Kohn et al., 2005).

Rumen fluid samples were collected orally 3 h post feeding near the end of period 4 from 7 animals per treatment using the method described by Paz et al. (2016). A 3-m tube was passed through a speculum that was inserted via the mouth into the ventral sac. The other end of the tube was connected to a 1000 mL Nalgene polypropylene vacuum flask (Thermo Scientific Inc., Waltham, MA, USA). A suction pump (model DD195, Precision Scientific, Niagara Falls, NY) connected to the vacuum flask was applied for a few seconds to free any blockage and facilitate the collection of rumen samples. To avoid cross contamination between animals, the speculum and tubing were thoroughly washed with warm water and the first 200 mL sample was discarded to avoid saliva contamination. The following 200 mL collected was strained through a PECAP polyester screen (355-µm pore size; B & S H Thompson, Ville Mont-Royal, QC, Canada) and retained for analysis of VFA, NH_3 concentration and protozoa enumeration. The pH of the unfiltered rumen fluid was immediately measured using a pH meter (Orion Model 260A, Fisher Scientific, Toronto, ON, Canada). For VFA determination 5 mL of the filtered ruminal fluid was added to 1 mL of 25% meta-phosphoric acid (wt/vol). Another 5 mL of filtered ruminal fluid was added to 1 mL of 1% sulfuric acid (vol/vol) for NH_3 determination. Also, for protozoa enumeration, 5 mL of the filtrate was mixed with 5 mL of methyl green-formalin-saline solution and stored in the dark at room temperature until analyzed.

Enteric CH_4 production from all control, 1.5% CN, and 1.5% CNQ animals was measured using the SF_6 tracer gas technique as described by McGinn et al. (2006). To facilitate the measurements, the animals were first assigned to 3 groups with 5 animals per treatment in each group (15 animals per group) with each group measured sequentially. At least 7 d prior to CH_4 measurement, a permeation tube was introduced into the rumen of each animal using a tube within a speculum that was introduced via the mouth into the ventral sac. The permeation tubes were stored for at least 1 mo at 39 °C to determine the release rate of SF_6 (mean ± SD release rate; 4.79 ± 0.559 mg/d). Prior to CH_4 measurement, animals were adapted to the wearing the halters and yokes. Background levels of SF_6 and CH_4 were measured daily by suspending 3 yokes in the barn. The yokes were replaced every 24 h for 6 days for each animal. Three 20 mL gas subsamples were taken from each yoke with a syringe and injected into 6.8 mL exetainer vials (Labco Ltd, Wycombe, Bucks, UK) for further analysis. During the CH_4 measurement, TMR and orts were collected daily to determine DM content and calculate daily DMI. Ingredients were sampled once for each group and used for chemical analysis.

Laboratory Analyses

Samples of TMR, orts and ingredients were dried in a forced-air oven at 55°C for 72 h to determine DM content. The dried ingredients were ground through a 1-mm screen (Wiley mill; A.H. Thomas, Philadelphia, PA). The ground ingredients were used to determine analytical DM (method 930.15; AOAC, 2005), which was used to correct the chemical analysis to a DM basis. Ash (method 942.05; AOAC, 2005), NDF (heat-stable amylase and sodium sulfite were used; Ankom A200, Ankom Technology, Fairport, NY) and acid detergent fiber (**ADF**; Ankom Technology) were analysed. The ingredients were further ground using a ball grinder (Mixer Mill MM 2000; Retsch, Haan, Germany) and analyzed for starch (Koenig et al., 2013) and total

N concentration using flash combustion and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). The CP content of the ingredients was calculated by multiplying N content by 6.25.

Concentrations of VFA in ruminal fluid were analyzed as described by Romero-Perez et al. (2015) using gas chromatography (model 5890; Hewlett Parkard, Wilmington, DE) with crotonic acid as an internal standard. Ruminal NH_3-N concentration was determined by the salicylate–nitroprusside–hypochlorite method using segmented flow analyzer (Rhine et al., 1998). Ruminal protozoa were enumerated under a light microscope using a counting chamber (Neubauer Improved Bright-Line counting cell, 0.1 mm depth; Hausser Scientific, Horsham, PA) as described by Veira et al. (1983).

The PUN concentrations were determined using micro-segmented flow analysis (model Astoria2; Astoria Pacific Inc., Clackamas, OR). The PUN was used to estimate the quantity of urine N excreted (**UNE**) using the equation of Kohn et al. (2005), UNE = CR × BUN × BW; where **CR** is N clearance rate of kidney in beef cows (1.3 L • d^{-1} • kg^{-1}) and **BUN** is blood urea N. The PUN was presumed to be equivalent to BUN because urea diffuses freely into and out of blood cells.

The CH_4 and SF_6 concentrations were analyzed using gas chromatography as described by McGinn et al. (2006). Standard curves were generated throughout the study using 8 gas standards (1.64 to 248 μmol/mol for CH_4 and 18.3 to 1540 nmol/mol for SF_6 with correlation coefficient exceeding 99.9% for all curves).

Statistical Analyses

All data were analyzed using a mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Animal was the experimental unit for all variables. Normality of distribution and homogeneity of

variance was determined using the Univariate procedure of SAS. For the DMI, weekly data were averaged for each animal in each period. A $\log_{10}$ transformation was applied to the protozoa data before analysis because the data were not normally distributed and inverse $\log_{10}$ of the least square means were reported. The initial and final BW, VFA, rumen pH, ruminal NH_3-N concentration, and protozoa data were analyzed using a model that included treatment as fixed effect and animal as random effect. The DMI, ADG, G:F, PUN, and UNE data were analyzed using a statistical model that included treatment, period and their interaction as fixed effects and the random effect of animal within treatment with period as a repeated measure. For PUN and UNE, the data included their baseline measurements (d 0) as covariates.

The model used to analyze CH_4 production and DMI during gas measurements included fixed effect of treatment with group as random effect and day of sampling (d 1 to 6) within each group as a repeated measure. For all data, covariance structure (autoregressive) that yielded the smallest Akaike and Bayesian information criteria value was used and means were separated at $P < 0.05$ while tendencies were indicated at $0.05 \leq P < 0.10$. The Tukey-Kramer was used to determine significant differences among means.

3.3. Results

The DMI during the growth performance phase averaged 7.51 ± 0.198 kg/d and did not differ ($P = 0.10$) among treatments (Table 3.2). However, there was a treatment × period interaction for DMI ($P < 0.01$); during wk 10 to 12, the DMI of steers fed 1.5% CNQ was greater ($P < 0.05$) than that of steers fed 0.25% CN and 0.25% CNQ (8.66 vs. 7.49 and 7.45 kg/d, respectively), although it did not differ ($P > 0.05$) from that of steers fed control and 1.5% CN diets (8.04 and 8.36 kg/d, respectively). There was a treatment × period interaction ($P = 0.04$) for ADG, whereby during wk 10 to 12, the ADG of steers fed 1.5% CN and 1.5% CNQ was greater

($P < 0.05$) than that of steers fed 0.25% CN (1.075 and 1.085 vs. 0.778 kg/d, respectively). The G:F was greater ($P < 0.05$) for 0.25% CN compared with 0.25% CNQ in wk 4 to 6, while the opposite occurred during wk 10 to 12. Regardless of these treatment differences, the ADG and G:F of animals fed the tannin treatments did not differ ($P > 0.05$) from those of the control in any of the periods and there was no effect ($P \geq 0.80$) of treatment on ADG and G:F when examined over the entire study.

The PUN concentration averaged over the entire study for animals receiving tannin treatments was greater ($P < 0.05$) for 1.5% CN and 1.5% CNQ than those fed 0.25% CNQ (120.9 and 120.4 vs. 111.7 mg/L, respectively), but not different ($P > 0.05$) from animals fed control and 0.25% CN (117.2 and 117.5 mg/L, respectively; Table 3.3). However, the treatment × period interaction ($P < 0.01$) indicated the differences among treatments differed among periods, with differences occurring later in the study. From wk 7 to 9, the PUN of animals fed 1.5% CN was greater ($P < 0.05$) than that of animals fed control, 0.25% CN and 0.25% CNQ (128.9 vs. 110.3 mg/L) but not different ($P > 0.05$) from that of animals fed 1.5% CNQ (119.9 mg/L).

Table 3.2. Effects of hydrolyzable tannin with or without condensed tannin on growth performance of beef steers (n = 15) fed an alfalfa:barley silage (50:50) diet**.**

	Treatment[1]						
	Control	CN		CNQ			
Item[2]		0.25%	1.5%	0.25%	1.5%	SEM	*P*-value
BW, kg							
Initial	292	290	293	293	293	4.1	0.98
Final	352	345	353	353	357	4.1	0.78
DMI, kg/d							
Wk 1 to 3	6.98	6.44	6.96	6.90	6.88	0.220[3]	<0.01[3]
Wk 4 to 6	7.65	7.06	7.54	7.55	7.69		
Wk 7 to 9	7.68	7.33	7.62	7.77	8.12		
Wk 10 to 12	8.04[ab]	7.49[b]	8.36[ab]	7.45[b]	8.66[a]		
Overall	7.59	7.08	7.62	7.41	7.84	0.198[4]	0.10[4]
ADG, kg/d							
Wk 1 to 3	0.551	0.454	0.620	0.635	0.635	0.0822[3]	0.04[3]
Wk 4 to 6	0.875	0.913	0.901	0.755	0.847		
Wk 7 to 9	0.526	0.516	0.329	0.512	0.437		
Wk 10 to 12	0.913[ab]	0.778[b]	1.075[a]	0.959[ab]	1.085[a]		
Overall	0.716	0.665	0.733	0.715	0.751	0.0501[4]	0.80[4]
G:F							
Wk 1 to 3	0.077	0.069	0.089	0.089	0.090	0.0099[3]	0.04[3]
Wk 4 to 6	0.114[ab]	0.127[a]	0.120[ab]	0.099[b]	0.107[ab]		
Wk 7 to 9	0.067	0.070	0.043	0.065	0.055		
Wk 10 to 12	0.111[ab]	0.099[b]	0.125[a]	0.126[a]	0.125[a]		
Overall	0.092	0.092	0.094	0.095	0.094	0.0058[4]	0.99[4]

[a–b]Within a row, means without a common superscript letter differ at $P < 0.05$.
[1]CN, chestnut; CNQ, chestnut and quebracho mix.
[2]Body weight, BW; dry matter intake, DMI; average daily gain, ADG; gain:feed, G:F
[3]The SEM and *P*-values of treatment × period interaction for DMI, ADG, and G:F.
[4]The SEM and *P*-values of the overall effect of treatment for DMI, ADG, and G:F.

These differences decreased over time, such that from wk 10 to 12, the PUN of animals fed 1.5% CN and 0.25% CNQ was actually less ($P < 0.05$) than that of animals fed control (95.2 and 97.4 vs. 110.6 mg/L, respectively) but not different ($P > 0.05$) from those fed 0.25% CN and 1.5% CNQ (101.1 and 105.9 mg/L, respectively). The overall estimated UNE was less ($P < 0.05$) for 1.5% CN, 0.25% CNQ, and 1.5% CNQ compared with the control (51.1, 49.1, 43.0 vs. 54.8 g/d) with 0.25% CN being intermediate (52.1 g/d). The treatment × period interaction ($P = 0.01$) occurred because 1.5% CNQ differed ($P < 0.05$) from control in all periods, 0.25% CNQ differed

($P < 0.05$) from control in all periods except the first, while 1.5% CN differed ($P < 0.05$) from control in wk 7 to 9 only.

Table 3.3. Effects of hydrolyzable tannin with or without condensed tannin on plasma urea N and urine N excretion of beef steers ($n = 15$) fed an alfalfa:barley silage (50:50) diet**.**

	Treatment[1]						
	Control	CN		CNQ			
Item[2]		0.25%	1.5%	0.25%	1.5%	SEM	*P*-value
PUN, mg/L							
Wk 1 to 3	128.6	130.8	132.4	122.9	131.8	4.59[3]	<0.01[3]
Wk 4 to 6	118.8	119.2	126.9	125.3	123.8		
Wk 7 to 9	110.7^{bc}	118.8^{b}	128.9^{a}	101.5^{c}	119.9^{ab}		
Wk 10 to 12	110.6^{a}	101.1^{ab}	95.2^{b}	97.4^{b}	105.9^{ab}		
Overall	117.2^{ab}	117.5^{ab}	120.9^{a}	111.7^{b}	120.4^{a}	2.78[4]	0.047[4]
UNE, g/d[5]							
Wk 1 to 3	50.8^{a}	51.1^{a}	49.8^{a}	51.2^{a}	40.4^{b}	2.09[3]	0.01[3]
Wk 4 to 6	55.0^{a}	49.2^{b}	52.0^{ab}	42.3^{c}	40.1^{c}		
Wk 7 to 9	57.2^{a}	51.3^{b}	47.1^{bc}	51.9^{b}	42.1^{c}		
Wk 10 to 12	56.0^{a}	56.7^{a}	55.6^{a}	51.0a^{b}	49.2^{b}		
Overall	54.8^{a}	52.1^{ab}	51.1^{b}	49.1^{b}	43.0^{c}	1.17[4]	<0.01[4]

[a–c]Within a row, means without a common superscript letter differ at $P < 0.05$.
[1]CN, chestnut; CNQ, chestnut and quebracho mix.
[2]Plasma urea nitrogen, PUN; and urine nitrogen excretion, UNE.
[3]The SEM and *P*-values of treatment × period interaction for PUN and UNE.
[4]The SEM and *P*-values of the overall effect of treatment for PUN and UNE.
[5]Estimated using the BW and PUN measured and the N clearance rate of kidney in beef cattle (1.3 L • d^{-1} • kg^{-1}), using the equation by Kohn et al. (2005).

The ruminal fluid pH averaged 7.3 ± 0.89 and was not affected by tannin addition ($P = 0.35$; Table 3.4). Total ruminal VFA concentration averaged 81.2 ± 7.78 m*M* was also not affected ($P = 0.16$) by adding tannins to the diet. Also, tannin inclusion did not affect the molar proportions of acetate, propionate, and isobutyrate ($P \geq 0.19$) and thus, acetate:propionate ratio was not affected ($P = 0.62$). However, butyrate proportion was greater ($P < 0.05$) for 1.5% CNQ compared with control, 0.25% CN, and 0.25% CNQ (11.29 vs. 10.31, 9.18 and 9.57 mol/100 mol, respectively). Tannin supplementation at 1.5% CN and 1.5% CNQ decreased ($P < 0.05$) valerate and isovalerate proportions compared with control. Ammonia-N concentration was decreased ($P < 0.01$) with the addition of tannin, irrespective of tannin source or dose compared

with the control (mean; 6.64 vs. 11.96 m*M*). Total protozoa numbers in rumen fluid were not affected by tannin source or dose (mean; 4.20× 10^5/mL; $P = 0.14$).

Table 3.4. Effects of hydrolyzable tannin with or without condensed tannin on ruminal fermentation of beef steers ($n = 7$) fed an alfalfa:barley silage (50:50) diet[1].

	Treatment[2]						
	Control	CN		CNQ			
Item		0.25%	1.5%	0.25%	1.5%	SEM	*P*-value
pH	7.2	7.3	7.4	7.4	7.2	0.89	0.35
Total VFA, m*M*	94.7	86.3	69.4	72.5	82.9	7.78	0.16
VFA, mol/100 mol							
Actate (A)	68.5	70.1	69.4	69.5	68.6	0.60	0.31
Propionate (P)	14.1	14.6	14.0	14.7	14.2	0.24	0.19
Butyrate	10.31^{bc}	9.18^{d}	10.69^{ab}	9.57^{cd}	11.29^{a}	0.298	<0.01
Valerate	1.81^{a}	1.54^{ab}	1.36^{b}	1.44^{b}	1.41^{b}	0.109	0.049
Isobutyrate	1.76	1.64	1.64	1.69	1.57	0.065	0.34
Isovalerate	2.64^{a}	2.24^{b}	2.19^{b}	2.48^{ab}	2.18^{b}	0.124	0.049
A:P	4.88	4.82	4.96	4.73	4.83	0.104	0.62
NH_3-N, m*M*	11.96^{a}	8.07^{b}	5.56^{b}	7.16^{b}	5.76^{b}	1.287	<0.01
Protozoa, cells × 10^5/mL [3]	3.17	3.79	4.10	3.18	6.74	0.093	0.14

[a–d]Within a row, means without a common superscript letter differ or tend to differ at $P < 0.05$ or $0.05 \leq P < 0.10$, respectively.
[1]Samples were taken on wk 12 in period 4.
[2]CN, chestnut; CNQ, chestnut and quebracho mix.
[3]Data were $\log_{10}$ transformed before statistical analysis and inverse $\log_{10}$ least squares mean reported herein.

During CH_4 measurement, the DMI averaged 7.27 ± 0.322 kg/d and was not affected ($P = 0.54$) by treatment (Table 3.5). The daily CH_4 produced did not differ ($P = 0.84$) among treatments (154 ± 5.94 g/d). However, adding tannin at 1.5% CNQ tended ($P = 0.094$) to decrease CH_4 yield by 6.4% compared with the control (20.6 vs. 22.0 g/kg DMI).

Table 3.5. Effects of hydrolyzable tannin with or without condensed tannin on intake and methane (CH_4) emission of beef steers ($n = 15$) fed an alfalfa:barley silage (50:50) diet.

	Treatment[1]				
Item	Control	1.5% CN	1.5% CNQ	SEM	*P*-value
DMI, kg/d	7.01	7.27	7.52	0.32	0.54
CH_4, g/d	152	156	155	5.94	0.84
CH_4, g/kg DMI	22.0^{a}	21.7^{ab}	20.6^{b}	0.47	0.094

[a–b]Within a row, means without a common superscript letter tend to differ at $0.05 \leq P < 0.10$.
[1]CN, Chestnut; CNQ, chestnut and quebracho mix.

3.4. Discussion

This study examined the effects of supplementing HT alone or in combination with CT as a means of improving animal performance and N use status, while reducing CH_4 emission of growing beef cattle fed a high forage diet consisting of blend of alfalfa and barley silages. The basal diet was high in CP (17.1%) to maximize animal performance; however, silage diets high in soluble protein increase the amount of N excreted in feces and urine (Dijkstra et al., 2013). As tannins have the ability to bind to proteins, they may decrease soluble protein degradation in the rumen and increase protein flow to the small intestine leading to improved animal performance, if MP supply is limiting. An improvement in animal performance is generally attributed to an increase in MP supply to the small intestine increasing absorption of amino acids to the blood. Using the Empirical Level of Solution of the Beef Cattle Nutrient Requirements Model (NASEM, 2016), MP required was estimated at 447.5 g/d for cattle gaining 1.0 kg/d, while it was estimated that the control diet supplied 553.9 g/d of MP. Thus, the lack of increase in ADG for cattle fed the tannin treatments is consistent with MP supply not being limiting for growth.

The effect of added tannin sources was examined at low (0.25% DM) and high (1.5% DM) concentrations of HT alone or in combination with CT, with CN used as the source of HT and Q as the source of CT. Accounting for the tannin concentrations of the CN and Q, the 0.25% CN, 0.25% CNQ, 1.5% CN and 1.5% CNQ treatments contained 0.19, 0.20 (0.09% HT and 0.11% CT), 1.11, and 1.24% (0.56% HT and 0.68% CT) tannin, respectively. These tannin concentrations are below the concentration of tannin (i.e. < 2% of dietary DM) reported by Jayanegara et al. (2012) that may depress DMI and animal performance. Supplementing

ruminant diets with tannins at high concentration may decrease DMI due to a reduction in palatability and digestibility, which negatively affect ADG of the animal (Waghorn, and Shelton, 1997; Mueller-Harvey, 2006; Waghorn, 2008). In the present study, the overall lack of effect of tannin supplementation on DMI was consistent with the overall lack of effect of treatment on animal performance (ADG and G:F). Beauchemin et al. (2007) also reported no effects of Q extract at 0, 1 and 2% dietary DM on DMI or ADG in growing cattle (initial BW; 223 kg) fed a diet of 70% forage DM. Also, Krueger et al. (2010) showed no difference in DMI, ADG, or G:F when 1.5% of HT extract was added to a 64% corn grain diet (DM basis) compared with a control diet fed to finishing beef steers (initial BW; 414 kg). In contrast, adding a combination of HT and CT at 0.6% CNQ (50:50) dietary DM increased DMI and ADG of feedlot Holstein steers (initial BW; 392 kg) compared with the control, while no effect occurred when the tannins were applied separately (Rivera-Méndez et al., 2016). In the present study the greater DMI intake in period 4 for the high concentration of CNQ compared with the low dosage of CN coincided with greater ADG and improved G:F of cattle fed CNQ during this period. The effect of tannin supplementation of diets on animal performance may depend on the dosage and type of tannin, nutrient composition of the diet and animal nutrient requirements.

Ruminal fluid pH was similar to expectations for cattle fed forage diets (Kiro, 2017). The lack of effect of tannin treatments on pH supports the findings of other studies with tannins fed to cattle or sheep (Krueger et al., 2010; Liu et al., 2011; Aguerre et al., 2016). The lack of treatment effect on total VFA and the main VFA proportions (acetate and propionate) indicated that there were likely no effects of tannins on rumen digestibility of the diet. Similarly, regardless of CP concentration of the diet (15.3 vs. 16.6%), tannin supplemented as CNQ (0, 0.45, 0.9 and 1.8 % dietary DM) did not affect total VFA or proportions of acetate and

propionate in dairy cattle (Aguerre et al., 2016). Beauchemin et al. (2007) reported a linear reduction in total VFA and acetate:propionate ratio with increasing levels of CT tannin from Q extract (0, 1 or 2% dietary DM) added to a high forage diet fed to growing beef cattle. The present study sampled ruminal fluid 3 h after feeding only in wk 12 of the experiment while Beauchemin et al. (2007) sampled 2 h after feeding every 21 days. In the present study the rumen microbiota may have been changed to favour microbes that can hydrolyze tannin but the changes were not marked and therefore, did not show any substantial effect on total VFA and the main VFA proportions. Bacterial richness decreased in Holstein steers fed CNQ mix, but the overall population structure of rumen microbiota was not significantly disturbed by tannins (Carrasco et al., 2017). Thus, at the greater dose of 1.5% CNQ the proportions of butyrate (4-carbon) increased and valerate (5-carbon) decreased relative to the control. Greater hydrolysis of tannins in animals fed the 1.5% CNQ by rumen microbes may have increased the butyrate proportion. Similarly, the butyrate proportion increased on d 42 in the study of Krueger et al. (2010) who fed 1.5% CN or Q to feedlot cattle. Both HT and CT can be hydrolyzed to butyrate through a series of enzymatic action using 3-hydroxy-5-oxohexanoate pathways (Krumholz and Bryant, 1986; Bhat et al., 1998).

Tannins bind to proteins and as such decrease ruminal degradation of protein (Reed, 1995) and consequently decrease urinary N excretion (Carulla et al., 2005; Grainger et al., 2009). In the present study, irrespective of dosage or type, tannin decreased ruminal NH_3-N concentration relative to the control with a 53% decrease for the greater dosage and 36% for the lower dosage. Similarly, other studies with tannin supplementation *in vitro* (Getachew et al., 2008) and *in vivo* (Beauchemin et al., 2007) have shown a reduction in NH_3 concentration. Isovalerate is a branched chain VFA from the deamination of leucine and in the present study its

proportion decreased with a greater dose of tannin. The decrease in isovalerate with a greater dose of tannin may indicate a reduction in ruminal protein degradation which could partly explain the observed decrease in ruminal NH_3-N concentration for the greater dose tannin.

Plasma urea N, which indicates the protein status of the animal, ranged between 95 and 132 mg/L, and was below the value of 150 mg/L reported by Byers and Moxon (1980) for growing beef cattle fed a corn silage diet with dietary CP content of 16.5% DM. However, Johnson and Preston (1995) suggested that an optimal PUN concentration for protein deposition by beef steers is between 60 and 80 mg/L. Surprisingly, there was no overall effect of tannins on PUN relative to the control. Thus, the greater average PUN reported in this study compared with 80 mg/L is an indication of excess dietary protein relative to the protein and energy requirements of the animal. Again, surprisingly, the higher dose of tannin inclusion for CN and CNQ increased PUN compared with the lower level CNQ. Usually as ruminal NH_3-N concentration decreases, PUN concentration decreases as reviewed by Kennedy and Milligan (1980). However, is possible that the decrease in ruminal NH_3-N concentration by tannins especially for the higher dose tannins which was 13 percentage point more than the lower dose CNQ may have shifted protein digestion post-ruminally and consequently increased PUN relative to the lower dose CNQ. Also the decrease in ruminal NH_3-N concentration coupled with an increase in PUN for the higher dose tannin relative to the lower CNQ may partly be due to an increase in the rate of urea transfer through the blood to the rumen to ensure efficient use of N (Kennedy and Milligan, 1978). Both PUN and UNE increase in cattle when they are fed high protein diets (Ruiz et al., 2002; Kohn et al., 2005; Wickersham, 2008). However, estimated UNE, which accounts for both PUN and BW of animals, was decreased for 1.5% CN, 0.25% CNQ and 1.5% CNQ by 6.8%, 10.4% and 21.5% respectively. Also, CNQ at 1.8% or 0.9% vs. 0% DM has been shown to shift

the partitioning of N from urine to feces, thereby decreasing UNE (Aguerre et al., 2016). In the present study feces and urine excretion was not measured. However, the decreased estimated UNE for 1.5% CNQ tannin relative to the control in all periods, combined with a consistent decrease in rumen NH_3-N, suggests that this tannin treatment decreased protein degradation in the rumen leading to decreased absorption of NH_3 from the rumen, and a shift in route of N excretion with proportionately less excreted in urine and more excreted in feces. Hence, for diets containing excess N, feeding a high dose of HT and CT in combination may be beneficial for the environment, because decreasing urinary excretion of N would be expected to decrease the volatilization of N in the form of NH_3-N.

A meta-analysis from 30 *in vivo* experiments showed that decrease in CH_4 production is consistent with tannin concentration greater than 2% but tannin at this dietary concentration can negatively affect DMI (Jayanegara et al., 2012). Thus, CH_4 was not measured at the lower doses of tannins in the present study as a decrease in CH_4 was not expected. Although daily CH_4 produced was not affected by tannin supplementation, CH_4 yield (g/kg DMI) tended to decrease by 6% with supplementation of 1.5% CNQ relative to the control. Other studies have reported a 13% decrease in CH_4 yield for sheep (Carulla et al., 2005) and a 22% decrease for dairy cattle (Grainger et al., 2009) with CT applied at 2.5% and 1.8% of dietary DM, respectively. On the contrary, other studies in beef cattle supplemented with CT at 1.8% (Beauchemin et al., 2007) and 1% (Ebert et al., 2017) dietary DM reported no effect of tannin on CH_4 yield. It has been proposed that tannins decrease CH_4 emissions by directly inhibiting methanogens (Field et al. 1989) and ruminal microbes that produce H_2 and are associated with methanogens such as protozoa (Bhatta et al., 2009; Cieslak et al., 2012), or indirectly by decreasing fiber degradation (Carulla et al., 2005) in the rumen. The lack of effect of tannins on DMI, total VFA

concentration and the major VFA profile in the present study would suggest that fiber degradation may not have been affected. Although the methanogens were not measured, the lack of effect of tannin on the protozoa population indicates that the decrease in CH_4 production for 1.5% CNQ relative to the control was not directly related to protozoa. However, a decrease in CH_4 production is not always concomitant with a decrease in protozoa (Bhatta et al., 2013), as some tannins may decrease methanogens that are not associated with protozoa. The discrepancies between the present study and that of Beauchemin et al. (2007) who did not observe any effect of tannin on CH_4 when beef cattle were fed a high forage diet supplemented with 1.8% CT may be due to the differences in the type of tannin used. *In vitro*, HT combined with CT was more potent in terms of reducing CH_4 compared with CT applied alone with a decrease in the methanogen population when 6 commercial sources of tannins containing HT or CT were compared with control without tannin (Bhatta et al., 2009). Also, Carrasco et al. (2017) reported a decrease in methanogenic archaea in Holstein steers fed a diet supplemented with HT and CT blend from CN and Q extracts respectively. Thus the decrease in CH_4 production by the 1.5% CNQ combination may be due to the greater tannin concentration in this treatment relative to the1.5% CN.

In conclusion, adding CN to supply HT, alone or in combination with Q to supply CT, to a high forage diet (95% DM) at a low (0.25% DM) or greater dose (1.5% DM) decreased ruminal NH_3-N concentration without adversely affecting animal performance of growing beef cattle. Adding HT from CN alone to a high forage diet at a concentration of 1.5% (50:50) dietary DM did not affect CH yield but a combination of HT and CT from CN and Q respectively, at a concentration of 1.5% (50:50) dietary DM (1.24% DM total tannin) tended to decrease CH_4 yield from growing beef cattle, without negative effects on animal performance.

3.5. Literature Cited

Aguerre, M. J., M. C. Capozzolo, P. Lencioni, C. Cabral, and M. A. Wattiaux. 2016. Effect of quebracho-chestnut tannin extracts at 2 dietary crude protein levels on performance, rumen fermentation, and nitrogen partitioning in dairy cows. J. Dairy Sci. 99: 4476–4486. doi: 10.3168/jds.2015-10745.

Aguerre, M. J., B. Duvall, J. M. Powell, P. Vadas, and M. A. Wattiaux. 2015. Effects of dietary tannin extracts levels during a thirteen-week period on lactating cow performance and N use efficiency. J. Anim. Sci. 93, (Suppl. 3) / J. Dairy Sci. 98(Suppl.2):775 (Abstr.).

Beauchemin, K. A., S. M. McGinn, T. F. Martinez, and T. A. McAllister. 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. J. Anim. Sci. 85:1990–1996. doi:10.2527/jas.2006-686.

Bhat, T. K., B. Singh, and O. P. Sharma.1998. Microbial degradation of tannins – a current perspective. Biodegradation 9:343–357. doi:10.1023/A:100839750696.

Bhatta, R., L. Baruah, M. Saravanan, K. P. Suresh, and K. T. Sampath. 2013. Effect of medicinal and aromatic plants on rumen fermentation, protozoa population and methanogenesis in vitro. J. Anim. Physiol. Anim. Nutr. 97:446–456 doi:10.1111/j.1439-0396.2012.01285.x.

Bhatta, R., Y. Uyeno, K. Tajima, A. Takenaka, Y. Yabumoto, I. Nonaka, O. Enishi, and M. Kurihara. 2009. Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. J. Dairy Sci. 92:5512–5522. doi:10.3168/jds.2008-1441.

Byers, F. M., and A. L. Moxon. 1980. Protein and selenium levels for growing and finishing beef cattle. J. Anim. Sci. 50:1136–1144.

Canadian Council on Animal Care (CCAC). 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. Ottawa, ON, Canada.

Carrasco, J. M. D., C. Cabral, L. M. Redondo, N. D. P. Viso, D. Colombatto, M. D. Farber, and M. E. F. Miyakawa. 2017. Impact of chestnut and quebracho tannins on rumen microbiota of bovines. BioMed. Res. Int. 2017:11. doi:10.1155/2017/961081.

Carulla, J. E., M. Kreuzer, A. Machmüller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Austr. J. Agric. Res. 56:961–970.

Cieslak, A., P. Zmora, E. Pers-Kamczyc, and M. Szumacher-Strabel. 2012. Effects of tannins source (*Vaccinium vitis idaea* L.) on rumen microbial fermentation *in vivo*. Anim. Feed Sci. Technol. 176:102–106. doi:10.1016/j.anifeedsci.2012.07.012.

Dijkstra, J., O. Oenema, J. W. van Groenigen, J. W. Spek, A.M. van Vuuren, A. Bannink. 2013. Diet effects on urine composition of cattle and N_2O emissions. Animal 7 (Suppl. 2), 292–302. doi:10.1017/S1751731113000578.

Ebert, P. J., E. A. Bailey, A. L. Shreck, J. S. Jennings, and N. A., Cole. 2017. Effect of condensed tannin extract supplementation on growth performance, nitrogen balance, gas emissions, and energetic losses of beef steers. J. Anim. Sci. 95:1345–1355. doi:10.2527/jas2016.0341.

Field, J. A., S. Kortekaas, and G. Lettinga. 1989. The tannin theory of methanogenic toxicity. Biol. Wastes. 29:241–262.

Getachew, G., W. Pittroff, D. H. Putnam, A. Dandekar, S. Goyal, and E. J. DePeters. 2008. The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on *in*

vitro rumen fermentation and microbial protein synthesis. Anim. Feed Sci. Technol. 140:444–461. doi:10.1016/j.anifeedsci.2007.03.011.

Grainger, C., T. Clarke, M. J. Auldist, K. A. Beauchemin, S. M. McGinn, G. C. Waghorn, and R. J. Eckard. 2009. Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Can. J. Anim. Sci. 89:241–251. doi:10.4141/CJAS08110.

Jayanegara A., F. Leiber, and M. Kreuzer. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments. J. Anim. Physiol. Anim. Nutr. 96:365–375. doi:10.1111/j.1439-0396.2011.01172.x.

Jayanegara, A., G. Goel, H. P. S. Makkar, and K. Becker. 2015: Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol. 209:60–68. doi:10.1111/jpn.12531

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

Johnson, J. W., and R. L. Preston. 1995. Minimizing nitrogen waste by measuring plasma urea-N levels in steers fed different dietary crude protein levels. In: Texas Tech Univ. Res. Rep. T-5-355. Texas Tech Univ., Lubbock, TX. p. 62–63.

Kennedy, P. M., and L. P. Milligan. 1978. Transfer of urea from the blood to the rumen of sheep. Br. J. Nutr. 40:149–154. doi:10.1079/BJN19780105.

Kennedy, P. M., and L. P. Milligan. 1980. The degradation and utilization of endogenous urea the gastrointestinal tract of ruminants: A review. Can. J. Anim. Sci. 60:205–221. doi:10.4141/cjas80-030.

Kiro, R. P. 2017. Assessment of the rumen fluid of a bovine patient. Dairy Vet. Sci. J. 2:555588. doi:10.19080/JDVS.2017.02.555588.

Koenig, K. M., S. M. McGinn, and K. A. Beauchemin. 2013. Ammonia emissions and performance of backgrounding and finishing beef feedlot cattle fed barley-based diets varying in dietary crude protein concentration and rumen degradability. J. Anim. Sci. 91:2278–2294. doi:10.2527/jas.2012–5651.

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

Krueger, W. K., H. Gutierrez-Bañuelos, G. E. Carstens, B. Min, W. Pinchak, R. Gomez, R. C. Anderson, N. K. Krueger, and T. D. A. Forbes. 2010. Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain. Anim. Feed Sci. Technol. 159:1–9. doi:10.1016/j.anifeedsci.2010.05.003.

Krumholz, L. R., and M. P. Bryant.1986. *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. Arch. Microbiol. 144:8–14. doi:10.1007/BF00454948.

Liu, H., V. Vaddella, and D. Zhou. 2011. Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. J. Dairy Sci. 94:6069–6077. doi:10.3168/jds.2011-4508.

MacAdam, J. W., and J. J. Villalba. 2015. Beneficial effects of temperate forage legumes that contain condensed tannins. Agriculture. 5:475–491. doi:10.3390/agriculture5030475.

McGinn, S. M., K. A. Beauchemin, A. D. Iwaasa, and T. A. McAllister. 2006. Assessment of the sulfur hexafluoride (SF_6) tracer technique for measuring enteric methane emissions from cattle. J. Environ. Qual. 35:1686–1691. doi:10.2134/jeq2006.0054.

McLeod, N. M. 1974. Plant tannins - their role in forage quality. Nutr. Abstr. Rev. 44:803–815.

Mueller-Harvey, I. 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86:2010–2037. doi:10.1002/jsfa.2577.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient Requirements of Beef Cattle, Eighth Revised Edition. Washington, DC: The National Academies Press. doi:177226/19014.

Patra, A.K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91:24–37. doi:10.1002/jsfa.4152.

Paz, H. A., C. L. Anderson, M. J. Muller, P. J. Kononoff, and S. C. Fernando. 2016. Rumen bacterial community composition in Holstein and Jersey cows is different under same dietary condition and is not affected by sampling method. Front. Microbiol. 7:1206. doi:10.3389/fmicb.2016.01206.

Reed, J. D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73:1516–1528.

Rhine, E. D., G. K. Sims, R. L. Mulvaney, and E. J. Pratt. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Sci. Soc. Am. J. 62:473–480. doi:10.2136/sssaj1998.03615995006200020026x.

Rivera-Méndez, C., A. Plascencia, N. Torrentera, and R. A. Zinn. 2016. Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. J. Appl. Anim. Res. 45:199–203. doi:10.1080/09712119.2016.1141776.

Romero-Perez, A., E. K. Okine, S. M. McGinn, L. L. Guan, M. Oba, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2015. Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. J. Anim. Sci. 93:1780–1791. doi:10.2527/jas.2014-8726.

Ruiz, R., L. O. Tedeschi, J. C. Marini, D. G. Fox, A. N. Pell, G. Jarvis, and J. B. Russell. 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: Evaluation of the Cornell Net Carbohydrate and Protein System ruminal N deficiency adjustment. J. Dairy Sci. 85:2986–2999. doi:10.3168/jds.S0022-0302(02)74384-1.

Veira, D.M., M. Ivan, and P.Y. Jui. 1983. Rumen ciliate protozoa: effects on digestion in the stomach of sheep J. Dairy Sci. 66:1015–1022. doi:10.3168/jds.S0022-0302(83)81896-7.

Waghorn, G. C. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production – progress and challenges. Anim. Feed Sci. Technol. 147:116–139. doi:10.1016/j.anifeedsci.2007.09.013.

Waghorn, G. C., and I. D. Shelton. 1997. Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep. J. Agric. Sci. Camb.128:365–372.

Wang, Y., G. C. Waghorn, W. C. McNabb, T. N. Barry, M. J. Hedley, and I. D. Shelton. 1996. Effect of condensed tannins in *Lotus corniculatus* upon the digestion of methionine and cysteine in the small intestine of sheep. J. Agric. Sci. Camb. 127, 413–421. doi:10.1017/S0021859600078576.

Wickersham, E. E., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and D. P. Gnad. 2008. Effect of rumen degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low quality forage. J. Anim. Sci. 86:3079–3088. doi:10.2527/jas.2007-0326.

CHAPTER 4- Use of gallic acid and hydrolyzable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage[3]

4.1. Introduction

There is growing concern that livestock production contributes significantly to anthropogenic GHG emissions, mainly due to CH_4 and N_2O from manure. Globally, enteric fermentation from ruminants contributes about 39% of the livestock sector GHG emissions (Gerber et al., 2013). In addition to contributing to GHG emissions, ruminant animals utilize dietary N with low efficiency with only 10 to 40% of consumed N retained in products (Calsamiglia et al., 2010). The high loss of N in feces and urine leads to an increase in NH_3-N and N_2O emissions from manure (Dijkstra et al., 2013). Eliminating livestock production and adoption of a vegan diet is often promoted as a means of reducing environment impact (Veeramani et al., 2017). However, a recent modeling study of US agriculture without animal production showed that adoption of a plant-derived diet decreased CH_4 emission but the food supply did not support the population's nutritional requirements (White and Hall, 2017).

The negative environmental impacts of ruminant livestock production have stimulated interest in finding suitable mitigation strategies. One such possible mitigation approach is incorporation of tannins in the diets consumed by ruminants. Hydrolyzable tannin and CT are secondary compounds in plants with the ability to form complexes with protein and carbohydrate fractions through hydrogen bonds. Tannins have been shown to improve N utilization and decrease CH_4 production from ruminants (Patra and Saxena, 2011). The inhibitory effects of tannins on CH_4 production have been suggested to result from direct effects on methanogens,

[3]A version of this chapter is in press. Aboagye, I. A., M. Oba, K. M. Koenig, G. Y. Zhao, and K. A. Beauchemin. 2019. Use of gallic acid and hydrolyzable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage. Journal of Animal Science

indirect effects on protozoal-associated CH_4 production, and reduction of fiber digestion (Patra and Saxena, 2011). However, most of the research on feeding tannins to ruminants has focused on CT rather than HT because it is assumed that HT would have negative effects on OM digestibility and animal performance (Beauchemin et al., 2008).

Unlike CT, HT are complexes of low molecular weight (500-3000 Da) formed from a monosaccharide (glucose or glucitol) at the central core, that is partially or totally esterified with GA or ellagic acid (Patra and Saxena, 2011). Thus, HT is either a gallotannin (i.e. glucose core surrounded by several GA units, with more GA attached through depside bonding of additional galloyl residues) or ellagitannin (sugar core often a glucose unit surrounded by hexahydroxydiphenic acid formed from oxidative coupling of galloyl groups; Hagerman, 2011). However, the effects of tannins on microbes and CH_4 emissions may depend on the unit structure (McAllister et al., 2005; Tavendale et al., 2005). An *in vitro* study showed that HT did not decrease OM degradability and was more effective in reducing enteric CH_4 emission than CT (Jayanegara et al., 2010). Tannic acid, a HT with about 10 molecules of GA, decreased CH_4 production of beef cattle by 11.1, 14.7 and 33.6%, respectively, when applied at 0.65, 1.3 and 2.6% of dietary DM to a 50:50 forage:concentrate diet (Yang et al., 2016).

Hydrolyzable tannins can bind to microbes thereby affecting their function and to proteins decreasing their degradation in the rumen and consequently altering N excretion. For example, feeding GA to beef cattle altered the pattern of N excretion by increasing the ratio of fecal N:urinary N and decreasing the ratio of urinary urea N:urinary N (Wei et al., 2016). Also, HT extract from CN applied at 1 to 3% dietary DM in sheep (Liu et al., 2011) or combined with Q (CT extract) at 1.5% dietary DM in beef cattle (Aboagye et al., 2018) lowered ruminal NH_3 concentration and CH_4 production. Thus, differences in unit structure and molecular weight of

HT may affect rumen microbes differently and consequently CH_4 production and N excretion differently. However, little is known about the effect of the different sources (TA or CN) and components of HT (GA) on diet digestibility, CH_4 production and N excretion.

Alfalfa, which does not contain tannin, is widely used as a source of forage for ruminants, particularly grazing cattle and dairy cows (Berard et al., 2011). Alfalfa has high CP soluble protein contents such that its dietary inclusion can elevate ruminal NH_3-N concentration and excretion of excess N into the environment. Feeding a source of HT to ruminants fed alfalfa-based diets may decrease methanogenesis and N excretion without negatively affecting feed digestibility. Thus, we hypothesized that feeding HT or a component of HT to cattle fed a high protein diet based on alfalfa silage would decrease both urinary N excretion and enteric CH_4 production, and that the response to HT would depend on the source of HT or its sub-unit. Therefore, the objective of this study was to determine the effects of different forms of HT on CH_4 production, N utilization, diet digestibility, protozoal populations, ruminal fermentation, and blood metabolite profile in beef cattle fed a high-protein diet mainly containing alfalfa silage.

4.2. Materials and Methods

The Animal Care Committee of the LeRDC reviewed the experimental protocol (ACC 1633) and throughout the experiment the animals were cared for according to the guidelines of the Canadian Council on Animal Care (2009).

Alfalfa Silage Preparation, Animals, Diets and Experimental Design

Alfalfa (*Medicago sativa L.*) was from a stand grown in Lethbridge County, Alberta, Canada and harvested at 9% bloom as second-cut on 04 Aug. 2016. The fresh forage was wilted to 34% DM and the windrows were raked (FELLA, TS880; Jackson, MN) and chopped to a 10-mm theoretical length (John Deere 6810; John Deere, Moline, IL) into a truck. An inoculant (11

GFT; Pioneer Hi-Bred Ltd., Chathan, ON, Canada) was applied at the manufacturer's recommended rate of 1 g/T fresh forage during chopping. Loaded trucks delivered the chopped forages to the LeRDC (15 km from the harvested site) where they were packed in a horizontal plastic silo bag (Hyplast; RKW Klerks Inc., St. Lenaartseweg 26, 2320 Hoogstraten, Belgium) with a silage bagger (Ag-bagger 7000; Ag-Bag, St. Nazianz, WI). After 60 d of ensiling the bag was opened and the alfalfa silage was used to formulate a basal diet consisting of 75% alfalfa silage, 20% barley silage (from a bunker silo at LeRDC) and 5% supplement containing minerals and vitamins to meet or exceed the nutrients requirement of beef cattle gaining 1 kg/d (NASEM, 2016; Table 4.1). The diet was formulated to reflect a high-protein forage diet such as lush pasture for grazing animals.

Eight ruminally cannulated beef heifers with an initial BW of 480 ± 29.2 kg (mean ± SD) were used in a double 4 × 4 Latin square experiment. Before the start of the experiment, the animals were adapted to the basal (control) diet for 14 d. The experiment consisted of four 28-d periods (14 d adaptation, 14 d measurement) and a 7-d washout between periods during which all animals were fed the basal diet. The heifers were housed in individual tie-stalls fitted with rubber mats and bedded with wood shavings and they were permitted access to a group exercise pen for 1 h daily (except during measurements). The BW of heifers was measured at the beginning of the experiment, and before and after digestibility and CH_4 measurements. The heifers were assigned to 2 groups (4 animals per group) based on their initial BW and the 4 periods were staggered by 1 wk between groups 1 and 2 to facilitate measurements.

Table 4.1. Feed ingredients and chemical composition of the dietary treatments.

	Treatment[1]			
Item	Control	GA	TA	CN
Ingredients, % DM				
Alfalfa silage[2]	75.0	75.0	75.0	75.0
Barley silage[3]	20.0	18.5	18.5	18.0
Supplement[4]	5.0	5.0	5.0	5.0
Barley ground	4.9	4.9	4.9	4.9
Salt (sodium chloride)	0.05	0.05	0.05	0.05
Vitamin and mineral premix[5]	0.05	0.05	0.05	0.05
Gallic acid[6]	–	1.5	–	–
Tannic acid[6]	–	–	1.5	–
Chestnut[6]	–	–	–	2.0
Chemical composition[7], % of DM				
DM (as is)	34.4 ± 0.13	34.4 ± 0.15	34.3 ± 0.19	34.4 ± 0.29
OM	85.1 ± 1.14	85.1 ± 0.92	85.2 ± 1.08	84.8 ± 1.04
CP	19.8 ± 0.79	19.7 ± 1.00	20.2 ± 0.30	19.3 ± 0.90
NDF	42.2 ± 0.94	41.7 ± 0.70	42.1 ± 0.57	41.3 ± 0.81
ADF	33.1 ± 0.52	32.4 ± 1.03	32.2 ± 0.91	32.6 ± 1.45
Starch	5.9 ± 1.41	5.4 ± 1.66	4.3 ± 1.13	5.9 ± 2.25
GE, Mcal/kg DM	5.2 ± 0.07	5.2 ± 0.12	5.2 ± 0.16	5.3 ± 0.18

[1]GA, gallic acid; TA, tannic acid; CN, chestnut.
[2]Contained 31.4 ± 0.19% DM, 80.6 ± 2.35% OM, 21.6 ± 0.93% CP (soluble CP, 25.2% CP), 43.7± 1.35 % NDF, and 35.0 ± 1.24% ADF on a DM basis using pooled samples from each period during digestibility measurement (mean ± SD; n = 8).
[3]Contained 37.0 ± 0.40% DM, 91.7 ± 1.00% OM, 13.2 ± 0.61% CP, 46.4 ± 1.45% NDF, 25.5 ± 1.07% ADF, and 17.8 ± 5.57% starch on a DM basis using pooled samples from each period during digestibility measurement (mean ± SD; n = 8).
[4]Contained 90.1 ± 0.51% DM, 96.2 ± 0.83% OM, 10.8 ± 0.23% CP, 19.7 ± 3.39% NDF, 6.13 ± 1.19% ADF, and 59.9 ± 4.44% starch on a DM basis using samples from each period during digestibility measurement (mean ± SD; n = 8); provided as mash.
[5]Contained 35.01% $CaCO_3$, 10.37% $CuSO_4$, 28.23% $ZnSO_4$, 0.15% ethylenediamine dihydriodide (80% concentration), 5.01% Se (10,000 mg Se/kg), 0.1% $CoSO_4$, 14.54% $MnSO_4$, 1.71% vitamin A (500,000,000 IU/kg), 0.17% vitamin D (500,000,000 IU/kg), and 4.7% vitamin E (500,000 IU/kg).
[6]Gallic acid (99% GA; *Rhus chinensis*; J & K Scientific Ltd., Beijing, China); tannic acid (95% TA; J & K Scientific Ltd., Beijing, China); chestnut (74% tannin; *Castanea sativa*; Tannin Sevnica, Hermanova 1, 8290 Sevnica, Slovenia); all in powdered forms; CN was applied such that 2% CN and 1.5% TA added to the diet both supplied 1.43% HT.
[7]Determined using samples pooled by diet for each period during digestibility measurement (mean ± SD; n=8).

Each heifer received a unique sequence of 4 dietary treatments over time. The dietary treatments were: control (basal diet, no tannin), GA (1.5% of diet DM; 99% GA; extracted from *Rhus chinensis* mill; J & K Scientific Ltd., Beijing, China), TA (1.5% of diet DM; 95% TA; J & K Scientific Ltd., Beijing, China) and CN (2% of diet DM; 74% HT; extracted from *Castanea*

sativa; Tanin Sevnica, Hermanova 1, 8290 Sevnica, Slovenia). The GA, TA and CN were substituted for barley silage in the diet. They were mixed into the diet in powdered form using a feed mixer (Data Ranger; American Calan Inc., Northwood, NH), and the diets were fed as TMR. Chestnut was applied on a HT equivalent basis such that the 2% CN and 1.5% TA added to the diet both supplied 1.43% HT. The level of 1.5% GA or 1.5% TA was chosen based on 2 dose studies in beef cattle, where a maximum concentration of GA (2.1%; Wei et al., 2016) or TA (2.6%; Yang et al., 2016) added to the dietary DM did not cause toxicity in the beef cattle.

At the start of each period the heifers were gradually adapted to the experimental diets. Animals fed GA or TA received a diet containing 0.75% GA or 0.75% TA, respectively, for 5 d of the adaptation phase before stepping up to 1.5% for the remainder of the period. Animals fed CN received a diet with 0.75% CN for 5 d, 1.5% CN for the next 5 d, and 2% CN for the rest of the period. The heifers were fed once daily at 1030 h and had free access to water. Animals were fed for ad libitum intake during the adaptation phase, and 95% of their ad libitum intake during the measurement phase to minimize feed sorting.

Feed Sampling

Diets offered and orts (when available) were weighed daily for individual animals. During the digestibility and methane measurements DMI was calculated using the DM contents of the dietary treatments and ort samples (if any). Orts were sampled, composited for each animal to provide representative samples corresponding to the digestibility and CH_4 measurements. Samples were stored at –20°C until analyzed for DM and chemical composition including: OM, NDF, ADF, CP, and gross energy (**GE**). Sampling of the dietary treatments and feed ingredients (alfalfa silage, barley silage and supplement) was done weekly to monitor DM content and where DM content of the silages varied by more than 3%, an adjustment in diet

composition was made. A subsample of the ingredients was composited by period and stored at –20°C until analyzed for chemical composition. Daily dried samples of dietary treatments were pooled by period and stored for chemical analysis to provide representative samples corresponding to the digestibility and CH_4 measurements.

Rumen Fermentation and Plasma Urea Nitrogen Measurements

Ruminal contents (1 L of fluid and solids) were collected from multiple sites in the rumen on d 15 and d 25 of each period before feeding (0 h), and at 3 h intervals after feeding for 12 h (i.e., 3, 6, 9, and 12 h). The ruminal contents were sieved through a polyester screen (355-µm pore size; B & S H Thompson, Ville Mont-Royal, QC, Canada) and retained for analysis of VFA, NH_3-N concentration, and protozoa enumeration. For VFA determination 5 mL of the filtered ruminal fluid was added to 1 mL of 25% meta-phosphoric acid (wt/vol) and for NH_3-N determination another 5 mL was added to 1 mL of 1% sulfuric acid (vol/vol). The collected samples were immediately frozen with liquid N and stored at -80ºC until analyzed. For protozoa enumeration, 5 mL of the filtrate from the 0, 6, and 12 h samples was mixed with 5 mL of methyl green-formalin-saline solution and stored in the dark at room temperature until analyzed.

On d 15 and 25, as rumen contents were sampled, dissolved hydrogen (**dH_2**) concentration was measured (0, 3, 6, 9, and 12 h) using a polarized H_2-sensor (1000 mV; H_2-500; Unisense, Aarhus, Denmark) connected to a glass flow-through cell (2 mm internal diameter, 6 mm external diameter) as described by Guyader et al. (2017). Briefly, the H_2-sensor was connected to a micro-sensor multi-meter (Unisense), which was controlled by the Unisense logger computer software (SensorTrace Suite; Version 2.5.0) recording dH_2 concentration every second. The sensor was standardized each day before the first measurement using a 2-point calibration curve (0 and 592.7 µ*M*), which was created using water without and with H_2 gas

bubbling (80% H_2 and 20% CO_2 gas mixture). For standardization, the flow cell was connected through a closed system using H_2-impermeable chemical tubing (Masterflex Tygon®; Cole-Parmer Instrument Co., Vermon Hills, IL), beginning and ending in an Erlenmeyer flask filled with water and kept in a 39°C-water bath. The water in the flask circulated in the system via a peristaltic pump (Model 1001, Medical Technology Products, Inc., Huntington Station, NY). At each time-point of sampling, a 15 cm long polyvinyl chloride pipe (18 mm internal diameter and 20 mm external diameter) with closed ends and a 2.5 cm cut on the side (covered with a mesh) was connected at one end to H_2-impermeable chemical tubing (Masterflex Tygon®; Cole-Parmer Instrument Co., Vermon Hills, IL) and inserted into the rumen. A 40-mL syringe was used to sample the ruminal fluid (40 mL) and injected directly into the flow cell of the H_2-sensor for dH_2 concentration.

Starting on d 18, pH data loggers (LeRDC pH data logger system, Dascor, Escondido, CA; Penner et al., 2006) were placed in the ventral sac of the rumen. The ruminal pH was recorded every min continuously for 6 d, coinciding with digestibility measurements. The loggers were standardized in buffers pH 4 and 7 at the start and end of each measurement.

Blood samples were collected from heifers on d 15 and 25 at 0 and 6 h after feeding. Blood samples were taken from the jugular vein into sterile evacuated tubes containing an anticoagulant (10 mL, lithium heparin, Vacutainer, Becton Dickinson, Oakville, ON). The blood was centrifuged at 3000 × g and 4°C for 20 min to obtain plasma and stored at -20°C until analysis of PUN concentration.

Digestibility and Nitrogen Excretion

On d 18, heifers were housed in metabolism stalls (without bedding) for 4 days (d 18 to 21) for apparent total-tract digestibility and N excretion determination. The animals were fitted

with urinary indwelling balloon catheters (Bardex Lubricath Foley catheter, balloon size: 75 cm^3, catheter diameter: 8.7 mm; Bard Canada Inc., Oakville, ON, Canada) to enable separate collection of urine and feces. Urine was collected into a container containing 4 *N* H_2SO_4 to ensure the pH < 2 to prevent microbial activity and volatilization of NH_3. Total feces and urine were collected and weighed daily. A subsample (1 kg) of the daily fecal output was dried at 55°C in an oven for 72 h to determine the DM content. A composite sample of dried feces for each animal within period was obtained by pooling daily samples based on DM contents and these were stored at an ambient temperature until ground and analyzed for OM, NDF, ADF, CP, and GE. Total urine output from each animal was measured daily and samples of diluted (15 mL to 60 mL of deionized water) urine were stored frozen (-20 °C). The diluted urine was composited (10 mL) by animal within period based on daily output until analyzed for total N, urea, allantoin and uric acid.

Methane Emission Measurement

Near the end of each period (d 26 to 28), heifers were moved to 4 environmentally controlled chambers to obtain 72 h of continuous CH_4 measurement. Prior to starting the experiment the animals had been adapted to the chambers to minimize stress. The dimensions of the chambers were 4.4 m wide × 3.7 m deep × 3.9 m tall (C1330, Conviron Inc., Winnipeg, MB) and the methodology used for CH_4 measurement was previously described by Beauchemin and McGinn (2006). Briefly, CH_4 concentrations in the intake and exhaust ducts were measured in succession (3 or 4 min from the intake or from the exhaust ducts per chamber) using a CH_4 analyzer (model Ultramat 5E; Siemens Inc., Karlsruhe, Germany). For each chamber, intake and exhaust airflow was monitored (FE-1500-FX-12; Paragon Controls Inc., Santa Rosa, CA) and airflow CH_4 concentration was sampled every 30 min (i.e., 27 min plus 3 min of zero reference

gas measurement using pure N gas). The difference between the incoming and outgoing mass and air flow of CH_4 was used to calculate the amount generated in each chamber.

Before and after the experiment the chambers were calibrated by releasing a known quantity of CH_4 into each chamber and the recovered amount (i.e., to adjust each chamber to 100% recovery) were then used to correct chamber CH_4 emission data from the experiment. The chambers were opened daily for feeding and cleaning and corresponding CH_4 fluxes were removed from the analysis. As the time required for gas concentration to reach steady-state was 5 min, the interruptions from daily feeding and cleaning had limited impact on emissions.

Laboratory Analyses

Samples of TMR, ingredients, orts and feces were dried in a forced-air oven at 55°C for 72 h to determine DM content. The dried samples were ground through a 1-mm screen (Wiley mill; A.H. Thomas, Philadelphia, PA) and duplicate samples were used to determine analytical DM (method 930.15; AOAC, 2005), which was used to correct the chemical analysis to a DM basis. Ash (OM = 100 – ash; method 942.05; AOAC, 2005), NDF (with heat-stable amylase and sodium sulfite; Ankom A200, Ankom Technology, Fairport, NY) and ADF (Ankom Technology) were analyzed. Gross energy content was determined using a bomb calorimeter (model E2k; CAL2k, Johannesburg, South Africa). The 1-mm size samples were further ground using a ball grinder (Mixer Mill MM 2000; Retsch, Haan, Germany) and analyzed for total N (CP = N × 6.25) concentration using flash combustion and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). Total urinary N was analyzed the same way using freeze dried urine samples. The ball ground samples of the ingredients were analyzed for starch content (Koenig et al., 2013).

Urea N concentrations in the blood and urine were determined using micro-segmented flow analysis (model Astoria2; Astoria Pacific Inc., Clackamas, OR). Uric acid N was determined using uric acid standard (5 mg/dL), reagent and control set obtained from the manufacturer (Pointe Scientific Inc., Canton, MI). Briefly, 1 mL of uric acid reagent (U7581; Pointe Scientific Inc., Canton, MI) was added to 25 µl each of the following: urine sample, uric acid standard (U7580; Pointe Scientific Inc., Canton, MI) and control set (Levels 1 and II; P7582-CTL; Pointe Scientific Inc., Canton, MI). After mixing completely, the mixture was kept at 37ºC for 10 min and the absorbance of the mixture (duplicate of 200 µL) was read at a wavelength of 520 nm (Thermo Scientific Appliskan; Thermo Fisher Scientific, Vantaa, Finland).

Concentrations of VFA in ruminal fluid were analyzed using gas chromatography (model 5890; Hewlett Parkard, Wilmington, DE) with crotonic acid as an internal standard. Ruminal NH_3-N concentration was determined by the salicylate–nitroprusside–hypochlorite method using segmented flow analyzer (Rhine et al., 1998). Ruminal protozoa were enumerated under a light microscope using a counting chamber (Neubauer Improved Bright-Line counting cell, 0.1 mm depth; Hausser Scientific, Horsham, PA) as described by Veira et al. (1983).

Statistical Analyses

All data were analyzed using a mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Animal was the experimental unit for all variables. The 6 d of continuous ruminal pH data were summarized by day for mean, minimum, maximum and range values. Data for ruminal fermentation variables, protozoa and blood samples were averaged across days for time-point and animal within period before analysis (i.e. for VFA and dH_2 at 0, 3, 6, 9, and 12 h; protozoa at 0, 6, and 12 h; NH_3-N and PUN at 0 and 6 h). Data for the apparent digestibility and N excretion

were averaged for each animal for each period before analysis. Daily CH_4 production was determined for each animal within period and CH_4 yield was calculated as CH_4 production expressed relative to DMI, GE and DE intakes using daily intakes when animals were in the chambers. Therefore, the fixed effects in the model included treatment, interval (time-point or day) and treatment × interval interaction with time-point interval as a repeated measure to analyze ruminal fermentation variables, protozoa and blood samples data, and day interval as repeated measure for CH_4 production data, respectively. However, the fixed effect in the model used to analyze data for apparent digestibility and N excretion was treatment. All data were analyzed using the random effects of group, animal nested within group and period nested within group.

Normality of distribution and homogeneity of variance was determined using the Univariate procedure of SAS. For normality of the protozoa data, a $\log_{10}$ transformation was applied and an inverse $\log_{10}$ of the least square means were reported. For all data, covariance structure (autoregressive) that yielded the smallest Akaike and Bayesian information criteria value was used and means were separated at $P < 0.05$ while tendencies were indicated at $0.05 \leq P < 0.10$. Least square differences were used to determine significant differences among means.

4.3. Results

Nutrient Intakes and Apparent Nutrient Digestibilities

The restricted DMI during the apparent digestibility and N excretion measurements averaged 10.79 ± 1.076 kg/d and did not differ ($P = 0.23$) among treatments (Table 4.2). Organic matter, CP, NDF, ADF, and GE content were comparable across dietary treatments. Therefore, nutrient intakes were not affected by treatment (mean ± SEM; 9.16 ± 0.878, 2.13 ± 0.219, 4.53 ± 0.430, 3.53 ± 0.378 kg/d, and 56.72 ± 5.646 Mcal/d, respectively; $P \geq 0.22$). Similarly, apparent

DM digestibility was not affected by treatment (60.3 ± 0.86%; $P = 0.20$) and apparent OM, NDF, ADF, and GE digestibilities also did not differ among treatments (64.0 ± 0.92%, 45.7 ± 1.48%, 39.0 ± 3.20%, 59.6 ± 1.46%, respectively; $P \geq 0.13$). However, apparent CP digestibility decreased for TA and CN treatments compared with the control and GA treatments (63.1 vs. 69.0%; $P < 0.001$), with no difference ($P = 0.26$) between control and GA.

Table 4.2. Effects of different forms of hydrolyzable tannin on nutrient intake and apparent digestibility of heifers (n = 8) fed an alfalfa silage-based high forage diet.

	Treatment[1]					
Item[2]	Control	GA	TA	CN	SEM	*P*-value
Intake						
DM, kg/d	10.55	10.58	11.19	10.82	1.076	0.23
OM, kg/d	8.95	8.98	9.54	9.18	0.878	0.22
CP, kg/d	2.09	2.10	2.20	2.12	0.219	0.55
NDF, kg/d	4.46	4.44	4.73	4.48	0.430	0.63
ADF, kg/d	3.50	3.44	3.62	3.55	0.378	0.63
GE, Mcal/d	54.98	55.67	58.83	57.39	5.646	0.23
Digestibility, %						
DM	61.6	60.1	58.9	60.0	0.86	0.20
OM	65.2	64.7	62.7	63.5	0.92	0.13
CP	69.6[a]	68.3[a]	62.2[b]	64.0[b]	0.87	<0.001
NDF	48.3	45.8	44.2	44.3	1.48	0.20
ADF	40.3	38.1	37.5	40.1	3.20	0.77
GE	60.6	60.2	57.1	60.4	1.46	0.24

[a–b]Within a row, means without a common superscript letter differ at $P < 0.05$.
[1]GA, gallic acid; TA, tannic acid; CN, chestnut.
[2]Nutrient intakes and apparent total tract digestibility at 95% ad libitum intake

Ruminal Fermentation and Plasma Urea Nitrogen Measurements

Ruminal pH in terms of minimum, mean, maximum, and range values did not differ among treatments (6.21 ± 0.069, 6.66 ± 0.042, 7.07 ± 0.057, 0.87 ± 0.078, respectively; $P \geq 0.13$; Table 4.3). Also, ruminal pH variables did not differ among days and there was no treatment × day interaction ($P \geq 0.22$). Total VFA differed among treatments at specific time-points ($P =$

0.048), with an increase for the different forms of HT compared with control at 3 h after feeding (124 vs. 101 ± 5.98 m*M*; $P < 0.01$; Figure 4.1). The increase in total VFA remained at 9 h after feeding for GA compared with the control, TA and CN (171 vs. 153 m*M*; $P \leq 0.04$). However, at 12 h after feeding, total VFA did not differ among treatments (153 ± 5.98 m*M*; $P \geq 0.96$). Consequently, over all time points, total VFA tended ($P = 0.089$) to increase for GA compared with the control and TA (134 vs. 125 and 126 m*M*) with CN not different from the other treatments (129 m*M*). The proportion of isobutyrate tended ($P = 0.06$) to decrease for tannin treatments compared with control (1.73 vs. 1.84 mol/100 mol) but treatment or treatment × time interaction effects were not observed for the molar proportions of acetate, propionate, butyrate, valerate, and isovalerate and acetate:propionate ratio ($P \geq 0.13$). However, sampling time affected ($P \leq 0.01$) the molar proportion of VFA, whereby acetate proportion and acetate:propionate ratio decreased after feeding while the proportions of propionate, butyrate, valerate, isobutyrate, and isovalerate, increased after feeding.

The dH2 concentration was not affected by treatment or treatment × time (32.7 ± 4.24 μM; $P \geq 0.65$). Also, total protozoa numbers in rumen contents were not affected by treatment (mean; 4.63 × 105/mL; $P = 0.59$). The ruminal NH3-N concentration tendered to decrease in animals fed TA compared with control (11.8 vs. 14.4 mM; $P = 0.05$) with GA and CN not different from the other treatments (13.0 and 12.7 mM, respectively). However, PUN was reduced for GA, TA, and CN treatments compared with the control (196 vs. 213 mg/L; $P = 0.02$).

Figure 4.1. Mean daily pattern of total volatile fatty concentration (m*M*) averaged over two d (d 15 and 25) for heifers (n = 8) fed an alfalfa silage-based high forage diet. Error bars indicate the SEM. Treatment significance for each time point is indicated by the ns = not-significant, * = $P < 0.05$, and ** = $P < 0.01$.

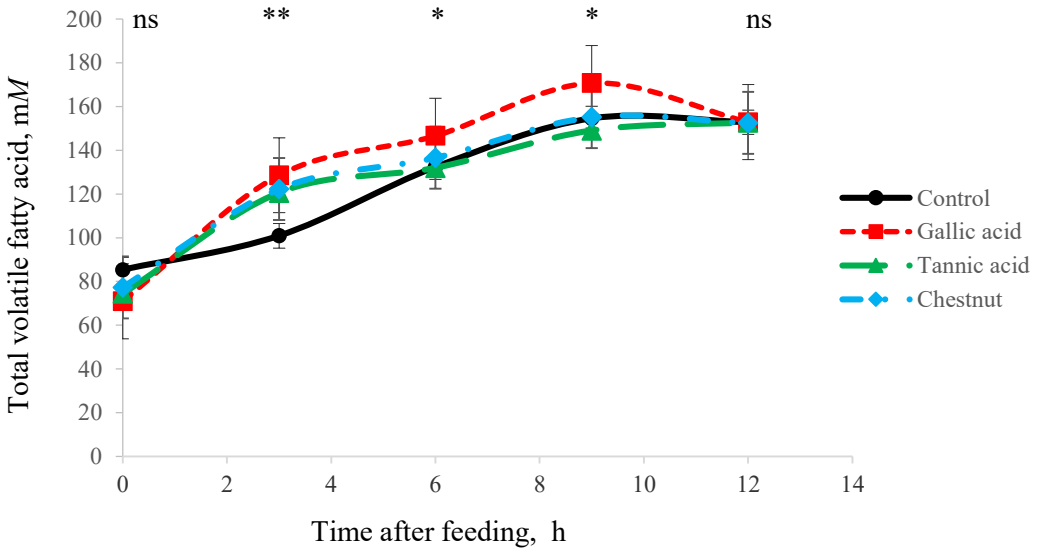


Table 4.3. Effects of different forms of hydrolyzable tannin on ruminal fermentation, protozoa enumeration and plasma urea N concentration of heifers (n = 8) fed an alfalfa silage-based high forage diet.

	Treatment[1]					*P*-value[2]		
Item	Control	GA	TA	CN	SEM	Trt	I	Trt × I
Ruminal pH[3]								
Minimum	6.26	6.21	6.16	6.19	0.069	0.23	0.55	0.42
Mean	6.74	6.66	6.60	6.64	0.042	0.13	0.22	0.70
Maximum	7.14	7.08	7.01	7.05	0.057	0.19	0.65	0.99
Range	0.88	0.87	0.85	0.86	0.078	0.96	0.39	0.70
Total VFA[4], m*M*	125^{y}	134^{x}	126^{y}	129^{xy}	3.3	0.089	<0.001	0.048
VFA[4], mol/100 mol								
Acetate	69.0	69.7	69.8	69.1	0.47	0.13	<0.001	0.21
Propionate	14.5	14.1	14.1	14.5	0.46	0.39	0.01	0.84
Butyrate	9.75	9.68	9.62	9.75	0.68	0.96	<0.001	0.99
Valerate	1.83	1.76	1.78	1.84	0.068	0.58	<0.001	0.29
Isobutyrate	1.84^{x}	1.73^{y}	1.72^{y}	1.74^{y}	0.044	0.06	<0.001	0.13
Isovalerate	2.41	2.29	2.23	2.28	0.084	0.23	<0.001	0.31
Acetate:Propionate ratio	4.82	5.03	4.98	4.83	0.15	0.13	<0.001	0.32
dH_2[4], µ*M*	34.4	31.0	31.4	34.1	4.24	0.67	<0.001	0.65
Protozoa[5], cells × 10^5/mL	5.23	4.08	4.68	4.51	0.07	0.59	0.45	0.91

NH_3-N[6], m*M*	14.4^{x}	13.0^{xy}	11.8^{y}	12.7^{xy}	0.80	0.05	<0.001	0.51
PUN[6], mg/L	213^{a}	195^{b}	195^{b}	198^{b}	14.6	0.02	<0.001	0.76

$^{a–b, x–y}$Within a row, means without a common superscript letter differ or tend to differ at $P < 0.05$ or $0.05 \leq P < 0.10$, respectively.
[1]GA, gallic acid; TA, tannic acid; CN, chestnut.
[2]Trt, treatment; I, interval (day or time); Trt × I, treatment × interval interaction.
[3]Determined for 6 d during digestibility measurements; range = maximum ruminal pH – minimum ruminal pH.
[4]Total VFA, total volatile fatty acids; dH_2, dissolved hydrogen; determined at 0, 3, 6, 9, and 12 h using average values of d 15 and 25 samples.
[5]Data were $\log_{10}$ transformed before statistical analysis and inverse $\log_{10}$ least squares mean reported herein for 0, 6 and 12 using average values of d 15 and 25 of each period.
[6]NH_3-N, ammonia-nitrogen; plasma urea nitrogen, PUN; determined at 0 and 6 h using average values of d 15 and 25 samples of each period.

Nitrogen Retention, Excretion and Urinary N Fraction

Nitrogen intake and estimated microbial N flow were not affected by treatment (341 ± 35.4 g/d and 109 ± 14.40 g/d, respectively; $P \geq 0.23$; Table 4.4). Fecal output of animals fed TA and CN increased compared with control animals (4.49 vs. 4.07 kg DM/d, $P = 0.02$) but fecal output of GA animals (4.20 kg DM/d) only differed from that of TA animals. In contrast total urinary output was not affected by treatment (22.2 ± 1.23 L/d; $P = 0.15$). Consequently, urinary N excretion was not affected by treatment (171 ± 25.5 g/d; $P = 0.24$) but fecal N excretion was greatest for animals fed TA, followed by those fed CN, compared with animals fed control and GA diets (134, 124 vs. 104 g/d, respectively; $P < 0.001$). Thus, total N excreted tended ($P = 0.07$) to increase for TA fed animals compared with control animals (300 vs. 270 g/d). However, the proportion of total N excreted, increased in feces and decreased in urine for TA and CN compared with control and GA (43.9 vs. 37.8% and 56.1 vs. 62.2%; respectively; $P < 0.001$). Similarly, the proportion of N excreted as a proportion of N consumed increased in feces for TA and CN compared with the control and GA (37.3 vs. 31.0%; $P < 0.001$). However, the proportion of N excreted as a proportion of N consumed in urine for animals fed GA compared with animals fed control, TA and CN diets (53.5 vs. 48.2%; $P = 0.02$). Urea N excreted in urine was not different among treatments (80.8 ± 9.23 g/d; $P = 0.15$); however, the proportion of urea N in

urinary N was reduced for all tannin treatments compared with the control (47.2 vs. 51.2%; P = 0.02). Allantoin N excreted in urine or its proportion in urinary N was not affected by treatment (21.7 ± 2.97 g/d or 12.9 ± 0.60%, respectively; $P \geq 0.13$) The amount of uric acid N excreted in urine and the proportion of uric acid N in urinary N was reduced for GA compared with control, TA and CN (1.57 vs 2.28 g/d and 0.91 vs. 1.32%, respectively; $P < 0.001$). The N retained did not differ among treatments irrespective of how it was expressed (54.2 g/d, 16.3% of N intake and 24.5% N digested; $P \geq 0.21$).

Table 4.4. Effects of different forms of hydrolyzable tannin on N intake, excretion, and retention, and urinary N fractions of heifers (n = 8) fed an alfalfa silage-based high forage diet.

	Treatment[1]					
Item[2]	Control	GA	TA	CN	SEM	*P*-value
N intake, g/d	335	336	352	340	35.4	0.53
Microbial N flow[3], g/d	110	109	113	102	14.4	0.23
Output						
Feces, kg DM/d	4.07^{c}	4.20^{bc}	4.59^{a}	4.39^{ab}	0.42	0.02
Urine, L/d	23.1	22.2	22.0	21.3	1.23	0.15
N excretion, g/d						
Feces	102^{c}	106^{c}	134^{a}	124^{b}	13.1	<0.001
Urine	168	181	166	167	25.5	0.24
Total	270^{y}	287^{xy}	300^{x}	291^{xy}	38.3	0.07
N excretion, % of total N excretion						
Feces	38.3^{b}	37.3^{b}	44.7^{a}	43.1^{a}	1.05	<0.001
Urine	61.7^{a}	62.7^{a}	55.3^{b}	56.9^{b}	1.05	<0.001
N excretion, % of N intake						
Feces	30.3^{b}	31.7^{b}	37.8^{a}	36.8^{a}	0.09	<0.001
Urine	49.3^{b}	53.5^{a}	46.8^{b}	48.6^{b}	0.03	0.02
Urinary N fraction, g/d						
Urea N	82.8	85.2	78.1	77.1	9.23	0.15
Allantoin N	21.8	22.2	22.5	20.3	2.97	0.20
Uric acid N	2.28^{a}	1.57^{b}	2.21^{a}	2.02^{a}	0.21	<0.001
Urinary N fraction, % of urine N						
Urea N	51.2^{a}	47.8^{b}	47.4^{b}	46.5^{b}	2.03	0.02
Allantoin N	13.1	12.3	13.6	12.4	0.60	0.13
Uric acid N	1.32^{a}	0.91^{b}	1.36^{a}	1.26^{a}	0.12	<0.001
N retention						
g/d	65.0	50.1	52.7	48.8	7.59	0.30

% of N intake	20.4	15.0	15.4	14.5	0.03	0.21
N retention, % N digested	29.0	21.9	24.6	22.6	4.27	0.28

[a–c, x–y]Within a row, means without a common superscript letter differ or tend to differ at $P < 0.05$ or $0.05 \leq P < 0.10$, respectively.
[1]GA, gallic acid; TA, tannic acid; CN, chestnut.
[2]Nitrogen intakes and excretion were sampled over 4 d and averaged before analysis.
[3]Estimated based on Allantoin and uric acid N (Chen andGomes,1992).

Methane Emission Measurement

During CH_4 measurement, the DMI averaged 10.06 ± 1.109 kg/d and was not affected by treatment ($P = 0.95$; Table 4.5). The daily CH_4 produced did not differ among treatments (213 ± 21.3 g/d; $P = 0.31$). However, adding GA to the diet tended to decrease CH_4/DMI ($P = 0.07$) and it decreased the proportion of GE intake emitted as CH_4 ($P = 0.04$) by 9% compared with the control (20.4 vs. 22.3 g/kg DMI; 5.16 vs. 5.71%). Also, the proportion of DE intake emitted as CH_4 decreased by 9% for animals fed GA diet compared with the control and TA (8.57 vs. 9.42%; $P = 0.02$) while it was intermediate for CN (8.83%).

Table 4.5. Effects of different forms of hydrolyzable tannin on methane (CH_4) emission of beef steers (n = 8) fed an alfalfa silage-based high forage diet.

	Treatment[1]					
Item[2]	Control	GA	TA	CN	SEM	*P*-value
DMI, kg/d	9.92	10.10	10.15	10.07	1.11	0.95
Methane[3]						
g/d	220	204	216	213	21.3	0.31
g/kg of DMI	22.3^{x}	20.4^{y}	21.2^{xy}	21.2^{xy}	0.87	0.07
% of GE intake	5.71^{a}	5.16^{b}	5.39^{ab}	5.34^{ab}	0.21	0.04
% of DE intake	9.42^{a}	8.57^{b}	9.42^{a}	8.83^{ab}	0.35	0.02

[a–b, x–y]Within a row, means without a common superscript letter differ or tend to differ at $P < 0.05$ or $0.05 \leq P < 0.10$, respectively.
[1]GA, gallic acid; TA, tannic acid; CN, chestnut.
[2]Treatment means reported herein because the effects of day and treatment × day interaction did not differ ($P > 0.10$).
[3] DMI, dry matter intake; GE, gross energy; DE, digestible energy at 95% ad libitum intake.

4.4. Discussion

Nutrient Intakes and Apparent Nutrient Digestibilities

Alfalfa is a forage crop cultivated for silage production by beef and dairy producers due to its high digestibility and high CP content (Berard et al., 2011). However, the use of high protein alfalfa silage to improve animal performance has the potential to negatively affect the environment, because high intake of soluble protein can lead to an increase in N excreted in feces and urine (Dijkstra et al., 2013). Thus, this study examined the effects of supplementing different forms of HT as a means of improving N utilization and reducing CH_4 emission of beef cattle fed a high protein forage-based diet. Hydrolyzable tannins have the ability to bind to proteins and, so, they may decrease CP degradation in the rumen (Getachew et al., 2008). Therefore, the alfalfa used for this study was at 9% bloom (21.1% CP) and used to formulate a diet high in CP (19.8%) to evaluate the hypothesis that adding HT to a high protein diet would decrease enteric CH_4 and urinary N excretion, and that the response to HT would depend on the form of HT.

The effect of added HT was examined using different sources of HT (TA; 1.5% DM and CN; 2% DM) and a component of HT from TA (GA; 1.5% DM). The GA was extracted from the *Rhus chinensis* plant, and is a sub-unit of gallotannin (i.e. a glucose core surrounded by several GA units, with more GA attached through depside bonding of additional galloyl residues; Djakpo and Yao, 2010). Tannic acids from commercial sources are usually gallotannins (Hagerman, 2011) and the GA and TA were obtained from the same commercial source. The HT extracted from CN is usually a mix of gallotannins and ellagitannins (a sugar core often a glucose unit surrounded by hexahydroxydiphenic acid formed from oxidative coupling of galloyl groups; Hagerman, 2011; Chiarini et al., 2013). Thus, accounting for the HT concentrations of the TA and CN in the diets, both supplied 1.43% HT.

Adding tannins to ruminant diets may have a negative effect on palatability and digestibility and consequently decrease DMI as reviewed by Waghorn (2008). In the present study, limit-feeding (95% ad libitum) of the animals may have obscured any palatability issues associated with tannin-feeding. The lack of effect of HT treatments on apparent total-tract DM, OM, NDF, ADF, and GE digestibilities indicates no negative effects of tannin treatments on energy availability of the diets. On the contrary, the observed 9% decrease in apparent CP digestibility for TA and CN compared with GA and control indicates that only the more complex form of HT, decreased CP digestibility. This effect of HT is consistent with Frutos et al. (2004) who reported that CN did not affect *in situ* DM degradability of soybean meal but it decreased CP degradability by 12%. Also similar to our findings for total-tract digestibility, supplementing alfalfa hay with TA reduced *in vitro* CP degradability compared with a control but CP degradability was not affected by GA addition (Getachew et al., 2008). The capacity of different types of HT to form complexes with proteins is generally greater for those with greater molecular weight (Kawamoto et al., 1995). Thus, the lack of effect of GA on apparent CP digestibility can be attributed to its rapid and complete hydrolyzation in the rumen compared with HT (Murdiati et al., 1992). Thus, the complex form of HT (TA and CN) were more effective in decreasing CP digestibility than the sub-unit of HT.

Ruminal Fermentation and PUN

The lack of effect of the HT treatments on ruminal pH agrees with the results of other studies with HT fed to cattle (Krueger et al., 2010; Aboagye et al., 2018) or sheep (Liu et al., 2011). The tendency for GA to increase VFA concentration in the present study is similar to the study by Getachew et al. (2008) who compared GA and TA *in vitro* and found that GA incubated with alfalfa hay produced more total VFA compared with a control while there was no effect for

TA. As GA is easily hydrolyzed in the rumen to pyrogallol, resorcinol and phloroglucinol, it may have been used as an energy source by the microbes (Murdiati et al., 1992; McSweeney et al., 2001), which would account for the increase in VFA concentration. The lack of effect of the HT treatments on the main VFA proportions (acetate and propionate) is consistent with the lack of effect of treatments on apparent DM and fiber digestibility.

Usually, greater ruminal NH_3-N concentration is associated with greater PUN concentration because of excess dietary protein intake relative to the dietary energy. However, in the present study there was inconsistency between ruminal NH_3-N and PUN concentrations. While all HT treatments decreased PUN concentration, only TA lowered NH_3-N concentration, indicating that the forms of HT functioned differently. Tannins complex with dietary protein and prevent ruminal degradation of protein, thereby, decreasing ruminal NH_3-N concentration. This effect may may increase amino acid absorption from undegraded feed protein or cause an offset by decreasing microbial protein synthesis rendering no net effect of MP flow to the small intestine. The HT-protein complex occur ideally between pH 3 and 5, but also up to pH 7 (Murdiati et al., 1991; Osawa and Walsh, 1993), which may account for how the different sources of HT affected CP digestibility differently in the gut. It appears that TA decreased the ruminal degradation of dietary CP, as evidenced by a lower rumen NH_3-N concentration, which led to decreased total-tract CP digestion and lower PUN. However, the decreased ruminal degradation of dietary CP did not negatively affect estimated microbial N flow to the lower tract. The HT from CN may have decreased the digestion of dietary CP post-ruminally, as rumen NH_3-N concentration and estimated microbial N flow were not affected even though total tract CP digestibility and PUN decreased. Similarly, Hagerman et al. (1992) showed that both TA and HT extracted from forage differed in their protein binding abilities in the gut of sheep and deer. In

contrast with the results for TA and CN in our study, the decrease in PUN for animals fed GA did not correspond to a decrease in apparent CP digestibility and ruminal NH_3-N concentration, but a tendency for a decrease in isobutyrate proportion. Isobutyrate is formed through oxidative deamination and decarboxylation of the amino acid valine (Allison and Peel, 1971). As the gallotannin sub-unit is easily hydrolyzed in the rumen, it may be toxic to microbes that are not able to utilize its metabolites in contrast to CN and TA (Murdiati et al., 1992). Thus, decreased PUN concentration in animals fed a high CP diet supplemented with GA may have been due to inactivation of microbial deaminases by the toxic metabolites (Goel et al., 2005). Therefore, while the effect of complex forms of HT appears be directed towards dietary CP, a component of the gallotannin may target the rumen microbes without affecting microbial N flow.

Nitrogen Retention, Excretion and Urinary N Fraction

An increase in N intake can lead to increased N excreted in feces and urine (Dijkstra et al., 2013). Although N intake was not affected by the forms of HT included in the diet, N excretion was altered. The observed 24% greater amount of N excreted in feces for animals fed TA and CN, compared with the control and GA diets, resulted from decreased CP digestibility as well as a shift in N excretion from urine to feces. The shift in route of N excretion is reflected by a 6% unit greater fecal N proportion and a 6% unit lower urinary N proportion. Similarly, other studies have shown that adding HT extract from CN at 1.53% dietary DM to a mixed diet increased fecal N excretion and decreased urinary N excretion in sheep (Wischer et al., 2014). Also, Yang et al. (2016) supplemented a mixed diet with TA at 1.3% and 2.6% dietary DM and reported decreased urinary N proportion (15% and 23% respectively) and increased fecal N proportion (23% and 36%, respectively). However, contrary to the present study, GA added to a mixed diet increased the ratio of fecal N:urinary N in beef cattle fed at maintenance level (Wei et

al., 2016). The authors attributed this shift in ratio to the binding effect of GA to protein and a possible decrease in protein digestibility, although CP digestibility was not measured. Gallic acid has been shown to be completely hydrolyzed in the rumen (Murdiati et al., 1992) and to lack effects on *in vitro* protein degradability (Getachew et al., 2008), which agrees with the findings from the present study where there was no effect of GA on apparent CP digestibility and estimated microbial N flow.

The decreased percentage of urea N in urinary N for both the complex (TA and CN) and sub-unit (GA) forms of HT and the decreased amount of uric acid N in urine for GA indicate that the different sources of HT and especially the sub-unit of TA, affected the N fractions of the urine. The urinary N of cattle contains various compounds but urea-N represents the greatest proportion (52 to 94%) as reviewed by Dijkstra et al. (2013). Urinary urea is hydrolyzed rapidly in the environment to form NH_3 and later ammonium, which through nitrification and denitrification processes forms N_2O, a potent GHG. The decrease in urea-N (% of urinary N) for all forms of HT treatments shows that HT, including a sub-unit of gallotannin has the ability to reduce N_2O emission from urine. The N in feces is in a less volatile form compared with that of urinary N because it is present mainly as indigestible organic N (proteins or nucleic acids; Hristov et al., 2011). The shift in route of N excretion from urine to feces for CN and TA suggests that these 2 sources of HT have the potential to reduce NH_3 emissions, and eventually N_2O emissions from manure, in contrast with control and GA.

Methane Emission Measurement

During feed fermentation, metabolic H_2 is used for VFA production and also serves as an important substrate for reducing CO_2 to CH_4 by methanogens (Hegarty, 1999; Cottle et al., 2011). The metabolic H_2 produced in the rumen is either gaseous H_2 or dH_2, but the dH_2 is

readily available for methanogens and influences fermentation pathways (Janssen, 2010; Wang et al., 2016). Thus, protozoa associated with methanogens have been reported to influence CH_4 formation through interspecies transfer of dH_2 from protozoa to CH_4 (Morgavi et al., 2010). However, the CH_4 reducing effect of GA was likely not due to protozoa because of the lack of effect of the different forms of HT inclusion on protozoa enumeration and dH_2 concentration. Bhatta et al. (2013) showed that CH_4 production may decrease without a decrease in protozoa with tannin addition, indicating that some tannins may directly affect methanogens that are not associated with protozoa. The tendency for GA to decrease CH_4 yield and decrease CH_4 relative to GE and DE intakes by 9% may have been partly due to a direct effect on methanogens. Similarly, supplementing a diet of 50% concentrate:50% corn silage with GA at 1%, 2% and 4% linearly decreased *in vitro* CH_4/substrate degraded (Wei et al., 2018). Contrary to the present study, adding TA to a mixed diet at 0.65%, 1.3% and 2.6% dietary DM decreased CH_4 production (L/kg DM intake) by 11.1%, 14.7% and 33.6%, respectively in beef cattle (Yang et al., 2016). Also, HT extract from CN added to a mixed diet at 0.5, 0.75 and 1.0 mg/ml decreased *in vitro* CH_4 production quadratically (Jayanegara et al., 2015). Those studies (Jayanegara et al., 2015; Yang et al., 2016; Wei et al., 2018) showed that HT has the potential to decrease CH_4 production; however, no study compared multiple sources of HT as in the present study. The mechanism by which tannins reduce CH_4 production could be due to direct or indirect effects on methanogenesis. Tannins may bind to surface membranes of methanogens and decrease their growth (Naumann et al., 2017), decrease fiber degradation (Carulla et al., 2005), and act as a hydrogen sink (Becker et al., 2014). However, as total-tract NDF digestibility and dH_2 in ruminal fluid were not affected by treatments in the present study, it appears that the rapid hydrolyzation of the HT component relative to the complex HT forms may have caused GA to be more toxic to

methanogens than TA and CN. The metabolites of GA (pyrogallol, resorcinol, and phloroglucinol) can be hydrolyzed to acetate and butyrate by rumen microbes to generate energy (McSweeney et al., 2001). In the present study GA tended to increase VFA compared with the control and TA and this may partly explain the 9% decrease in CH_4 emission (% per DE intake) for animals fed the GA diet compared with animals fed the control and TA diets.

In summary, adding different complex forms of HT (TA or CN) and a component of HT (GA) to a high protein alfalfa silage diet fed to heifers had no effects on nutrient intakes or apparent total-tract DM and fiber digestibilities, but TA and CN decreased apparent CP digestibility. Only TA decreased NH_3-N concentration in rumen fluid, but all three forms of HT and GA tended to reduce isobutyrate proportion in the rumen and decreased PUN concentration. Both complex forms of HT increased fecal N excretion and shifted N excretion from urine to feces compared with the control and GA treatments. However, regardless the form of HT, including its sub-unit from gallotannin, urea N as a proportion of urinary N decreased compared with the control. Also, the results indicate that GA altered the N fractions in urine by decreasing the proportion of uric acid in urinary N compared with TA, CN and control. Dietary supplementation with complex forms of HT (TA and CN) did not affect CH_4 production but a sub-unit of HT applied as GA tended to decrease (g/kg DMI) or decreased CH_4 (% GE and DE intakes) by 9% compared with the control.

In conclusion this study demonstrates that the response to HT by beef cattle fed a high protein forage diet depends on composition of HT. Gallic acid, which is a component of HT, was more efficient in altering the N fractions of urine by reducing both urea and uric acid N without negatively affecting CP digestibility. However, because GA did not shift N excretion from feces to urine but regulated the urinary N fraction components, it may not reduce the overall NH_3

emission in manure relative to TA and CN. On the contrary, GA also reduced CH_4 production from beef cattle, unlike TA and CN. Therefore by this study, GA has the potential to lower CH_4 and N_2O emissions from beef cattle, without reducing feed digestibility in cattle consuming a high protein forage diet.

4.5. Literature Cited

Aboagye, I. A., M. Oba, A. R. Castillo, K. M. Koenig, and K. A. Beauchemin. 2018. Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high forage diet. J. Anim. Sci. 96:5276-5286. doi: 10.1093/jas/sky352.

Allison, M. J., and J. L. Peel. 1971 The biosynthesis of valine from isobutyrate by *Peptostreptococcus elsdenii* and *Bacteroides ruminicola*. Biochem. J. 121:431–437 doi:10.1042/bj1210431.

Association of Official Analytical Chemists. 2005. Official methods of analysis, 18th ed. AOAC Int., Gaithersburg, MD.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48:21–27 doi:10.1071/EA07199.

Beauchemin, K. A., and S. M. McGinn. 2006. Enteric methane emissions from growing beef cattle as affected by diet and level of intake. Can. J. Anim. Sci. 86:401–408. doi:10.4141/A06-021.

Becker, P. M., P. G. Wikselaar, M. C. R. Franssen, R. C. H. Vos, R. D. Hall, and J. Beekwilder. 2014. Evidence for a hydrogen-sink mechanism of (+)catechin-mediated emission reduction of the ruminant greenhouse gas methane. Metabolomics 10:179–189. doi:10.1007/s11306-013-0554-5.

Berard, N. C., Y. Wang, K. M. Wittenberg, D. O. Krause, B. E. Coulman, T.A McAllister, and K. H. Ominski. 2011. Condensed tannin concentrations found in vegetative and mature forage legumes grown in western Canada. Can. J. Plant Sci. 91:669–675. doi:10.4141/cjps10153.

Bhatta, R., M. Saravanan, L. Baruah, K. Sampath, and C. S. Prasad. 2013. Effect of plant secondary compounds on *in vitro* methane, ammonia production and ruminal protozoa population. J. Appl. Microbiol. 115:455–465. doi:10.1111/jam.12238.

Calsamiglia, S., A. Ferret, C. K. Reynolds, N. B. Kristensen, and A. M. Van Vuuren. 2010. Strategies for optimizing nitrogen use by ruminants. Animal 4:1184–1196. doi: 10.1017/S1751731110000911.

Canadian Council on Animal Care (CCAC). 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. Ottawa, ON, Canada.

Carulla, J. E., M. Kreuzer, A. Machmüller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Austr. J. Agric. Res. 56:961–970.

Chen, X. B, and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on purine derivatives: an overview of technical details. Int. Feed Res. Unit. Rowett Res. Inst. Bucksburn Aberdeen, UK. p. 1–21.

Chiarini A, M., Micucci, M. Malaguti, R. Budriesi, P. Ioan, M. Lenzi, C. Fimognari, , T. G. Toschi, P. Comandini, and S. Hrelia. 2013. Sweet chestnut (Castanea sativa Mill.) bark extract: Cardiovascular activity and myocyte protection against oxidative damage. Oxid. Med. Cell Longev. 2013:471790. doi:10.1155/2013/471790.

Cottle, D. J., J. V. Nolan, and S. G. Wiedemann. 2011. Ruminant enteric methane mitigation: A review. Anim. Prod. Sci. 51:491–514. doi:10.1071/AN10163.

Dijkstra, J., O. Oenema, J. W. van Groenigen, J. W. Spek, A.M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N_2O emissions. Animal 7 (Suppl. 2):292–302. doi:10.1017/S1751731113000578.

Djakpo, O, W. R. Yao. 2010. Rhus chinensis and Galla chinensis —folklore to modern evidence: review. Phytother. Res. 24:1739–1747. doi:10.1002/ptr.3215.

Field, J. A., S. Kortekaas, and G. Lettinga. 1989. The tannin theory of methanogenic toxicity. Biol. Wastes. 29:241–262.

Frutos, P., M. Raso, G. Hervás, A. Mantecón, V. Pérez, and F. J. Giráldez. 2004. Is there any detrimental effect when a chestnut hydrolysable tannin extract is included in the diet of finishing lambs? Anim. Res. 53:127–136. doi:10.1051/animres:2004001.

Gerber, P. J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, and G. Tempio. 2013. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. FAO, Rome, Italy.

Getachew, G., W. Pittroff, D. H. Putnam, A. Dandekar, S. Goyal, and E. J. DePeters. 2008. The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis. Anim. Feed Sci. Technol. 140:444–461. doi:10.1016/j.anifeedsci.2007.03.011.

Goel, G., A. K. Puniya, C. N. Aguilar, and K. Singh. 2005. Interaction of gut microflora with tannins in feeds. Naturwissenschaften 92:497–503. doi:10.1007/s00114-005-0040-7.

Guyader, J., E. M. Ungerfeld, and K. A. Beauchemin. 2017. Redirection of metabolic hydrogen by inhibiting methanogenesis in the rumen simulation technique (RUSITEC). Front. Microbiol. 8:1–16. doi: 10.3389/fmicb.2017.00393.

Hagerman, A. E. 2011. Tannin Handbook, Department of Chemistry and Biochemistry, Miami University, USA. http://www.users.muohio.edu/hagermae (Accessed 17 October 2017)

Hagerman, A. E., C. T. Robbins, Y. Weerasuriya, T. Wilson, and C. McArthur. 1992. Tannin chemistry in relation to digestion. J. Range Manag. 45:57–62.

Hegarty, R. S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. Aust. J. Agric. Res. 50:1321–1327. doi:10.1071/AR99008.

Hristov, A. N., M. Hanigan, A. Cole, R. Todd, T. A. McAllister, P. M., Ndegwa, and A. Rotz. 2011. Review: Ammonia emissions from dairy farms and beef feedlots. Can. J. Anim. Sci. 91:1–35. doi:10.4141/CJAS10034.

Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160:1–22. doi:10.1016/j.anifeedsci.2010.07.002.

Jayanegara, A., G. Goel, H. P. S. Makkar, and K. Becker. 2010. Reduction in methane emissions from ruminants by plant secondary metabolites: effects of polyphenols and saponins. In N. E. Odongo, M. Garcia, G. J. Viljoen, editor, Sustainable improvement of animal production and health. FAO, Rome, Italy. p. 151–157.

Jayanegara, A., G. Goel, H. P. S. Makkar, and K. Becker. 2015: Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol. 209:60–68. doi:10.1111/jpn.12531.

Kawamoto H., F. Nakatsubo, and K. Murakami. 1995. Quantitative determination of tannin and protein in the precipitates by high-performance liquid chromatography. Phytochemistry 40:1503-1505. doi:10.1016/0031-9422(95)00451-C.

Koenig, K. M., S. M. McGinn, and K. A. Beauchemin. 2013. Ammonia emissions and performance of backgrounding and finishing beef feedlot cattle fed barley-based diets varying in dietary crude protein concentration and rumen degradability. J. Anim. Sci. 91:2278–2294. doi:10.2527/jas.2012–5651.

Krueger, W. K., H. Gutierrez-Bañuelos, G. E. Carstens, B. Min, W. Pinchak, R. Gomez, R. C. Anderson, N. K. Krueger, and T. D. A. Forbes. 2010. Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain. Anim. Feed Sci. Technol. 159:1–9. doi:10.1016/j.anifeedsci.2010.05.003.

Liu, H., V. Vaddella, and D. Zhou. 2011. Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. J. Dairy Sci. 94:6069–6077. doi:10.3168/jds.2011-4508.

McAllister, T.A., T. Martinez, H. D. Bae, A. D. Muir, L. J. Yanke, and G. A. Jones. 2005. Characterization of condensed tannins purified from legume forages: chromophore production, protein precipitation and inhibitory effects on cellulose digestion. J. Chem. Ecol. 31:2049–2068.

McSweeney, C. S., B. Palmer, D. M. McNeill, and D. O. Krause. 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Anim. Feed Sci. Technol. 91:83–93. doi:10.1016/S03778401(01)00232-2.

Morgavi, D. P., E. Forano, C. Martin, and C. J. Newbold. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal. 4:1024–1036. doi:10.1017/S1751731110000546.

Murdiati, T. B., C. S. McSweeney, and J. B. Lowry. 1991. Complexing of toxic hydrolysable tannins of yellow-wood (*Terminalia oblongata*) and harendong (*Clidemia hirta*) with reactive substances: An approach to preventing toxicity. J Appl. Toxicol. 11:333–338. doi:10.1002/jat.2550110506.

Murdiati, T. B., C. S. McSweeney, and J. B., Lowry. 1992. Metabolism in sheep of gallic acid, tannic acid, and hydrolysable tannin from *Terminalia oblongata*. Aust. J. Agric. Res. 43:1307–1319. doi:10.1071/AR9921307.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient Requirements of Beef Cattle, Eighth Revised Edition. Washington, DC: The National Academies Press. doi:177226/19014.

Naumann, H. D., L. O. Tedeschi, W. E. Zeller, and N. F. Huntley. 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. R. Bras. Zootec. 46: 929–949. doi:10.1590/s1806-92902017001200009.

Osawa R., and T. P. Walsh. 1993. Effects of acidic and alkaline treatments on tannic acid and its binding property to protein. J. Agric. Food Chem. 41:704–7.

Patra, A.K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91:24–37. doi:10.1002/jsfa.4152.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132–2140. doi:10.3168/jds.S00220302(06)72284-6.

Rhine, E. D., G. K. Sims, R. L. Mulvaney, and E. J. Pratt. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Sci. Soc. Am. J. 62:473–480. doi:10.2136/sssaj1998.03615995006200020026x.

Tavendale, M. H., L. P. Meagher, D. Pacheco, N. Walker, G. T. Attwood, and S. Sivakumaran. 2005. Methane production from in vitro rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. Anim. Feed Sci. Technol. 123:403–419.

Veeramani A., G. M. Dias, and S. Kirkpatrick. 2017. Carbon footprint of dietary patterns in Ontario, Canada: A case study based on actual food consumption. J. Clean. Prod. 162:1398–1406. doi:10.1016/j.jclepro.2017.06.025.

Veira, D. M., M. Ivan, and P. Y. Jui. 1983. Rumen ciliate protozoa: effects on digestion in the stomach of sheep J. Dairy Sci. 66:1015–1022. doi:10.3168/jds.S0022-0302(83)81896-7.

Waghorn, G. C. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production – progress and challenges. Anim. Feed Sci. Technol. 147:116–139. doi:10.1016/j.anifeedsci.2007.09.013.

Wang, M., R. Wang, P. H. Janssen, X. M. Zhang, X. Z. Sun, D. Pacheco, and Z. L. Tan. 2016. Sampling procedure for the measurement of dissolved hydrogen and volatile fatty acids in the rumen of dairy cows. J. Anim. Sci. 94:1159–1169. doi:10.2527/jas2015-965.

Wei, C., J. Guyader, L. Collazos, K. A. Beauchemin, and G. Y. Zhao. 2018. Effects of gallic acid on *in vitro* rumen fermentation and methane production using rumen simulation (Rusitec) and batch-culture techniques. Anim. Prod. Sci. doi:10.1071/AN17365.

Wei, C., K. Yang, G. Zhao, S. Lin, and Z. Xu. 2016. Effect of dietary supplementation of gallic acid on nitrogen balance, nitrogen excretion pattern and urinary nitrogenous constituents in beef cattle. Arch. Anim. Nutr. 70:416–423. doi:10.1080/1745039X.2016.1214345.

White R. R., and M. B. Hall. 2017. Nutritional and greenhouse gas impacts of removing animals from US agriculture. Proc. Natl. Acad. Sci. USA 114:E10301–E10308. doi:10.1073/pnas.1707322114.

Wischer, G., A. M. Greiling, J. Boguhn, H. Steingass, M. Schollenberger, K. Hartung, and M. Rodehutscord. 2014. Effects of long-term supplementation of chestnut and valonea extracts on methane release, digestibility and nitrogen excretion in sheep. Animal. 8:938–948. doi: 0.1017/S1751731114000639.

Yang, K., C. Wei, G. Y. Zhao, Z. W. Xu, and S. X. Lin. 2016. Effects of dietary supplementing tannic acid in the ration of beef cattle on rumen fermentation, methane emission, microbial flora and nutrient digestibility. J. Anim. Physiol. Anim. Nutr.101:302–310. doi: 10.1111/jpn.12531.

CHAPTER 5-Gerneral Discussion and Conclusions

5.1. Main Findings and Integration of Results

This thesis focused on understanding the variation in nutritional quality of short-season CS grown in AB and tannin supplementation of alfalfa silage based diets as ways of reducing the environmental impact of forage-fed ruminants.

For the short-season CS, it was hypothesized that harvesting after frost would increase DM and starch concentrations and reduce CH_4 emission of CS hybrids adapted to different locations in AB compared with harvesting before frost. To test this hypothesis, an *in vitro* experiment using batch culture (2 runs, 3 replicates/run) and the Daisy incubator were conducted. The first experiment (Chapter 2), compared CH_4 production of short-season WPCH (≤ 2600 CHU for grain maturity). Four WPCH with CHU rating of 2000 to 2200 or 2200 to 2600 were grown in 2 years in cooler central Alberta (AB) or warmer southern AB, respectively, with 2 field replications. Hybrids were harvested before and after light frost (-1.5ºC). The study showed that CH_4 production (mg CH_4/g DM degraded) decreased for central AB short-season CS hybrids harvested late after frost compared with before frost but the effect of harvest time did not affect southern AB hybrids. The observed reduction in CH_4 production for central AB hybrids harvested after frost was associated with greater concentration of propionate upon incubation. Harvesting southern AB hybrids after frost, increased NDFD compared with before frost but with no change in nutrient concentration and thus no associated effect with CH_4 production.

Harvesting corn after frost is common practice in AB that ensures proper CS plant DM concentration is obtained for ensilage. The freezing temperature can facilitate moisture loss by rupturing cells to increase DM concentration, especially in the stalk of the plant (Lee and

Herbek, 2004). However, severity of frost can damage crops depending on the intensity or duration of frost, and corn growth at the time of frost (White et al., 1976; Lee and Herbek, 2004). A light frost, similar to the frost that occurred in central AB (-0.2 to -2.0°C) may freeze leaf tissues, reduce photosynthesis, and movement of sugars from leaves to kernels, but as the air warms, and if stalk and ear are not frozen then carbohydrates stored temporarily in the stalk may be translocated as sucrose to grain, increasing starch concentration (White et al., 1976; Slewinski, 2012). This effect may partly explain the greater starch concentration and the associated reduction in CH_4 production reported for central AB hybrids harvested after frost compared with befor frost harvest. However, when frost occurs below -2.0°C, similar to the frost in southern AB (-0.2 to -2.2°C), a lot of plant leaves may be lost and translocation of carbohydrates from the stalk to grain may not occur and this may terminate grain filling. This effect may partly explain the lack of effect that harvesting after frost had on starch concentration for southern AB hybrids. Also, because of the relatively greater accumulated CHU for southern AB, it appears that the short-season hybrids were near the end of grain-filling before frost, and so, harvesting after frost had no significant change in starch concentration and associated CH_4 production. However, when frost occurred, matured leaves (mainly NDF concentration) were lost, which may have increased the NDFD for southern AB hybrids harvested after frost. This thesis shows that harvesting corn plants after frost in a relatively warm area may over-dry the material beyond the recommended DM concentration (320 to 380 g kg^{-1}) with no improvement in starch concentration or reduction in CH_4 production. Potentially this might decrease silage quality and animal performance.

An increase in starch concentration is associated with a decrease in enteric CH_4 production (Moe and Tyrrell, 1979). This effect was reflected among the hybrids harvested in the

cooler central AB, where the earliest hybrids also produced lower CH_4 production than the latest hybrids due to their greater starch concentration of the early hybrid. This result indicates the possibility of growing CS hybrids with lower CH_4 production in central AB. Therefore, the hypothesis that harvesting after frost would increase DM and starch concentrations and reduce CH_4 emission of short-season CS hybrids is only true for very early maturing short-season hybrids adapted to the cooler regions of AB.

For the alfalfa silage diets supplemented with tannins, it was hypothesized that a combination of HT and CT compared with the control (no tannin) at an optimum dose may lower rumen NH_3 concentration (an indicator of N excretion), decrease CH_4 emissions and improve animal performance (Chapter 3). Additionally, it was hypothesized that feeding HT or a component of HT to cattle fed a high protein diet based on alfalfa silage would decrease both urinary N excretion and enteric CH_4 production, and that the response to HT would depend on the form of HT (Chapter 4). To test these hypotheses, two animal studies using growing beef cattle were conducted.

The two animal experiments were conducted to determine the potential of tannins in reducing enteric CH_4 production and N excretion in steers (Chapter 3) or heifers (Chapter 4) fed an alfalfa silage based diets. Enteric CH_4 was measured using SF_6 and respiration chambers for Experiments 2 and 3, respectively. Beef cattle fed an alfalfa silage based diet supplemented with a combination of HT and CT at 1.5% CNQ mix (greater tannin dose) showed a tendency for a lower CH_4 yield (g/kg DMI) compared with the control (no tannin; Chapter 3) with no effect on BW gain. However, when the form of HT on CH production was assessed in a subsequent study, it was the subunit of HT (in the form of GA) that tended to decrease CH_4 yield and decreased the proportion of GE intake emitted as CH_4 by 9% compared with control (Chapter 4). Theoretically,

a decline in enteric CH_4 production means greater portion of DE can be available to the animal for use (i.e. greater metabolizable energy), which means animal productivity may increase. However, the energy lost as heat during fermentation from metabolizable energy is not accounted for (i.e. the efficiency of converting metabolizing energy to net energy for productivity) during digestion and metabolism is not accounted for and may cause inefficiency in energy utilization rendering no additional BW gain by the animal. Also, all possible H_2 sinks in the rumen when H_2 is not used to reduce CO_2 to CH_4 are not fully known and some of these sinks may not be used as energy substrates by animals.

In the second experiment (Chapter 3) the proportion of butyrate increased for 1.5% CNQ, because both HT and CT can be hydrolyzed into their subunits and fermented by rumen microbes to butyrate (Krumholz and Bryant, 1986; Bhat et al., 1998), which may or may not increase VFA concentration. In Chapter 4, although butyrate concentration was not affected by GA, TA or CN treatments, the HT subunit, GA, which decreased the proportion of GE emitted as CH_4, also tended to increase VFA relative to the control. It has been proposed that tannins decrease CH_4 emissions by directly inhibiting methanogens (Field et al. 1989) and ruminal microbes that produce H_2 and are associated with methanogens such as protozoa (Bhatta et al., 2009; Cieslak et al., 2012), or indirectly by decreasing fiber degradation (Carulla et al., 2005) in the rumen. However, in both the second and third experiments there was lack of effect of tannins on DMI, fiber digestion or protozoa. Though methanogens were not measured in either experiment, a decrease in CH_4 production is not always related to a decrease in protozoa (Bhatta et al., 2013), as some tannins may decrease methanogens that are not associated with protozoa. The metabolites of tannins may be toxic to microbes that are not able to utilize them (Murdiati et al., 1992), so, the greater quantity of tannins in the 1.5% CNQ mix, may have produced a

quantity of metabolites similar to those from GA (HT subunit) to directly affect methanogens and decrease CH_4 emission. This result demonstrates that the metabolites of tannins may be responsible for the decline in CH_4 yield with tannin supplementation in ruminants diets. Additionally, there was no toxicity observed for any of the forms of HT and the doses at which they were applied. The lack of effect of HT toxicity on the animals in this study also shows that at optimum tannin application, the metabolite of HT may target the diet digestibility and the rumen microbes rather than the animal itself. Thus the hypotheis that a combination of HT and CT at an optimum dosage would decrease CH_4 production is true but this effect may be due to the metabolites of tannins such as GA.

In Experiments 2 and 3, the effects of HT on N use in beef cattle fed an alfalfa silage based diet were examined. In Experiment 2, although there was no overall effect of tannins on PUN relative to the control, irrespective of dosage (0.25% or 1.5%) or type (HT or HT combined with CT in the form of CNQ) of tannin. However, tannin treatments decreased ruminal NH_3-N concentration compared with the control, with a 53% decrease for the greater dosage and 36% decrease for the lower dosage. This result indicates that tannins may have decreased ruminal dietary CP degradation and shifted CP digestion to the lower tract with a possibility of increasing the rate of CP digestion in the lower tract such that PUN did not change for tannin treatments relative to the control. This effect may have been possible due to the overall greater PUN concentration for animals fed the greater dosage, compared with the lower dosage, of CNQ. For Experiment 3, inclusion of the complex forms of HT as TA and CN both decreased apparent total CP digestion and decreased PUN concentration, but the location in the gastrointestinal tract where CP digestion is decreased may differ between TA and CN. While TA appeared to have decreased ruminal degradation of dietary CP as evidenced by the lower ruminal NH_3-N

concentration, CN seemed to have led to a reduction in CP digestion in the lower tract. However, in Experiment 2, there was a lower ruminal NH_3-N concentration reported for CN, which means ruminal degradation of dietary CP may have been decreased by CN. The contrasting effect of CN in Experiments 2 and 3 could be due to the associative effect of CN concentration and dietary composition and rumen microbial adaptation to CN. The HT-subunit in Experiment 3, GA, did not affect CP digestion but decreased PUN concentration and tended to decrease isobutyrate concentration compared with the control, which suggests that deamination of valine may have been inhibited. The decreased PUN concentration shows that GA may have inhibited the activity of some ruminal microbes, while the different complex forms of HT (TA and CN) prevented dietary CP digestion at different locations in the gut, without decreasing microbial N flow.

In Experiment 3, the effect of HT or its subunit on N utilization was further studied. Unsurprisingly, the decreased CP digestibility for the different sources of HT compared with the control and GA led to a 24% greater amount of N excreted in feces. The different sources of HT also shifted N excretion from urine to feces and this was evident by a 6%-unit greater fecal N proportion and a 6%-unit lower urinary N proportion compared with the control and GA treatments. It is generally known that CT shifts N excretion from urine to feces (Somada et al., 2003; Waghorn, 2008; Grainger et al., 2009), but this study shows that HT is also capable of diverting excess N from urine to feces and this effect only occurs for complex forms of HT, which can bind to dietary CP and prevent its digestion in the gut of the animal. This effect of the complex forms of HT was also evident in Experiment 2, where both ruminal NH_3-N and estimated UNE decreased for the high dose of HT or its combination with CT. As discussed in Chapter 1, the N in feces is less volatile, but urinary N, which is largely in the form of urea, upon contact with soil can be rapidly transformed into NH_3 and contributes to eutrophication,

formation of particulate matter, and indirect N_2O emission (Hristov et al., 2011; Ciais et al., 2013). The subunit of HT may not have the ability to bind to dietary CP but it may penetrate or disrupt the cells of microbes that are not able to utilize it and decrease their activity in the rumen. This effect may explain the potential of GA to decrease enteric CH_4 emission, and concentration of urea and uric acid fractions in urinary N. Feeding high dietary CP to animals may help maximize animal productivity, but it comes with a cost to the environment through an increase in N excretion (Hristov et al., 2011). However, as shown in this study the different forms of complex HT including a component of gallotannin has the ability to reduce NH_3 emissions from manure. Thus, feeding different forms of HT in beef cattle diets containing excess N can be beneficial to the environment without affecting animal productivity.

5.2. Challenges in the Thesis and Future Research

In Chapter 2, the WPCH were grown in AB based on their different CHU rating, which is generally practiced by producers to maximize yield; however, to be able to compare the effects of location on hybrids and hybrid across locations, the same hybrids would need to be planted in both locations. In this study the effect of location is confounded by hybrid because very few hybrids are adapted to both locations, and so, it was not possible to grow the same hybrids in both locations, which is the reality of growing corn in a cold environment. The study showed that hybrids that are adapted to both locations in AB produced less CH_4 in southern AB than in central AB, as evident by hybrid P7632HR (22 vs. 25 mg CH_4/g DM degraded), which was grown in both locations. This result suggests that hybrids adapted to cooler regions may have potential in warmer regions, but their capacity to maximize nutrient composition with a lower CH_4 production in both locations for hybrid selection are not known. The use of a single hybrid adapted to both locations as shown in this thesis does not allow for selecting among others the

best hybrid that is adapted to both locations. Thus, future work should look into comparing the same short-season hybrids across locations in AB to indicate adaptation potential and true location effect. Also, NDFD increased for hybrids harvested after frost for southern AB hybrids, but these hybrids were relatively advanced in maturity (i.e. near the end of grain-filling) before frost and so there was no effect on CH_4 production by harvesting after frost. Even though, NDFD increased in corn plants grown in southern AB after frost this apparent gain is uncertain and in addition, frost may cause the corn crop to dry beyond recommended for ensilage and have negate effects on silage yield, quality and animal performance. So harvesting after frost is not recommended for corn hybrids that are already close to the optimum DM concentration for silage production. An in *vitro study* was conducted because it is considerably less expensive than an *in vivo* study, but animal effects are not accounted for by the in *vitro study*. An *in vitro* study on CH_4 production can be relevant to feeding cows at maintenance level (Danielsson et al., 2017). Also, non-ensiled CS hybrids were used for this study, although the effects of ensilage on the variables examined would likely have been trivial if ensiling was done properly. However, the effect of frost on palatability of short-season CS hybrids should also be explored. Therefore, research with beef cattle is needed to document the relationships reported in the present *in vitro* study that identified factors that may reduce CH_4 emissions of ruminants fed short-season hybrids.

Most research with tannins for cattle has focused on CT with little information being available on HT. However, in Experiments 2 and 3, HT was shown to decrease both CH_4 yield and N excretion in beef cattle fed alfalfa-silage based diet. In conducting Experiment 2, animals were kept during winter in a semi-enclosed area, where temperature was sometimes as low as -30ºC. This decline in temperature froze feed and water in the barn and this may have negatively

affected the DMI, ADG and G:F mainly in wk 1 to 3 and wk 7 to 9. In an attempt to minimize the impact of cold weather, the frozen feed in the animals' feed troughs was crushed often to make it accessible to the animals. However, animals in colder temperatures may also increase their feed intake due to increased passage rate, and decreased CP digestion in the rumen (Kennedy and Milligan, 1978), which may confound the effect of tannin. In the future, similar work should be conducted during spring or summer to decrease the possible confounding effects of low temperature on response variables.

Experiment 3 further explained the form of the tannin that may potentially decrease CH_4 production. The smaller molecular weight of HT, GA, decreased CH_4 production and increased VFA concentration; however, in Experiment 3, the 1.5% CNQ tannin treatment, which is from a larger molecular weight of HT and CT mix tended to decrease CH_4 production and increased butyrate concentration in the rumen. Both HT and CT can be hydrolyzed into their smaller units and fermented by rumen microbes to butyrate, and so the decrease in CH_4 emission and the corresponding increase in butyrate concentration in Experiment 2 by the 1.5% CNQ was due to greater amount of metabolites from tannin. The small molecular weight tannins may prevent binding to larger protein molecules but they may be able to penetrate through the cells of methanogens and disrupt their activities. Thus, future work should explore the effect of GA and other tannin metabolites or sub-units such as pyrogallol or catechin on methanogens and enteric CH_4 reduction in beef cattle. Again, the shift in N excretion from urine to feces and the decreased urea-N (% of urinary N) by the different complex forms of HT coupled with GA decreasing uric acid-N fractions of urine should be studied further for their potential to decrease CH_4 and N_2O emission from manure.

The goal of silage making is to harvest a forage crop and preserve its high nutrient contents (protein, vitamins and minerals), digestibility, and palatability. Alfalfa is a forage crop with high soluble CP content, and during long periods of storage (> 60 d), alfalfa silage is prone to heat damage and volatilization of NH_3 and other nitrogenous compound by microbes (Rotz and Muck, 1994), which can reduce the CP content of the silage. The alfalfa silage used for Experiments 2 and 3 was stored for 60 d during ensiling and CP content of the ensiled material was not different from the fresh alfalfa forage. Genetic differences in protein degradation by microbes have been detected in alfalfa germplasm (Broderick and Buxton 1991), suggesting the potential for genetic engineering to add tannin to alfalfa, which only has tannin in the seed coat, to alter proteolysis during storage as silage or protein degradation in the rumen (Grabber et al., 2002). This effect coupled with the ability of tannin to decrease CH_4 production may mean that genetic engineered alfalfa containing-tannin may improve animal productivity while protecting the environment.

5.3. Industry Perspective and Conclusion

Forages play an important role in the Canadian beef and dairy cattle industries. Barley is a widely grown crop for silage production in AB; however, many farmers are switching to corn for silage production to produce milk and meat, for a number of reasons. These include: 1) lodging and leaf diseases (e.g. net blotch and scald), which affect both grain and silage yields of barley (McCartney, 2015); 2) because of its higher starch and digestible energy content, CS improves the performance of animals in comparison to barley silage (Beauchemin and McGinn, 2005; Addah et al., 2011); 3) its higher starch content means CS has the potential to reduce CH_4 emissions (Benchaar et al., 2014); and 4) because mean temperature during the growing season is increasing in AB (Gray and Hamann, 2015) due to global warming, cultivation of corn, which is

a warm-season crop, is increasing in colder areas of AB. The increased acceptance of growing CS by farmers to feed cattle has predominantly influenced the introduction of short-seasoned CS hybrids with ≤ 2600 CHU in adapted areas of western Canada.

Alfalfa is also a forage of choice, especially when grown in mixtures with grasses, in many Canadian beef cattle diets. However, alfalfa-based diets can increase CH_4 production and increase soluble protein intake compared with starch containing forages. The high soluble protein intake can increase rumen NH_3 concentration due to high rumen CP degradation and increase N excretion in urine resulting in N-use inefficiency.

Through technological and management advancements, the carbon footprint (i.e., kg of CO_2e/kg animal product) of the Canadian beef industry has been reduced by 15% between 1981 and 2011 (Legesse et al., 2015). There is growing interest in decreasing the carbon footprint of animal agriculture, but farmers will not adopt mitigation strategies that call for an additional cost, unless there are economic benefits or government support. Nutritional strategies can be economical and effective methods of reducing CH_4 per unit of animal product, but lack of knowledge hinders implementation. With consumer preferences for forage fed-beef, gaining more knowledge on nutritional strategies related to forage based diets may expedite their implementation.

With the advent of carbon credits to beef producers (i.e. consumers willingness to buy one tonne of CO_2e that hasn't been emitted by beef producers), the carbon footprint of the beef industry could be reduced further. Feeding grain to beef cattle is an option producers may choose to reduce the environmental impact of beef cattle with the carbon credits they receive; however, with the increase in prices and competition for grain coupled with consumer preferences for forage-fed beef (Conner and Oppenheim, 2008), use of high energy CS hybrids with low CH_4

potential as shown by this thesis, is a suitable option for producers to reduce their opportunity cost while protecting the environment.

Inorganic N fertilizer application accounted for 22% of total agricultural GHG emission in 2016 (Environment and Climate Change Canada, 2018). Inorganic N fertilizer manufacturing coupled with transport and usage by producers, are indirect and direct sources of CO_2 that contribute to the carbon footprint of the Canadian beef industry. By switching to manure application with a low quantity of urinary urea-N from cattle, both the financial and environmental costs associated with inorganic N fertilizer may be reduced by producers. This effect may in turn, reduce the national GHG emission. This thesis has demonstrated that the urine of cattle fed an alfalfa-based diet supplemented with tannin contains lower urea-N fraction, and if adopted by producers may lower the carbon footprint of agricultural emissions relative to inorganic N fertilizer.

Therefore, for short-season CS, selecting hybrids that are adapted to a particular location in AB with the potential to decrease CH_4 production may decrease the carbon footprint of beef cattle production. For alfalfa, adding tannin as a nutritional supplement may decrease CH_4 production and N excretion in Canadian beef cattle.

The thesis demonstrates that harvesting short-seasoned WPCH adapted to the cooler central AB region after frost increased starch concentration without affecting NDF concentration and DMD, but it lowered CH_4 emissions. Again, among the hybrids harvested in the cooler central AB, the earliest hybrids produced lower CH_4 production than the latest hybrids. Harvesting southern AB hybrids after frost did not affect starch or NDF concentration, but increased NDFD, with no effect on CH_4 production. Thus, in conclusion for short-season CS, it was possible to select hybrids adapted in central AB that reduce the carbon footprint of animal

agriculture but hybrid selection had minimal effect on CH_4 emissions for southern AB hybrids. Adding HT to an alfalfa silage based diet decreased ruminal NH_3-N concentration and combining it with CT showed a potential to decrease CH_4 production. However, it was the smaller molecular weight of HT that decreased CH_4 production and decreased both the urea N and uric acid N fractions of urine. Therefore, for alfalfa silage based diets supplemented with tannin, the simplest unit or metabolite of tannin has the potential to lower CH_4 and NH_3 emissions without any detrimental effect on beef cattle performance.

5.4. Literature Cited

Addah, W., J. Baah, P. Groenewegen, E. K. Okine, and T. A. McAllister. 2011. Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. Can. J. Anim. Sci. 91:133–146.

Beauchemin, K. A., and S. M. McGinn. 2005. Methane emissions from feedlot cattle fed barley or corn diets. J. Anim. Sci. 83:653–661.

Benchaar, C., F. Hassanat, R. Gervais, P. Y. Chouinard, H. V. Petit, and D. I. Massé. 2014. Methane production, digestion, ruminal fermentation, nitrogen balance, and milk production of cows fed corn silage- or barley silage-based diets. J. Dairy Sci. 97:961–974.

Bhat, T. K., B. Singh, and O. P. Sharma.1998. Microbial degradation of tannins – a current perspective. Biodegradation 9:343–357.

Bhatta, R., L. Baruah, M. Saravanan, K. P. Suresh, and K. T. Sampath. 2013. Effect of medicinal and aromatic plants on rumen fermentation, protozoa population and *methanogenesis in vitro.* J. Anim. Physiol. Anim. Nutr. 97:446–456.

Bhatta, R., Y. Uyeno, K. Tajima, A. Takenaka, Y. Yabumoto, I. Nonaka, O. Enishi, and M. Kurihara. 2009. Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. J. Dairy Sci. 92:5512–5522.

Broderick, G. A., and D. R. Buxton. 1991. Genetic variation in alfalfa for ruminal protein degradability. Can. J. Plant Sci. 71:755–760.

Carulla, J. E., M. Kreuzer, A. Machmüller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Austr. J. Agric. Res. 56:961–970.

Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J. Galloway, M. Heimann, C. Jones, C. Le Quéré, R.B. Myneni, S. Piao and P. Thornton, 2013: Carbon and Other Biogeochemical Cycles. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Cieslak, A., P. Zmora, E. Pers-Kamczyc, and M. Szumacher-Strabel. 2012. Effects of tannins source (*Vaccinium vitis idaea* L.) on rumen microbial fermentation *in vivo*. Anim. Feed Sci. Technol. 176:102–106.

Conner, D. S., and D. Oppenheim. 2008. Demand for pasture-raised livestock products: results from Michigan surveys. J. Agribusiness. 26:1–20.

Danielsson R, M. Ramin, J. Bertilsson, P. Lund, and P. Huhtanen. 2017. Evaluation of a gas *in vitro* system for predicting methane production *in vivo*. J. Dairy Sci. 100:8881–8894.

Environment and Climate Change Canada. 2018. National Inventory Report 1990-2016: Greenhouse gas sources and sinks in Canada. http://www.publications.gc.ca/site/eng/9.506002/publication.html (Accessed 12 September 2018).

Field, J. A., S. Kortekaas, and G. Lettinga. 1989. The tannin theory of methanogenic toxicity. Biol. Wastes. 29:241–262.

Grabber J, C. Rotz, R. Muck, and D. Mertens. 2002. Tannin-containing alfalfa: a way to improve nitrogen use and profitability of dairy farms? U.S. Dairy Forage Research Center, Madison, WI. https://www.ars.usda.gov/research/publications/publication/?seqNo115=134825. (Accessed 03 December 2018).

Gray, L. K., and A. Hamann. 2015. Projected change in climate for Alberta and forest tree improvement program regions. https://sites.ualberta.ca/~lkgray/uploads/7/3/6/2/7362679/tsarm-climate-modeling-2015-july-16.pdf (Accessed 17 January, 2019)

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

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

Krumholz, L. R., and M. P. Bryant.1986. *Eubacterium oxidoreducen*s sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. Arch. Microbiol. 144:8–14.

Lee, C., and J. Herbek. 2004. Late-season frost damage to corn grown for silage: AGR-183. University of Kentucky. Available: https://www.uky.edu/Ag/Forage/frost%20damage%20to%20corn%20silage.pdf (Accessed 28 August 2017).

Legesse G, K. A. Beauchemin, K. H. Ominski, E. J. McGeough, R., Kroebel, D. MacDonald, S. M. Little, and T. A. McAllister. 2015. Greenhouse gas emissions of Canadian beef production in 1981 as compared with 2011. Anim. Prod. Sci. 56:153−168.

McCartney, D. 2015. What's all the fuss about corn on the prairies? Canadian Cattlemen, www.canadiancattlemen.ca. (Accessed, 28 June 2016).

Moe, P. W., and H. F. Tyrrell. 1979. Methane production in dairy cows. J. Dairy Sci. 62:1583–1586.

Murdiati, T. B., C. S. McSweeney, and J. B., Lowry. 1992. Metabolism in sheep of gallic acid, tannic acid, and hydrolysable tannin from *Terminalia oblongata*. Aust. J. Agric. Res. 43:1307–1319.

Narasimhalu, P, R. P. White, and K. B. MaCrae. 1986. The effect of harvesting before and after frost on corn silage composition and its intake and digestibility in sheep. Can. J. Plant Sci. 66:579–586.

Rotz, C. A., and R. E. Muck. 1994. Changes in forage quality during harvest and storage. In Fahey, G. C. J., M. Collins, D. R. Mertens, and L. E. Moser, eds. Forage quality, evaluation, and utilization, ASA, Madison, WI, USA, pp. 828–868.

Slewinski, T. L. 2012. Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. J. Exp. Bot. 63:4647–4670.

White, R. P., K. A. Winter, and H. T. Kunelius. 1976. Yield and quality of silage corn as affected by frost and harvest date. Can. J. Plant Sci. 56:481–486.

LITERATURE CITED

Aboagye, I. A., M. Oba, A. R. Castillo, K. M. Koenig, and K. A. Beauchemin. 2018. Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high forage diet. J. Anim. Sci. 96:5276-5286.

Addah, W., J. Baah, P. Groenewegen, E. K. Okine, and T. A. McAllister. 2011. Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. Can. J. Anim. Sci. 91:133–146.

Aguerre, M. J., M. C. Capozzolo, P. Lencioni, C. Cabral, and M. A. Wattiaux. 2016. Effect of quebracho-chestnut tannin extracts at 2 dietary crude protein levels on performance, rumen fermentation, and nitrogen partitioning in dairy cows. J. Dairy Sci. 99: 4476–4486.

Aguerre, M. J., B. Duvall, J. M. Powell, P. Vadas, and M. A. Wattiaux. 2015. Effects of dietary tannin extracts levels during a thirteen-week period on lactating cow performance and N use efficiency. J. Anim. Sci. 93, (Suppl. 3) / J. Dairy Sci. 98(Suppl.2):775 (Abstr.).

Allen, M. S. 2012. Adjusting concentration and ruminal digestibility of starch through lactation. In Proc. of the Four-State Dairy Nutr. Managt. Conf., Madison, WI. p. 24–30.

Allen, M. S., J. G. Coors, and G. W. Roth. 2003. Corn silage. In Silage Science and Technology, D. Buxton, R. Muck, and J. Harrison, eds. ASA, CSSA, and SSA, Madison, WI.

Allison, M. J., and J. L. Peel. 1971. The biosynthesis of valine from isobutyrate by *Peptostreptococcus elsdenii* and *Bacteroides ruminicola*. Biochem. J. 121:431–437

Alonso-Díaz, M. A., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Hoste, H., 2010. Tannins in tropical tree fodders fed to small ruminants: A friendly foe? Small. Ruminant Res. 89: 164-173.

Archimède, H., M. Eugène, C. M. Magdeleine, M. Boval, C. Martin, D. P. Morgavi, P. Lecomte, and M. Doreau. 2011. Comparison of methane production between C3 and C4 grasses and legumes. Anim. Feed Sci. Technol. 166:59–64.

Arndt, C., J. M. Powell, M. J. Aguerre, and M. A. Wattiaux. 2015. Performance, digestion, nitrogen balance, and emission of manure ammonia, enteric methane, and carbon dioxide in lactating cows fed diets with varying alfalfa silage-to-corn silage ratios. J. Dairy Sci. 98:418–430.

Association of Official Analytical Chemists. 2005. Official methods of analysis, 18th ed. AOAC Int., Gaithersburg, MD.

Baldwin, R. L., and M. J. Allison. 1983. Rumen metabolism. J. Anim. Sci. 57(Suppl. 2):461–477.

Baron, V. S., K. A. Beauchemin, P. Juskiw, and R. R. Doce. 2014. Managing short-season corn and small-grain silage in Western Canada. In Proc. of the 35th Western Nutrition Conference, 24 – 25 Sept. 2014, Edmonton, AB. p. 147–162.

Baron, V. S., T. B. Daynard, G. Dupuis, N. A. Fairey, D. J. Major, J. F. Muldoon, and R. P. White. 1987. Evaluation of early-maturing European and Canadian corn hybrids for grain and forage production in Canada. Maydica. XXXII: 33–48.

Baron, V. S., M. Oba, and A. Aasen. 2008. Challenges for growing corn silage suitable for the dairy industry in a northern climate. WCDS Advances Dairy Technol. 20:181–192.

Beauchemin, K. A., H. H. Janzen, S. M. Little, T. A. McAllister, and S. M. McGinn. 2010. Life cycle assessment of greenhouse gas emissions from beef production in western Canada: A case study. Agric. Syst. 103:371–379.

Beauchemin, K.A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48:21–27.

Beauchemin, K. A., and S. M. McGinn. 2005. Methane emissions from feedlot cattle fed barley or corn diets. J. Anim. Sci. 83:653–661.

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

Beauchemin, K. A., and S. M. McGinn. 2008**.** Reducing methane in dairy and beef cattle operations: What is feasible? Prairie Soil Crop 1:17–21.

Beauchemin, K. A., S. M. McGinn, T. F. Martinez, and T. A. McAllister. 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. J. Anim. Sci. 85:1990–1996.

Becker, P. M., P. G. Wikselaar, M. C. R. Franssen, R. C. H. Vos, R. D. Hall, and J. Beekwilder. 2014. Evidence for a hydrogen-sink mechanism of (+) catechin-mediated emission reduction of the ruminant greenhouse gas methane. Metabolomics 10:179–189.

Benchaar, C., F. Hassanat, R. Gervais, P. Y. Chouinard, H. V. Petit, and D. I. Massé. 2014. Methane production, digestion, ruminal fermentation, nitrogen balance, and milk production of cows fed corn silage- or barley silage-based diets. J. Dairy Sci. 97:961–974.

Berard, N. C., Y. Wang, K. M. Wittenberg, D. O. Krause, B. E. Coulman, T.A McAllister, and K. H. Ominski. 2011. Condensed tannin concentrations found in vegetative and mature forage legumes grown in western Canada. Can. J. Plant Sci. 91:669–675.

Bhat, T. K., B. Singh, and O. P. Sharma.1998. Microbial degradation of tannins – a current perspective. Biodegradation 9:343–357.

Bhatta, R., M. Saravanan, L. Baruah, K. Sampath, and C. S. Prasad. 2013. Effect of plant secondary compounds on *in vitro* methane, ammonia production and ruminal protozoa population. J. Appl. Microbiol. 115:455–465.

Bhatta, R., Y. Uyeno, K. Tajima, A. Takenaka, Y. Yabumoto, I. Nonaka, O. Enishi, and M. Kurihara. 2009. Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. J. Dairy Sci.

Blaxter, K. L., and J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19:511–522.

Blümmel M., D. I. Givens, and A. R. Moss. 2005. Comparison of methane produced by straw fed sheep in open-circuit respiration with methane predicted by fermentation characteristics measured by an *in vitro* gas procedure. Anim. Feed Sci. Technol. 123–124:379–390

Broderick, G.A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370–1381.

Brown, D.M. and Bootsma, A. 1993. Crop heat units for corn and other warm-season crops in Ontario. Ontario Ministry of Agriculture and Food Factsheet No. 93–119, Agdex 111/31. p. 4. [Online] Available: http://www.omafra.gov.on.ca/english/crops/facts/93-119.htm (Accessed 16 September 2016).

Byers, F. M., and A. L. Moxon. 1980. Protein and selenium levels for growing and finishing beef cattle. J. Anim. Sci. 50:1136–1144.

Cammell, S. B., J. D. Sutton, D. E. Beever, D. J. Humphries, and R. H. Phipps. 2000. The effect of crop maturity on the nutritional value of maize silage for lactating dairy cows. Anim. Sci. 71: 381–390.

Calsamiglia, S., A. Ferret, C. K. Reynolds, N. B. Kristensen, and A. M. Van Vuuren. 2010. Strategies for optimizing nitrogen use by ruminants. Animal 4:1184–1196.

Canadian Council on Animal Care (CCAC). 2009. CCAC guidelines on: The care and use of farm animals in research, teaching and testing. Ottawa, ON, Canada.

Capper, J. L. 2012. Is the grass always greener? Comparing the environmental impact of conventional, natural and grass-fed beef production systems. Animals. 2:127–143.

Capper, J. L., and D. E. Bauman. The role of productivity in improving the environmental sustainability of ruminant production systems. Annu. Rev. Anim. Biosci. 1:469–489

Carrasco, J. M. D., C. Cabral, L. M. Redondo, N. D. P. Viso, D. Colombatto, M. D. Farber, and M. E. F. Miyakawa. 2017. Impact of chestnut and quebracho tannins on rumen microbiota of bovines. BioMed. Res. Int. 2017:11.

Carulla, J. E., M. Kreuzer, A. Machmüller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Austr. J. Agric. Res. 56:961–970.

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

Chen, X. B, and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on purine derivatives: an overview of technical details. Int. Feed Res. Unit. Rowett Res. Inst. Bucksburn Aberdeen, UK. p. 1–21.

Chiarini A, M., Micucci, M. Malaguti, R. Budriesi, P. Ioan, M. Lenzi, C. Fimognari, , T. G. Toschi, P. Comandini, and S. Hrelia. 2013. Sweet chestnut (Castanea sativa Mill.) bark

extract: Cardiovascular activity and myocyte protection against oxidative damage. Oxid. Med. Cell Longev. 2013:471790

Chung, Y.-H., N. D. Walker, S. M. McGinn, and K. A. Beauchemin. 2011. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. J. Dairy Sci. 94:2431–2439.

Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J. Galloway, M. Heimann, C. Jones, C. Le Quéré, R.B. Myneni, S. Piao and P. Thornton, 2013: Carbon and Other Biogeochemical Cycles. In: Climate Change 2013: The physical science basis. contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and NY, USA.

Cieslak, A., P. Zmora, E. Pers-Kamczyc, and M. Szumacher-Strabel. 2012. Effects of tannins source (*Vaccinium vitis idaea* L.) on rumen microbial fermentation *in vivo*. Anim. Feed Sci. Technol. 176:102–106.

Cole, N. A., and R. W. Todd. 2009. Nitrogen and phosphorus balance of beef cattle feedyards. In: E. Jordan, ed. Proceedings of the Texas Anim. Manure Manage. Iss. Conf., Sept. 29-30. Round Rock, TX. p. 17–24.

Cottle, D. J., J. V. Nolan, and S. G. Wiedemann. 2011. Ruminant enteric methane mitigation: A review. Anim. Prod. Sci. 51:491–514.

Cyriac, J., A. G. Rius, M. L. McGilliard, R. E. Pearson, B. J. Bequette, and M. D. Hanigan. 2008. Lactation performance of mid-lactation dairy cows fed ruminally degradable protein

at concentrations lower than National Research Council recommendations. J. Dairy Sci. 91:4704–4713.

Danielsson R, M. Ramin, J. Bertilsson, P. Lund, and P. Huhtanen. 2017. Evaluation of a gas *in vitro* system for predicting methane production *in vivo*. J. Dairy Sci. 100:8881–8894.

Darby, H. M., and J. G. Lauer. 2002. Harvest date and hybrid influence on corn forage yield, quality, and preservation. Agron. J. 94:559–566.

Daynard, T. B. 1978. Practices affecting quality and preservation of whole plant corn silage. Can. J. Plant Sci 58:651–659.

Daynard, T. B., and R. B. Hunter. 1974. Relationships among whole-plant moisture, grain moisture, dry matter yield and quality of whole-plant corn silage. Can. J. Plant Sci. 55:77–84.

de Klein, C. A. M., and R. J. Eckard. 2008. Targeted technologies for nitrous oxide abatement from animal agriculture. Aust. J. Exp. Agric. 48:14–20.

Department for Environment, Food and Rural Affairs (DEFRA). 2010. Ruminant nutrition regimes to reduce methane and nitrogen emissions. Project AC0209 Report. DEFRA, Procurements and Contracts Division (Science R and D Team). http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=14952 (Accessed 11 November 2018).

Deppenmeier, U. 2002. The unique biochemistry of methanogenesis. Prog. Nucleic Acid Res. Mol. Biol. 71:223–283.

Deschamps A. M., G. Mohudeau, M. Conti, and J. M. Lebeault. 1980. Bacteria degrading tannic acid and related compounds. J. Ferment Technol. 58:93–97.

Dijkstra, J, E. Kebreab, A. Bannink, J. France, and S. López. 2005. Application of the gas production technique to feed evaluation systems for ruminants. Anim. Feed Sci. Technol. 123–124:561–578.

Dijkstra, J., O. Oenema, J. W. van Groenigen, J. W. Spek, A.M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N_2O emissions. Animal 7 (Suppl. 2):292–302.

Djakpo, O, W. R. Yao. 2010. Rhus chinensis and Galla chinensis —folklore to modern evidence: review. Phytother. Res. 24:1739–1747.

Dwyer, L. M., D. W. Stewart, L. Carrigan, B. L. Ma, P. Neave, and D. Balchin. 1999. Guidelines for comparisons among different maize maturity rating systems. Agron. J. 91: 946–949.

Ebert, P. J., E. A. Bailey, A. L. Shreck, J. S. Jennings, and N. A., Cole. 2017. Effect of condensed tannin extract supplementation on growth performance, nitrogen balance, gas emissions, and energetic losses of beef steers. J. Anim. Sci. 95:1345–1355.

Eckard, R. J., C. Grainger, and C. A. M. de Klein. 2010. Options for the abatement of methane and nitrous oxide from ruminant production: A review. Livest. Sci. 130:47–56.

Eisler, M. C., M. R. F. Lee, J. F. Tarlton, G. B. Martin, J. Beddington, J. A. J. Dungait, H. Greathead, J. Liu, S. Mathew, and H. Miller. 2014. Agriculture: Steps to sustainable livestock. Nature. 507:32–34.

Ellis, J. L., J. Dijkstra, E. Kebreab, A. Bannink, N. E. Odongo, B. W. McBride, and J. France. 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agr. Sci. 146:213–233.

Ellis, J. L., E. Kebreab, N. E. Odongo, B. W. McBride, E. K. Okine, and J. France. 2007. Prediction of methane production from dairy and beef cattle. J. Dairy Sci. 90:3456–3467

Environment and Climate Change Canada. 2018. National Inventory Report 1990-2016: Greenhouse gas sources and sinks in Canada. http://www.publications.gc.ca/site/eng/9.506002/publication.html (Accessed 12 September 2018)

Fanchone, A., P. Nozière, J. Portelli, B. Duriot, V. Largeau, and M. Doreau. 2013. Effects of nitrogen underfeeding and energy source on nitrogen ruminal metabolism, digestion and nitrogen partitioning in dairy cows. J. Anim. Sci. 91:895–906.

Fernandez, I., P. Noziere, and B. Michalet-Doreau. 2004. Site and extent of starch digestion of whole-plant maize silages differing in maturity stage and chop length, in dairy cows. Livest. Prod. Sci. 89:147–157.

Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2013. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. J. Dairy Sci. 96:533–550.

Ferraretto, L. F., and R. D. Shaver. 2012. Meta-analysis: Impact of corn silage harvest practices on intake, digestion, and milk production by dairy cows. Prof. Anim. Sci. 28:141–149.

Ferris, C. P., F. J. Gordon, D. C. Patterson, M. G. Porter, and T. Yan. 1999. The effect of genetic merit and concentrate proportion in the diet on nutrient utilisation by lactating dairy cows. J. Agric. Sci. 132:483–490.

Field, J. A., S. Kortekaas, and G. Lettinga. 1989. The tannin theory of methanogenic toxicity. Biol. Wastes. 29:241–262.

Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. J. Dairy Sci. 80:1426–1437.

Follett, R. F., and J. L. Hatfield. 2001. Nitrogen in the environment: sources, problems, and management. In optimizing nitrogen management in food and energy production and environmental protection: Proceedings of the 2nd International Nitrogen Conference on Science and Policy. The Scientific World 1(S2):920–926.

Food and Agriculture Organization (FAO). 2006. Livestock's long shadow: environmental issues and options. Rome, Italy.

Food and Agricultural Organization (FAO). 2009. The state of food and agriculture: livestock in the balance. Rome, Italy.

Food and Agricultural Organization (FAO). 2011. Mapping supply and demand for animal-source foods to 2030. In Robinson T. S, Pozzi F, ed. Animal Production and Health Working Paper. N. 2, Rome, Italy.

Food and Agriculture Organization (FAO). 2015. Environmental performance of large ruminant supply chains: guidelines for assessment. Rome, Italy.

Frutos, P., M. Raso, G. Hervás, A. Mantecón, V. Pérez, and F. J. Giráldez. 2004. Is there any detrimental effect when a chestnut hydrolysable tannin extract is included in the diet of finishing lambs? Anim. Res. 53:127–136.

Gabruch, M., and R. Gietz. 2014. The potential of grain corn in Alberta. Alberta Agriculture and Forestry. Available http://www1.agric.gov.ab.ca/4Department/deptdocs.nsf/all/bus15031 (Accessed 4 November 2017).

Gerber, P. J., A. Mottet, B. Henderson, A. Mottet, C. Opio, A. Falcucci, and F. Teillard. 2013. Environmental impacts of beef production: Review of challenges and perspectives for durability. Meat Sci. 109:2–12.

Gerber, P. J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, and G. Tempio. 2013.Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. FAO, Rome, Italy.

Getachew, G., W. Pittroff, D. H. Putnam, A. Dandekar, S. Goyal, and E. J. DePeters. 2008. The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis. Anim. Feed Sci. Technol. 140:444–461.

Gladine, C., E. Rock, C. Morand, D. Bauchart, and D. Durand. 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. Br. J. Nutr. 98:691–701.

Goel, G., and H. S. Makkar. 2012. Methane mitigation from ruminants using tannins and saponins. Trop. Anim. Health Prod. 44:729–739.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis. Agric. Handbook No. 379. Agricultural Research Service, USDA, Washington, DC.

Goopy J. P., C. Chang, and N. Tomkins. 2016. A Comparison of Methodologies for Measuring Methane Emissions from Ruminants. In: Rosenstock T., Rufino M., Butterbach-Bahl K., Wollenberg L., Richards M. eds. Methods for measuring greenhouse gas balances and evaluating mitigation options in smallholder agriculture. Springer, Cham.

Grabber J, C. Rotz, R. Muck, and D. Mertens. 2002. Tannin-containing alfalfa: a way to improve nitrogen use and profitability of dairy farms? U.S. Dairy Forage Research Center, Madison,

WI. https://www.ars.usda.gov/research/publications/publication/?seqNo115=134825. (Accessed 03 December 2018).

Grainger, C., T. Clarke, M. J. Auldist, K. A. Beauchemin, S. M. McGinn, G. C. Waghorn, and R. J. Eckard. 2009. Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Can. J. Anim. Sci. 89:241–251.

Grainger, C., T. Clarke, S. M. McGinn, M. J. Auldist, K. A. Beauchemin, M. C. Hannah, G. C. Waghorn, H. Clark, and R. J. Eckard. 2007. Methane emissions from dairy cows measured using the sulfurhexafluoride (SF_6) tracer and chamber techniques. J. Dairy Sci. 90:2755–2766.

Gray, L. K., and A. Hamann. 2015. Projected change in climate for Alberta and forest tree improvement program regions. https://sites.ualberta.ca/~lkgray/uploads/7/3/6/2/7362679/tsarm-climate-modeling-2015-july-16.pdf (Accessed 17 January, 2019)

Guyader, J., V. S. Baron, and K. A. Beauchemin. 2018. Corn forage yield and quality for silage in short growing season areas of the Canadian prairies. Agronomy 8:1-25.

Guyader, J., H. H. Janzen, R. Kroebel, and K.A. Beauchemin. 2016. Invited Review: Forage utilization to improve environmental sustainability of ruminant production. J. Anim. Sci. 94:3147–3158.

Guyader, J., E. M. Ungerfeld, and K. A. Beauchemin. 2017. Redirection of metabolic hydrogen by inhibiting methanogenesis in the rumen simulation technique (RUSITEC). Front. Microbiol. 8:1–16.

Hagerman, A. E. 2011. Tannin Handbook, Department of Chemistry and Biochemistry, Miami University, USA. http://www.users.muohio.edu/hagermae (Accessed 17 October 2017).

Hagerman, A. E., C. T. Robbins, Y. Weerasuriya, T. Wilson, and C. McArthur. 1992. Tannin chemistry in relation to digestion. J. Range Manag. 45:57–62.

Hammond, K. J., S. O. Hoskin, J. L. Burke, G. C. Waghorn, J. P. Koolaard, and S. Muetzel. 2011. Effects of feeding fresh white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*) on enteric methane emissions from sheep. Anim. Feed Sci. Technol. 166–167:398–404.

Hassanat, F., R. Gervais, C. Julien, D. I. Masse, A. Lettat, P. Y. Chouinard, H. V. Petit, and C. Benchaar. 2013. Replacing alfalfa silage with corn silage in dairy cow diets: Effects on enteric methane production, ruminal fermentation, digestion, nitrogen balance, and milk production. J. Dairy Sci. 96:4553–4567.

Hatew, B., A. Bannink, H. Van Laar, L. H. De Jonge, and J. Dijkstra. 2016. Increasing harvest maturity of whole-plant corn silage reduces methane emission of lactating dairy cows. J. Dairy Sci. 99:354−368

Hegarty, R. S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. Aust. J. Agric. Res. 50:1321–1327.

Hegarty, R. S., and R. Gerdes. 1999. Hydrogen production and transfer in the rumen. Rec. Adv. Anim. Nutr. Austr. 12:37–44.

Henry, B. K., R. J. Eckard, and K. A. Beauchemin. 2018. Review: Adaptation of ruminant livestock production systems to climate changes. Animal. 12(Suppl2):S445-S456.

Holter, J. B., and A. J. Young. 1992. Methane prediction in dry and lactating Holstein cows. J. Dairy Sci. 75:165–2175.

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

Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico. 2013. Special topics-Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069.

Huggins-Rawlins, N. 2015. Agroclimatic atlas of Alberta. Alberta Agriculture and Forestry. Available http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sag6301 (Accessed 01 November 2016).

Intergovernmental Panel on Climate Change (IPCC). 2006. Guidelines for national greenhouse gas inventories. Prepared by the National Greenhouse Gas Inventories Programme, IGES, Hayama, Kanagawa, Japan.

Intergovernmental Panel on Climate Change (IPCC). 2007. Changes in atmospheric constituents and in radiative forcing. in climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change.

Intergovernmental Panel on Climate Change (IPCC). 2013: Summary for policymakers. In: Climate Change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and NY, USA.

Ishler, V., and G. Varga. 2001. Carbohydrate nutrition for lactating dairy cattle. Dept. Dairy Anim. Sci. Pennsylvania State University. pp. 1–29.

Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160:1–22.

Jayanegara A., F. Leiber, and M. Kreuzer. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from *in vivo* and *in vitro* experiments. J. Anim. Physiol. Anim. Nutr. 96:365–375.

Jayanegara, A., G. Goel, H. P. S. Makkar, and K. Becker. 2015: Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol. 209:60–68.

Jerónimo, E., C. Pinheiro, E. Lamy, M. T. Dentinho, E. Sales-Baptista, O. Lopes, and F. C. Silva. 2016. Tannins in ruminant nutrition: Impact on animal performance and quality of edible products. In Tannins: Biochemistry, Food Sources and Nutritional Properties; Combs, C.A., Ed.; Nova Science Publisher Inc.: Hauppauge, NY, USA. pp. 121–168.

Johnson, K., M. Huyler, H. Westberg, B. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. Environ. Sci. Technol. 28:359–362.

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

Johnson, J. W., and R. L. Preston. 1995. Minimizing nitrogen waste by measuring plasma urea-N levels in steers fed different dietary crude protein levels. In: Texas Tech Univ. Res. Rep. T-5-355. Texas Tech Univ., Lubbock, TX. p. 62–63.

Kasuya, H., and J. Takahashi. 2010. Methane emissions from dry cows fed grass or legume silage. Asian-Australas. J. Anim. 23:563−566.

Kawamoto H., F. Nakatsubo, and K. Murakami. 1995. Quantitative determination of tannin and protein in the precipitates by high-performance liquid chromatography. Phytochemistry 40:1503−1505.

Kennedy, P. M., and L. P. Milligan. 1978. Transfer of urea from the blood to the rumen of sheep. Br. J. Nutr. 40:149–154.

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

Kiro, R. P. 2017. Assessment of the rumen fluid of a bovine patient. Dairy Vet. Sci. J. 2:555−588.

Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231–3261.

Koenig, K. M., S. M. McGinn, and K. A. Beauchemin. 2013. Ammonia emissions and performance of backgrounding and finishing beef feedlot cattle fed barley-based diets varying in dietary crude protein concentration and rumen degradability. J. Anim. Sci. 91:2278–2294.

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

Krueger, W. K., H. Gutierrez-Bañuelos, G. E. Carstens, B. Min, W. Pinchak, R. Gomez, R. C. Anderson, N. K. Krueger, and T. D. A. Forbes. 2010. Effects of dietary tannin source on

performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain. Anim. Feed Sci. Technol. 159:1–9.

Krumholz, L. R., and M. P. Bryant.1986. *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. Arch. Microbiol. 144:8–14.

Kumar, R., and M. Singh. 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food Chem. 32:447–453.

Lamy, E., G. da Costa, R. Santos, F. Capela e Silva, J. Potes, A. Pereira, A. V. Coelho, and E. Sales Baptista. 2011. Effect of condensed tannin ingestion in sheep and goat parotid saliva proteome. J. Anim. Physiol. Anim. Nutr.95:304–312.

Lee, C., and J. Herbek. 2004. Late-season frost damage to corn grown for silage: AGR-183. University of Kentucky. Available: https://www.uky.edu/Ag/Forage/frost%20damage%20to%20corn%20silage.pdf (Accessed 28 August 2017).

Lee, C., A. N. Hristov, C. J. Dell, G. W. Feyereisen, J. Kaye, and D. Beegle. 2012. Effect of dietary protein concentration on ammonia and greenhouse gas emissions from dairy manure. J. Dairy Sci. 95:1930–1941.

Legesse G, K. A. Beauchemin, K. H. Ominski, E. J. McGeough, R., Kroebel, D. MacDonald, S. M. Little, and T. A. McAllister. 2015. Greenhouse gas emissions of Canadian beef production in 1981 as compared with 2011. Anim. Prod. Sci. 56:153−168.

Leng, R. A. 1993. Quantitative ruminant nutrition—A green science. Aust. J. Agric. Res. 44:363–380.

Leng, R. A. 2018. Unravelling methanogenesis in ruminants, horses and kangaroos: the links between gut anatomy, microbial biofilms and host immunity. Anim. Prod. Sci. **58**:1175–1191.

Lesschaeve, I., and A. C. Noble. 2005. Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. Am J. Clin. Nutr. 81:330S-335S.

Li, L., J. Cyriac, K. F. Knowlton, L. C. Marr, S. W. Gay, M. D. Hanigan, and J. Arogo Ogejo. 2009. Effects of reducing dietary nitrogen on ammonia emissions from manure on the floor of a naturally ventilated free stall dairy barn at low (0-20ºC) temperatures. J. Environ. Qual. 38:2172−2181.

Liu, H., V. Vaddella, and D. Zhou. 2011. Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. J. Dairy Sci. 94:6069–6077.

MacAdam, J. W., and J. J. Villalba. 2015. Beneficial effects of temperate forage legumes that contain condensed tannins. Agriculture. 5:475–491.

Makkar, H. P. S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Res. 49:241−256.

Martin, N. P., D. R. Mertens, M. B. Hall, and J. G. Lauer. 2008. Fiber digestibility and starch content of corn silage. In Proc. of the Idaho Alfalfa and Forage Conference. p. 26–27.

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

Mauricio, R. M., F. L. Mould, M. S. Dhanoa, E. Owen, K. S. Channa, K.S. and M. Theodorou. 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. Anim. Feed Sci. Technol. 79:321–330.

McCartney, D. 2015. What's all the fuss about corn on the prairies? Canadian Cattlemen, www.canadiancattlemen.ca. (Accessed, 28 June 2016).

McCartney, C. A., I. D. Bull, and R. J. Dewhurst. 2012. Archaeol concentration in total rumen contents of cows offered diets based on ryegrass or white clover. In: Proc. of the Irish Agric. Res. Forum. Tullamore, Ireland. p. 99.

McGeough, E. J., P. O'Kiely, P. A. Foley, K. J. Hart, T. M. Boland, and D. A. Kenny. 2010. Methane emissions, feed intake, and performance of finishing beef cattle offered maize silages harvested at 4 different stages of maturity. J. Anim. Sci. 88:1479–1491.

McGinn, S. M., K. A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82:3346–3356.

McGinn, S. M, K. A. Beauchemin, T. K. Flesch, and T. Coates. 2009. Performance of a dispersion model to estimate methane loss from cattle in pens. J. Environ. Qual. 38:1796–1802.

McGinn, S. M., K. A. Beauchemin, A. D. Iwaasa, and T. A. McAllister. 2006. Assessment of the sulfur hexafluoride (SF_6) tracer technique for measuring enteric methane emissions from cattle. J. Environ. Qual. 35:1686–1691.

McLeod, N. M. 1974. Plant tannins - their role in forage quality. Nutr. Abstr. Rev. 44:803–815.

Menke, K. H., L. Raab, A. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs

from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. 93:217–222.

Min, B. R., T. N. Barry, G. T. Attwood, and W. C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperature forages. A review. Anim. Feed Sci. Technol. 106:3-19.

Moe, P. W., and H. F. Tyrrell. 1979. Methane production in dairy cows. J. Dairy Sci. 62:1583–1586.

Morgavi, D. P., E. Forano, C. Martin, and C. J. Newbold. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal. 4:1024–1036.

Mueller-Harvey, I. 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86:2010–2037.

Murdiati, T. B., C. S. McSweeney, and J. B., Lowry. 1992. Metabolism in sheep of gallic acid, tannic acid, and hydrolysable tannin from *Terminalia oblongata*. Aust. J. Agric. Res. 43:1307–1319.

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

Narasimhalu, P, R. P. White, and K. B. MaCrae. 1986. The effect of harvesting before and after frost on corn silage composition and its intake and digestibility in sheep. Can. J. Plant Sci. 66:579–586.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient requirements of beef cattle. 8th rev. ed. National Academies Press, Washington, DC.

National Research Council (NRC). 2001. Nutrient requirements of dairy cattle. 5th rev. ed. National Academy Press. Washington, DC.

Naumann, H. D., L. O. Tedeschi, W. E. Zeller, and N. F. Huntley. 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. R. Bras. Zootec. 46: 929–949.

Osawa R., and T. P. Walsh. 1993. Effects of acidic and alkaline treatments on tannic acid and its binding property to protein. J. Agric. Food Chem. 41:704–7.

Owens, F. 2008. Corn silage – Facts, fantasies and future. Proc. 19th Florida Ruminant Nutrition Symposium, Gainsville, Florida, US. Available: http://vffarms.com/Images/Resources/ProductionChoices/Pioneer_silage_data.pdf. Accessed [12 May 2017].

Patra, A., T. Park, M. Kim, and Z. Yu. 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. J. Anim. Sci. Biotechnol. 8:13.

Patra, A.K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91:24–37.

Paz, H. A., C. L. Anderson, M. J. Muller, P. J. Kononoff, and S. C. Fernando. 2016. Rumen bacterial community composition in Holstein and Jersey cows is different under same dietary condition and is not affected by sampling method. Front. Microbiol. 7:1206.

Pelletier, N., R. Pirog, and R. Rasmussen. 2010. Comparative life cycle environmental impacts of three beef production strategies in the Upper Midwestern United States. Agric. Sys. 103: 380–389.

Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132–2140.

Pinares-Patino, C. S., G. C. Waghorn, R. S. Hegarty, and S. O. Hoskin. 2009. Effects of intensification of pastoral farming on greenhouse gas emissions in New Zealand. N. Z. Vet. J. 57:252–261.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-126, <URL: http://CRAN.R-project.org/package=nlme>.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reed, J. D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73:1516–1528.

Reynolds, C. K., D. J. Humphries, P. Kirton, M. Kindermann, S. Duval, and W. Steinberg. 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. J. Dairy Sci. 97:3777–3789.

Rhine, E. D., G. K. Sims, R. L. Mulvaney, and E. J. Pratt. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Sci. Soc. Am. J. 62:473–480.

Rivera-Méndez, C., A. Plascencia, N. Torrentera, and R. A. Zinn. 2016. Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. J. Appl. Anim. Res. 45:199–203.

Romero-Perez, A., E. K. Okine, S. M. McGinn, L. L. Guan, M. Oba, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2015. Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. J. Anim. Sci. 93:1780–1791.

Rotz, C.A., and R. E. Muck. 1994. Changes in forage quality during harvest and storage. in: Fahey, G.C.J., Collins, M., Mertens, D.R. Moser, L.E. (Eds.), Forage Quality, Evaluation, and Utilization, ASA, Madison, WI, USA, pp. 828-868.

Ruiz, R., L. O. Tedeschi, J. C. Marini, D. G. Fox, A. N. Pell, G. Jarvis, and J. B. Russell. 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: Evaluation of the Cornell Net Carbohydrate and Protein System ruminal N deficiency adjustment. J. Dairy Sci. 85:2986–2999.

Sauvant, D., and S. Giger-Reverdin. 2009. Modélisation des interactions digestives et de la production de méthane chez les ruminants. (In French.) INRA Prod. Anim. 22:375–384.

Satter, L. D., T. J. Klopfenstein, and G. E. Erickson. 2002. The role of nutrition in reducing nutrient output from ruminants. J. Anim. Sci. 80:143–156.

Sere, C., and S. Steinfeld. 1996. World livestock production systems. Current status, issues and trends. FAO. Rome, Italy.

Slewinski, T. L. 2012. Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. J. Exp. Bot. 63:4647–4670.

Staerfl, S. M., J. O. Zeitz, M. Kreuzer, and C. R. Soliva. 2012. Methane conversion rate of bulls fattened on grass or maize silage as compared with the IPCC default values, and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca and lupine. Agric. Ecosyst. Environ. 148:111–120.

St. Pierre, N. R., R. Bouchard, G. St. Laurent, C. Vinet, and G. L. Roy. 1983. Effects of stage of maturity and frost on nutritive value of com silage for lactating dairy cows. J. Dairy Sci. 66:1466–1473.

Statistics Canada Agriculture Census, 2011. Highlights and analyses. http://www.statcan.gc.ca/pub/95-640-x/2011001/p1/prov/prov-48-eng.htm (Accessed 03 August 2017).

Steinfeld, H., C. de Haan, and H. Blackburn. 1996. Balancing Livestock and the Environment: Summary of the Results of a Multi-Donor Study. Mimeo. The World Bank, Washington, DC.

Tavendale, M. H., L. P. Meagher, D. Pacheco, N. Walker, G. T. Attwood, and S. Sivakumaran. 2005. Methane production from in vitro rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. Anim. Feed Sci. Technol. 123:403–419.

Thauer, R. 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson. Microbiology 144:2377–2406.

Thygesen, L. 2003. Annual total corn heat unit from 1971 to 2000. Alberta and Agriculture Forestry. 2003. Available:
https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sag6442?opendocument (Accessed 25 January 2019)

Torrent, J., and D. E. Johnson, D.E. 1994. Methane production in the large intestine of sheep. In: Energy metabolism of farm animals. Aquilera, J. F ed. CSIC Publishing Service. Granada, Spain. pp. 391–394.

Ungerfeld, E. M. 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. Front. Microbiol. 6:1–17.

United Nations (UN). 2017. Department of Economic and Social Affairs. Population Division. World Population Prospects, 2017. Available from:

http://esa.un.org/unpd/wpp/Graphs/Probabilistic/POP/TOT/. (Accessed 07 September, 2018).

van Dorland, H. A., H. R. Wettstein, H. Leuenberger, and M. Kreuzer. 2007. Effect of supplementation of fresh and ensiled clovers to ryegrass on nitrogen loss and methane emission of dairy cows. Livest. Sci. 111:57–69.

van Lingen, H. J., C. M. Plugge, J. G. Fadel, E. Kebreab, A. Bannink, and J. Dijkstra. 2016. Thermodynamic driving force of hydrogen on rumen microbial metabolism: A theoretical investigation. PLoS One 11:e0161362.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd rev. ed. Cornell University Press, Ithaca, NY.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. **7**4:3583–3597.

Veeramani A., G. M. Dias, and S. Kirkpatrick. 2017. Carbon footprint of dietary patterns in Ontario, Canada: A case study based on actual food consumption. J. Clean. Prod. 162:1398–1406.

Veira, D.M., M. Ivan, and P.Y. Jui. 1983. Rumen ciliate protozoa: effects on digestion in the stomach of sheep J. Dairy Sci. 66:1015–1022.

Waghorn, G. C. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production – progress and challenges. Anim. Feed Sci. Technol. 147:116–139.

Waghorn, G. C., and D. A. Clark. 2006. Greenhouse gas mitigation opportunities with immediate application to pastoral grazing for ruminants. Int. Congr. Ser. 1293:107–110.

Waghorn, G. C., and I. D. Shelton. 1997. Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep. J. Agric. Sci. Camb.128:365–372

Waghorn, G. C., M. H. Tavendale, and D. R. Woodfield. 2002. Methanogenesis from forages fed to sheep. Proc. N. Z. Grassland Assoc. 64:167–171.

Waghorn, G. C., M. J. Ulyatt, A. John, and M. T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on lotus. Br. J. Nutr. 57:115–126.

Wang, M., X. Z. Sun, P. H. Janssen, S. X. Tang, and Z. L. Tan. 2014. Responses of methane production and fermentation pathways to the increased dissolved hydrogen concentration generated by eight substrates in *in vitro* ruminal cultures. Anim. Feed Sci. Technol. 194:1–11.

Wang, M., R. Wang, P. H. Janssen, X. M. Zhang, X. Z. Sun, D. Pacheco, and Z. L. Tan. 2016. Sampling procedure for the measurement of dissolved hydrogen and volatile fatty acids in the rumen of dairy cows. J. Anim. Sci. 94:1159–1169.

Wang, Y., G. C. Waghorn, W. C. McNabb, T. N. Barry, M. J. Hedley, and I. D. Shelton. 1996. Effect of condensed tannins in *Lotus corniculatus* upon the digestion of methionine and cysteine in the small intestine of sheep. J. Agric. Sci. Camb. 127, 413–421.

Wei, C., K. Yang, G. Zhao, S. Lin, and Z. Xu. 2016. Effect of dietary supplementation of gallic acid on nitrogen balance, nitrogen excretion pattern and urinary nitrogenous constituents in beef cattle. Arch. Anim. Nutr. 70:416–423.

White R. R., and M. B. Hall. 2017. Nutritional and greenhouse gas impacts of removing animals from US agriculture. Proc. Natl. Acad. Sci. USA 114:E10301–E10308.

White, R. P., K. A. Winter, and H. T. Kunelius. 1976. Yield and quality of silage corn as affected by frost and harvest date. Can. J. Plant Sci. 56:481–486.

Wickersham, E. E., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and D. P. Gnad. 2008. Effect of rumen degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low quality forage. J. Anim. Sci. 86:3079–3088.

Williams, S. R. O., P. J. Moate, M. C. Hannah, B. E. Ribaux, W. J. Wales, R. J, Eckard. 2011. Background matters with the SF 6 tracer method for estimating enteric methane emissions from dairy cows: a critical evaluation of the SF 6 procedure. Anim. Feed Sci. Technol. 170:265–276.

Wischer, G., A. M. Greiling, J. Boguhn, H. Steingass, M. Schollenberger, K. Hartung, and M. Rodehutscord. 2014. Effects of long-term supplementation of chestnut and valonea extracts on methane release, digestibility and nitrogen excretion in sheep. Animal. 8:938–948.

Woodward, S. L., G. C. Waghorn, and P. G. Laboyrie. 2004. Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) reduce methane emissions from dairy cows. Proc. of the New Zealand Soc. Anim. Prod. 64:160−164.

Yan, T., R. E. Agnew, F. J. Gordon, and M. G. Porter. 2000. Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. Livest. Prod. Sci. 64:253–263.

Yan, W., and L. A. Hunt. 1999. An equation for modeling the temperature response of plants using only the cardinal temperatures. Ann. Bot. 84:607–614.

Yang, K., C. Wei, G. Y. Zhao, Z. W. Xu, and S. X. Lin. 2016. Effects of dietary supplementing tannic acid in the ration of beef cattle on rumen fermentation, methane emission, microbial flora and nutrient digestibility. J. Anim. Physiol. Anim. Nutr.101:302–310.

Zahiroddini, H., J. Baah, W. Absalom, and T. A. McAllister. 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Anim. Feed Sci. Technol. 117: 317–330.

Zom, R. L. G., A. Bannink, and R. M. A. Goselink. 2012. Effect of increased maturity of silage maize at harvest on conservation dairy cow performance and methane emission. Livestock Research Wageningen, Wageningen, the Netherlands. Report 578