5-2015

# Improvement of Nutrient Utilization Efficiency, Ruminal Fermentation and Lactational Performance of Dairy Cows by Feeding Birdsfoot Trefoil

Rachael G. Christensen
*Utah State University*

## Recommended Citation

Christensen, Rachael G., "Improvement of Nutrient Utilization Efficiency, Ruminal Fermentation and Lactational Performance of Dairy Cows by Feeding Birdsfoot Trefoil" (2015). *All Graduate Theses and Dissertations*. 4286.
https://digitalcommons.usu.edu/etd/4286

# IMPROVEMENT OF NUTRIENT UTILIZATION EFFICIENCY, RUMINAL FERMENTATION, AND LACTATIONAL PERFORMANCE OF DAIRY COWS BY USE OF BIRDSFOOT TREFOIL

By

Rachael G. Christensen

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

of

DOCTOR OF PHILOSOPHY

in

Animal, Dairy, and Veterinary Science

Approved:

| | |
|---|---|
| Dr. Jong-Su Eun<br>Major Professor | Dr. Allen J. Young<br>Committee Member |
| Dr. Jennifer MacAdam<br>Committee Member | Dr. J. Earl Creech<br>Committee Member |
| Dr. Juan Villalba<br>Committee Member | Dr. Mark R. McLellan<br>Vice President for Research and<br>Dean of the School of Graduate Studies |

UTAH STATE UNIVERSITY
Logan, Utah

2015

# ABSTRACT

## Improvement of Nutrient Utilization Efficiency, Ruminal Fermentation and Lactational Performance of Dairy Cows by Feeding Birdsfoot Trefoil

by

Rachael G. Christensen, Doctor of Philosophy

Utah State University, 2015

Major Professor: Dr. Jong-Su Eun
Department: Animal, Dairy, and Veterinary Science

Forages containing condensed tannins (**CT**) have potential to reduce the environmental impact of dairy farming. In 3 studies, I hypothesized that feeding CTcontaining birdsfoot trefoil (*Lotus corniculatus*, **BFT**) would result in improved nutrient utilization and lactational performance of dairy cows compared with control forages of the respective experiments.

Improved milk components, reduction in waste N, and overall improved N efficiency were hypothesized for BFT-fed cows compared to those cows fed alfalfa hay (Study 1) or grass-based diets (Study 2). In addition, a decrease in *in vitro* methane production and improved rumen fermentation due to diets based on BFT pasture and concentrate supplementation compared with grass pasture-based diets was the hypothesis of the third study.

Study 1 showed BFT-hay diets improved lactational performance through increased energy-corrected milk yield and increased milk protein yield, resulting in improved N utilization efficiency compared with the alfalfa hay diet. Total volatile fatty acids concentration tended to increase, and greater microbial protein yield was exhibited by cows fed BFT compared to other diets tested. Therefore, BFT can replace alfalfa hay in dairy diets and showed improved feed and N utilization efficiencies and lactational performance.

Study 2 determined that pasture nutrient content increased for BFT pasture compared to the mixed grass control, contributing to increases in milk yield most weeks during the 2-year study. Energy-corrected milk yield increased most weeks by BFT-grazed cows due to increased milk yield, although milk protein concentration was similar between treatments. Cows grazing BFT pasture increased N efficiency coupled with decreased milk urea N secretion in the first, but not the second year, suggesting an environmental advantage over traditional grass-based pastures depending on the effect of growing conditions on pasture quality at time of grazing.

Study 3 showed that offering BFT pasture to continuous cultures without or with barley grain or total mixed ration supplements reduced methane production and altered rumen microbial populations. The reduced methane production on the continuous cultures was likely due to direct and/or indirect effects of CT on rumen microbiota.

Overall, diets including BFT showed improved nutritive, lactational, and environmental benefits by decreasing N waste and methane production over typical alfalfa hay-based dairy diets and grass pastures.

(206 pages)

# PUBLIC ABSTRACT

## Improvement of Nutrient Utilization Efficiency and Lactational Performance of Dairy Cows by Feeding Birdsfoot Trefoil

Rachael G. Christensen

Condensed tannins (CT) are compounds that have shown potential to reduce the environmental impact of dairy farming waste products. In two live animal studies and a continuous culture study, it was hypothesized that feeding birdsfoot trefoil (*Lotus corniculatus*, **BFT**), a CT-containing legume, would improve nutrient utilization, milk, and component yield of dairy cows compared with feeding alfalfa hay or grass pasture in two studies, while feeding BFT forage would decrease methane production and improve rumen fermentation in addition to concentrate supplementation was the hypothesis of the third study. Reduction in milk urea nitrogen (**MUN**) and ruminal ammonia N nitrogen (**$NH_3$-N**) concentrations was used to indicate reduction in N waste.

The first study resulted in increased energy-corrected milk yield and increased milk protein yield for BFT-hay fed cows compared with the alfalfa diet, and improved N utilization for milk; however MUN and ruminal $NH_3$-N were not different between treatments.

A 2 year study showed that BFT-grazed cows increased milk yield, protein yield, and energy-corrected milk yield, but did not show a reduction in waste N compared to grass-based pastures.

A third experiment showed that feeding BFT forage pasture reduced methane production, altered rumen microbial populations and subsequent fermentation, and supplementation further improved nutrient yields and reduced methane.

Overall, diets including BFT showed improved nutritive and some reduction in N waste compared to typical alfalfa dairy diets and grass pastures. Further research is needed to understand interactive aspects of tannins and nutrient utilization with other feeds and microbial populations to reveal the full benefits of BFT.

# ACKNOWLEDGMENTS

There are many I need to thank: working with my committee was first and foremost the highlight of my time here at USU. I obtained many insights and encouragement to look outside the box regarding scientific advancement. Dr. Eun’s expert advice and attention to detail has taught me to slow down and notice the little things. Dr. Young was always the one to go to when things got too frustrating for this student to handle. Working with Dr. MacAdam, Dr. Creech, and Dr Villalba opened up a new dimension for me that has expanded my animal knowledge to that of animal-plant interactions through the effects of forages. A whole new world, with new opportunities and insights to gain.

There were many sources of financial support of this dissertation research. The 2-year pasture study funding was provided by the United States Department of Agriculture National Institute of Food and Agriculture Organic Agriculture Research and Extension Initiative Grant Number 2010-51300-21283, obtained through the great efforts of Dr. Jennifer MacAdam. The continuous culture study was supported also by the USDA-NIFA Grants number 2010-51300-21283 and the Utah State University Irrigated Pasture Grants Program. I am grateful to those willing to fund grants so students have the opportunity to expand scientific horizons.

The grazing study took place on commercial dairies, and I am grateful that dairymen were willing to open up their farms to a bunch of pesky plant and animal scientists, and willing to adapt and make changes that we asked to further the work and conform the pastures and cows to a feasible study.

I am grateful to Dr. Rusty Stott for his positive attitude and assistance with many protocols on the experimental animals for the hay study. Many thanks to John Wallentine, Jon Schumann, and the crew at the Caine Dairy Research Center for the extra time and effort to make sure the animals were well cared for and for smoothing out the wrinkles as we took ideas to the real world of a dairy farm and tried to make them work. I am grateful for the help of the “Skaggs Lab crew” and my undergraduate technicians who helped with some of the sampling, drying, grinding, and lab analyses. Especially Katie, whose organizational skills have been a great blessing (and envy) as she helped me sort out data and plan collection days, and make sure I had everything I needed.

Most importantly, I want to thank my loving husband, Clay, who carried the load at home and helped to encourage and support me through the early morning sampling, hours and hours of tough farm work, and some of the mechanical aspects of our experimental protocols. You are the best husband I could ever hope for. And to my sweet little kiddos, Taylor, Johnny, Zac, Josh, and baby Tessa whose world has been a little different in the past three years with Mom in school. You are all good troopers, and I love you.

Philippians 4:13

Rachael Grace Christensen

## CONTENTS

Page

CHAPTER

## LIST OF TABLES

Table Page

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

A = acetate

A: P = acetate to propionate ratio

AA = amino acid

ADF = acid detergent fiber

AF = alfalfa

AH = alfalfa hay

AIA = acid-insoluble ash

B = butyrate

BFT = birdsfoot trefoil

BFTH = birdsfoot trefoil hay

BFTP = birdsfoot trefoil pasture

BFTP-NS = birdsfoot trefoil pasture no supplement

BFTP-GB = birdsfoot trefoil pasture with ground barley

BFTP- TMR = birdsfoot trefoil pasture with total mixed ration

BH = biohydrogenation

BUN = blood urea nitrogen

BW = body weight

$CH_4$ = methane

CLA = conjugated linoleic acid

CP = crude protein

CT = condensed tannin

CTE = condensed tannin extract

DM = dry matter

DMI = dry matter intake

EAA = essential amino acids

ECM = energy corrected milk

FA = fatty acid

FO = flaxseed oil

FS = flaxseed

FCM = fat corrected milk

GHG = greenhouse gasses

GS = grass silage

ha = hectare

iNDF = indigestible neutral detergent fiber

$K_d$ = rate of degradation

LRCpH = Lethbridge research center ruminal pH measurement system

MCP = microbial crude protein

ME = metabolizable energy

MGP = mixed grass pasture

MJ = megajoules

MkN: MaN = milk nitrogen-to-manure nitrogen ratio

MkN: N intake = milk nitrogen-to-N intake ration

MNE = microbial nitrogen efficiency

MP = metabolizable protein

MUN = milk urea nitrogen

MY = milk yield

N = nitrogen

NAN = non-ammonia nitrogen

NDF = neutral detergent fiber

$NE_L$ = net energy lactation

NFC = non fiber carbohydrates

$NH_4$ = ammonia

$NH_3$-N = ammonia nitrogen

NPN = non-protein nitrogen

NRC = national research council

OGP = orchardgrass pasture

OGP-NS = orchardgrass pasture no supplement

OGP-GB = orchardgrass pasture with ground barley

OGP-TMR = orchardgrass pasture with total mixed ration

OM = organic matter

P = propionate

PD = purine derivatives

pdNDF = potentially digestible neutral detergent fiber

peNDF = physically effective neutral detergent fiber

PUFA = polyunsaturated fatty acids

RDP = ruminal degradable protein

RUP = ruminal undegradable protein

SFA = saturated fatty acids

TF = tall fescue grass

TFA = trans fatty acids

TMR = total mixed ration

UN: FN = urinary nitrogen excretion-to fecal nitrogen excretion ratio

Yb = ytterbium

VFA = volatile fatty acids

WC = white clover

# CHAPTER 1

# INTRODUCTION

Sustainability is defined as “meeting society’s present needs without compromising the ability of future generations to meet their own needs” and is made up of 3 interlinked pillars (Figure 1.1): environmental responsibility, economic viability and social acceptability (EPA, 2010). In consideration of these core pillars, the sustainability of animal production agriculture comes under extensive public scrutiny. Many studies have shown that production agriculture contributes greatly to environmental pollution or excess of nutrients to air and water and in the United States, Asia and Europe, increasing public attention has focused on animal agricultural production systems as a major nonpoint source of pollution affecting the quality of air, streams and groundwater resources (Wang et al., 2010; von Keyserlingk et al., 2013).

The 2 nutrients from animal production systems of the greatest concern are N and P because of their impact on air, water quality, and eutrophication. Nutrient management research has been conducted to identify strategies that reduce N and P pollution (EPA, 2012). In addition, methane (**$CH_4$**) has received critical attention as an agricultural pollutant, because it is a greenhouse gas (**GHG**) that contributes to climate change with an effect equivalent to 25 times that of $CO_2$ over a 100-yr period (IPCC, 2007). In perspective, on a world-wide basis, dairy animals, including cull cows and beef cattle from dairy breeds, are estimated to contribute only 4% to anthropogenic GHG emissions (FAO, 2010). In many developed countries, the contribution of dairy production to GHG emissions is estimated even lower, due to the higher productivity of livestock agriculture, the dilution by emissions from other sectors, and lack of significant land use change

(Hagemann et al., 2011; Knapp et al., 2014). However, the argument can be made that not all land needed for feed production is accounted for correctly in some models, and inference to all situations is not applicable.

Legume forages, including alfalfa (*Medicago sativa*: **AF**) and birdsfoot trefoil (*Lotus corniculatus L.:* **BFT**), are superb sources of both crude and true protein for livestock. However, most of the protein in alfalfa is degraded quickly in the silo when ensiled, or in the rumen when fed, impairing the efficient use of N by the cow. Protein nutrition influences productivity, profitability, and the efficiency of N use. For mature cows in zero N balance (requiring no weight gain, or under no demand for body condition use for energy), feed N that is not converted into milk N must be excreted. The efficiency of converting feed N to milk N seldom exceeds 30%; thus more than 70% of feed N is typically lost with approximately 30% lost in feces and about 40% lost in urine, mostly as urea (VandeHaar and St. Pierre, 2006). Efficient use of N for protein production (meat or milk) is difficult to improve, and better sustainability is reached if less CP is fed. However, too little protein will reduce production in early lactation and high genetic merit cows. Advancements in protein nutrition and precision feeding of amino acid balanced rations can improve N efficiency by reducing diet CP but ensuring essential amino acids are provided (NRC, 2001)

Feeding cows less protein can dramatically decrease urinary N excretion and increase the efficiency of N use. Excess feed N is deaminated and excreted as urea, a N waste compound, in urine and milk, while undigested ruminal undegradable protein and metabolic N (sloughed intestinal cells and hind gut fermentation products) are excreted in the feces (VandeHaar and St. Pierre, 2006). The route and amount of N excretion is of primary environmental concern; urinary N is more volatile than fecal N and is rapidly converted to ammonia. Simply reducing the amount of protein fed, however, can have negative impacts on productivity if the diet is not correctly balanced. Both nutrient intake and nutrient excretion (nutrient management) must be carefully considered.

Nutrient management is a complex issue because interrelationships among manure management, soil conservation, crop production, animal nutrition, and economic consequences must be considered. If economic viability cannot be achieved, then sustainability cannot be achieved. Researchers (Kohn et al., 1997; Jonker et al., 2002; Rotz, 2004) have proposed that home-grown forages, both as harvested hay and pasture, represent the best use of resources to minimize environmental impact of importing feeds on the farm. Because forages comprise the largest portion of dairy cow diets, improving the efficiency of product conversion from consumed forages is the best way of optimizing feed usage on individual farms, which leads to reduction of nutrient waste.

Corn silage and alfalfa (as hay and silage), closely followed by grass hay and grass silage are the most commonly utilized forages on US dairy farms. While recent improvements in plant breeding, agronomic management of forage stands, better equipment and harvesting techniques have all helped to improve forage quality, the underlying nature of plant cell wall composition and lignin content affecting digestibility in ruminant animals still remains under-researched. This has become the limiting and largest unknown factor in increasing the efficient use of dairy diets to optimize lactation performance. Additionally, there are some physiological factors of forages, such as the aforementioned highly rumen-degradable protein in alfalfa and the increased methane output from ruminants consuming high forage diets, which could potentially reduce the efficiency that might be gained from improved digestibility of fiber, undermining efforts to be sustainable.

Some forages, such as BFT, contain polyphenolic compounds with bioactivity that has proven beneficial to increase productivity or well-being of the animals that consume them (Patra and Saxena, 2011). In the case of BFT, condensed tannins (**CT**) bind to soluble proteins in the rumen, and then release those proteins once the complex reaches the acidic abomasum (Waghorn, 2008). This reduces the degradation of protein to ammonia in the rumen (Weiss et al., 2009). Diets containing modest levels of CT (around 2-4%) reduce proteolysis during ensiling and rumen fermentation by up to 50% (Grabber et al., 2001). This unique protein–binding feature of CT offers an alternative, naturally derived bioactive compound to help reduce N losses on dairy farms.

Another unique feature of BFT is that it is non-bloating, which enables it to be grazed in pure stands. Non-grazing varieties of AH and other legumes can cause bloat in grazing cattle, especially during the fast growing spring season when most grazing dairies want to maximize the use of fresh forage. Birdsfoot trefoil can be grazed as a fresh pasture, increasing its desirability as a forage for dairy cows. Recent studies suggest that in addition to CT and its non-bloating characteristics, BFT has unique fiber development and lignin growth patterns that may increase digestibility and improve utilization of nutrients in ruminant diets compared with other legumes (Hunt, 2013). Cast in this new light, BFT may prove to be even more valuable than once thought, though more research is needed regarding application of BFT on the farm, and managing the stand to overcome unique agronomic challenges associated with BFT.

The benefits of CT for improving protein utilization and ruminant performance are well-cited in New Zealand livestock systems for sheep and cattle fed pasture or green-chopped forages. Flow of feed protein to the small intestine increased by 30% in sheep fed increased tannin concentrations, up to 4% of dry matter intake (Waghorn and Shelton, 1997). Milk production of pastured, non-supplemented Holstein cows was increased by 2.7 kg per day due to the tannins in the birdsfoot trefoil (Woodward et al., 2009). The potential for CT to improve protein utilization and milk production of dairy cattle have not been evaluated in forage-concentrate rations typical of U.S. dairy farms (Grabber et al., 2001), particularly in hay-based diets. Research is needed to determine cattle performance on diets containing BFT and the effect of its digestibility on the total diet when fed in a traditional forage-concentrate ration utilizing dry hay and as a grazing forage in cows of typical US milk yield.

The overall hypothesis in a series of studies reported in this dissertation was that feeding BFT to lactating dairy cows would increase dairy efficiency, CT in BFT-containing diets would reduce milk urea N and urinary N excretion and improve N efficiency, and increase milk and milk protein yield compared to alfalfa hay or grass-based diets. In addition, *in vitro* $CH_4$ and fermentation end-products of BFT pasture forage compared to orchardgrass forage and effects of differing types of supplementation on methane production was also studied.

## REFERENCES

EPA (Environmental Protection Agency). 2010. Methane and Nitrous Oxide Emissions from Natural Sources. Publication 430-R-10-001. EPA, Washington, DC.

EPA. 2012. Science for a sustainable future: EPA research program overview 2012 - 2016. p.3 Accessed last on Jan. 9, 2015 at http://www.epa.gov/ord/docs/strap-overview2012.pdf.

FAO (Food and Agriculture Organization of the United Nations). 2010. Greenhouse Gas Emissions from the Dairy Sector: A Life Cycle Assessment. Prepared by P. Gerber, T. Vellinga, C. Opio, B. Henderson, and H. Steinfeld. FAO, Rome, Italy.

Grabber, J. H., G. A. Broderick, R. D. Hatfield, J. M. Powell, M. P. Russell, and R. E. Muck. 2001. Polyphenol-containing forages: A way to improve the profitability and nitrogen-use efficiency of dairy farms? US Dairy Forage Research Center 2000-2001 Research Report, USDA-ARS pp.1–7.

Hagemann, M., T. Hemme, A. Ndambi, O. Alqaisi, and M. N. Sultana. 2011. Benchmarking of greenhouse gas emissions of bovine milk production systems for 38 countries. Anim. Feed Sci. Technol. 166–167:46–58.

Hunt, S. 2013. Birdsfoot trefoil (*Lotus corniculatus)* as a forage crop of organic grazing-based dairy systems in western climates. MS Thesis. Utah State University, Logan.

IPCC (Intergovernmental Panel on Climate Change). 2007. Summary for Policy Makers. Page 23. Cambridge Press, Cambridge, UK and New York, NY.

Jonker, J. S, R. A Kohn, and J. High. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. J. Dairy Sci. 85:1218-1226.

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

Kohn, R. A., Z. Dou, J. D. Ferguson, and R. C. Boston. 1997. A sensitivity analysis of nitrogen losses from dairy farms. J. Environ. Manag. 50:417–428

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

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

Rotz, C. A. 2004. Management to reduce nitrogen losses in animal production. J. Anim. Sci. 82: E119–E137 suppl.

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

von Keyserlingk, M. A. G., N. P. Martin, E. Kebreab, K. F. Knowlton, R. J. Grant, M. Stephenson, and S. I. Smith. 2013. *Invited review*: Sustainability of the US dairy industry. J. Dairy Sci. 96:5405–5425.

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 I. D. Shelton. 1997. Effect of condensed tannins in Lotus corniculatus on the nutritive value of pasture for sheep. J. Agric Sci. 128:365–372

Wang, F., Z. Dou. L. Ma, W. Ma, J. T. Sims., and F. Zhang. 2010. Nitrogen mass flow in China's animal production system and environmental implications. J. Environ Qual. 35:1537–1544.

Weiss, W. P., L. B. Willett, N. R. St-Pierre, D. C. Borger, T. R. McKelvey, and D. J. Wyatt. 2009. Varying forage type, metabolizable protein concentration, and carbohydrate source affects manure excretion, manure ammonia, and nitrogen metabolism of dairy cows. J. Dairy Sci. 92:5607–5619.

Woodward, S. L., G. C. Waghorn, K. A. Watkins, and M. A. Bryant. 2009. Feeding birdsfoot trefoil (*Lotus corniculatus*) reduces the environmental impacts of dairy farming. Proc. NZ Soc. Anim. Prod. 69:1–5.

# CHAPTER 2

# REVIEW OF LITERATURE

## SUSTAINABILITY OF U.S. ANIMAL AGRICULTURE

The current US legal definition of sustainability (US Code Title 7, Section 3103) is as follows: “An integrated system of plant and animal production practices having a site-specific application that will over the long-term: satisfy human food and fiber needs, enhance environmental quality and the natural resource base upon which the agriculture economy depends, make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls, sustain the economic viability of farm operations, and enhance the quality of life for farmers and society as a whole.”

Hence, sustainability is made up of three interlinked facets: environmental responsibility, economic viability and social acceptability (Thompson, 2007; EPA, 2010; see Figure 1.1). In consideration of these core pillars, the sustainability of animal agriculture production comes under extensive public scrutiny. Many studies have shown that production agriculture contributes greatly to environmental pollution or excess of nutrients to air and water, and in the United States, Asia, and Europe, increasing public attention has focused on animal agricultural production systems as a major nonpoint source of pollution affecting the quality of streams and groundwater resources (Wang et al., 2010; von Keyserlingk et al., 2013).

Environmental issues and global food security are important concerns for governments and policy-makers who are conscious of both the prediction that the global population will increase to over 9.5 billion people by the year 2050 and also of the proportion of their national population that is currently food-insecure (FAO, 2009). Increased population growth will increase competition for resources such as water, land, and energy among agriculture, municipalities, and industrial enterprises. It is important to note, however, that ruminant livestock will play a crucial role in future global food security because far more grazing land exists, unusable for human food, than cropping land since most agricultural land can be grazed but only a small proportion is suitable for intensive crops (Gill et al., 2010). Ruminant animals can transform pasture into high quality human food and convert human-inedible by-products of food production into high-quality human food. If mitigation strategies are implemented that reduce GHG but also reduce production output, then the environmental benefits would be at least partly negated by increased food costs or reduced supply of animal-based foods (VandeHaar and St. Pierre, 2006; Knapp et al., 2014.)

Concurrent with increased use of water comes the increased risks associated with contamination. The 2 nutrients from animal production systems of the greatest concern are N and P because of their impact on air, water quality, and eutrophication. Salt and nitrates are the most widespread groundwater contaminants in the United States and salt contamination is a growing challenge in many regions of the United States. Public attention has focused on animal agricultural production systems as a major nonpoint source of pollution affecting the quality of streams and groundwater resources. Agricultural enterprises are striving to lower emissions and reduce amounts of polluting nutrients leaving farms. Nutrient management research has been conducted to identify strategies that reduce N and P pollution. In addition, methane (**$CH_4$**) has received critical attention as an agricultural pollutant because it is a greenhouse gas that effects the global surface temperature and has a global warming potential 21 times greater than that of carbon dioxide (IPCC, 2007).

## NUTRIENT METABOLISM AND DIGESTIBILITY

Feed ingredients provide the substrates for microbial fermentation within the rumen of a dairy cow, and differences in feed digestibility, dry matter intake, rate of passage, and chemical composition alter the amount of energy extracted by the microbes. The concentrations of metabolites formed by digestion can alter the proportions of fatty acids, protein, VFA and $CH_4$ produced. The proportions of individual metabolites affect the amount of $CH_4$ produced due to reducing equivalents, meaning that any dietary component or intervention that causes a shift in favor of propionate production will be accompanied by a reduction in $CH_4$ production per unit of feed fermented (Van Nevel and Demeyer, 1996). Rumen protein degradation and assimilation into microbial protein can result in either a net consumption or net production of $H_2$. Biohydrogenation (**BH**) of fatty acids (**FA**) in the rumen will result in a net consumption of $H_2$. Consequently, variations in rumen N metabolism and BH will affect $CH_4$ production, and since carbohydrate and protein substrates are also used for microbial maintenance and growth, theoretical predictions of VFA patterns and $CH_4$ formation do not always correlate to *in vitro* and *in vivo* observations (Knapp et al., 2014). Similarly, alterations in N metabolism by rumen microbes can increase or decrease efficiency in milk N:N intake and subsequent partitioning of excess N to waste products.

### *N Metabolism*

Metabolism of N compounds in ruminant animals is a complex pathway involving multiple mechanisms (Van Soest, 1994). There are several forms of N utilized by ruminants. Non-protein N (**NPN**) includes urea supplied by saliva, recycled from the rumen, ammonia from protein degradation in silages and wet feeds, and supplemented ammonia, in addition to fermentation of the most readily hydrolysable proteins (the “A” fraction) in the diet. True dietary protein or feed protein may be categorized as ruminal degradable (**RDP**) or undegradable protein (**RUP**; NRC, 2001). Rumen microbes utilize NPN and true protein from RDP to support their growth and reproduction, becoming an important source of protein to the animal called microbial crude protein (**MCP)**. Microbial protein provides between 50 and 80% of the total protein in dairy cows and is a high quality amino acid (**AA**) source including greater concentrations of methionine and lysine, the 2 most limiting AA for milk production (NRC, 2001). These protein sources, combined with RUP, and endogenous CP all contribute to passage of metabolizable protein (**MP**) to the small intestine, where it is enzymatically digested and the component AA and small peptides are available to the animal for absorption by the small intestine. These peptides and AA are used for the growth, maintenance, reproduction and production of ruminants (NRC, 2001).

### *Ruminal Undegraded Protein*

Ruminal undegraded protein is digested in the duodenum of the small intestine but not digested by rumen microbes, so it does not contribute to MCP synthesis, and instead provides a source of AA to the animal. The goal of feeding RUP is to complement the AA profile of MCP in order to maximize N use efficiency as well as to meet AA requirements of the animal because MCP alone cannot meet all the requirements. Ruminal undegradable protein is assumed to be 100% true protein, and digestibility varies among feedstuffs, ranging from 50 to 100% (NRC, 2001).

Ration RUP concentration and extent of N digestion in the rumen influences the flow of RUP to the duodenum. In addition to N concentration and source, dietary components such as condensed tannins (**CT**) may influence the proportion of RUP and MCP in MP. Condensed tannins can inhibit the degradation of protein the rumen (Barry and McNabb, 1999; Min et al., 2003) through a binding action of CT with protein which protects the bound protein from microbial degradation. Effects of CT on ruminal N metabolism are well documented and are influenced by tannin plant source and chemistry of the tannin (epicathecins and epigallocathecins, for example; Patra and Saxena, 2011) which affects bioactivity as well as overall diet CP%, and digestibility of N in the rumen (Min et al., 2003; Carulla et al., 2005; Waghorn, 2008). For example, a 27.8% reduction in $NH_3$–N concentration and a reduction in flow (26%) occurred with BFT compared to alfalfa in continuous cultures fed forage diets (Williams et al., 2010). In contrast, Williams et al. (2011) investigated TMR comprised of CT-containing forages compared to alfalfa TMR in vitro and found that the two diets containing low- and moderate-CT concentration BFT did not affect $NH_3$–N concentrations or flow. These contrasting results may have resulted from different CT contents between the two studies (20.8 g CT/kg DM in the BFT forage versus 3.75 or 7.44 g CT/kg DM, respectively in the concentrate-supplemented diets). Similarly, John and Lancashire (1981) used sheep to compare the BFT cultivars Empire and Maitland, containing low (i.e., 2.5 g CT/kg DM) and high (i.e., 14.5 g CT/kg DM) levels of CT, respectively, and found that the high CT cultivar bound more N and reduced NH3–N concentration in the rumen (John and Lancashire, 1981).

### ***Ruminal Metabolizable Energy***

Ruminal metabolizable energy is energy available in forms that can be utilized by rumen microbes for growth and reproduction. It is the most important and limiting factor determining MCP synthesis in lactating dairy cattle. Rumen microbes use carbon structures from carbohydrates and available ATP as energy and substrates for AA and protein synthesis. Microbial yield depends on microbial growth rate and the fractional degradation of the metabolizable energy available in the rumen, which is usually a reflection of the carbohydrate portion in the diet (Nocek and Tamminga, 1991). In addition to supporting microbial growth in the rumen, cows require energy for maintenance, growth, pregnancy and lactation, which is more difficult to differentiate and predict in ruminants. However, these energy sources are vitally important, and are the missing link in terms of full understanding of N utilization efficiency, as energy is required to synchronize with N sources for MCP production.

The metabolizable energy (**ME**) and net energy requirements for cow maintenance, activity, reproduction, and productive purposes have been estimated and are published in energy accounting systems (NRC, 2001). However, recommendations are not always consistent. For example, the NRC (2001) estimates the maintenance requirement to be approximately 0.54 MJ of ME/kg of $BW^{0.75}$ for mature lactating cows, assuming a conversion of ME to net energy of 0.62. This includes an additional allowance of 10% of maintenance for normal activity that is not expended when fasting heat production is measured (i.e., cows in calorimeters). One recent study (Mandok et al., 2013) was conducted to fine-tune energy requirements of non-lactating pregnant cows (i.e., to estimate maintenance energy requirements) suggested ME requirements for pregnancy to be 1.07 MJ of ME/kg of $BW^{0.75}$ (or 117 MJ of ME/d), which is greater than that published in the NRC (2001). The difference was potentially due to increased maintenance requirements in cattle selected for greater milk production, an underestimation of the ME requirements for pregnancy in mid to late gestation, an overestimation of the ME content of autumn pasture (the diet source used in the study), or low efficiency of use of ME from autumn pasture for maintenance or conceptus metabolism. Even if the estimated ME content of pasture used in these calculations was too high, it would not fully explain the discrepancy between predicted and measured values. Clearly, much more research into energy requirements and energy consumption and metabolism of dairy cows in relation to feed intake, digestibility, and feed source is needed.

### *Fecal and Urinary Losses of N*

The route and amount of N excretion is of primary environmental concern; urinary N is more volatile than fecal N and is rapidly converted to ammonia by ureases present in soil and on pen floors (Lee et al., 2014). Overall intake of N affects the total amount of N excreted via manure, but the type of carbohydrate and forage provided in the diet have greater impacts on the route (fecal or urinary) of excretion (Weiss et al., 2009). Protein in the diet directly affects the amount excreted, as shown in a recent study be Lee et al. (2014) where manure from cows consuming a 16.7 % CP diet had an increased ammonia emission rate, and urinary N contribution to nitrate-N was 100% greater than manure from cows consuming 14.8% CP diet.

Excess dietary N (from excess feed CP as well as AA from cell turnover and enzyme production) is converted to urea, which is a soluble compound that will diffuse into various body fluids, such as blood, milk, and urine (Kauffman and St-Pierre, 2001). About 80% of N consumed in excess of 500 g/d is believed to be excreted in urine in dairy cows (Castillo et al., 2001; Kebreab et al., 2001). Total N excretion as well as urinary N excretion can be decreased if overall dietary CP concentration can be reduced. Efficiency of N use from the diet (N output in milk divided by N intake) is low in dairy cattle, so research to increase efficiency continues to accumulate and is important if the environmental impacts of dairy production are to be reduced. Proper dietary balance is required to reduce the amount of dietary protein required by high producing cows and achieve optimal milk production, while still decreasing negative impacts on the environment. Because excessive N excretion is primarily caused by overfeeding RDP (Rotz, 2004), decreasing N intake by reducing the CP concentration in the diet can decrease total N excretion, including urinary N. Dairy cattle rations with 16.5% CP are recommended to support maximum milk and milk protein production, while decreasing N excretion compared with higher CP rations (Olmos-Colmenero and Broderick, 2006a). More recent studies (Lee et al., 2012a,b; Giallongo et al., 2014) have explored rations with CP concentrations even more reduced, but these rations require that essential amino acids (**EAA**) such as lysine, methionine and histidine be supplemented. As total protein in the diet decreases, EAA concentration tends to drop below required levels to sustain production (Lee et al., 2012a). Research in refining diets to meet EAA requirements is ongoing.

Undigested RUP and metabolic N (sloughed intestinal cells and hind gut fermentation products) are excreted in the feces (Tamminga, 1992). Dairy cows typically produce more ammonia in the feces per animal than other livestock due to overfeeding of protein in the diet, inefficient utilization of dietary protein in the rumen, realtively larger urinary excretion volume with a high concentration of urinary N, and inefficient conversion of dietary N to usable protein products. One study summarized data from 554 lactating cows from several experiments and estimated the average dairy cow weighing 625 kg, producing 40 kg/d milk with 25 kg/d DMI and 0.68 kg/d N intake would excrete 0.44 kg N/d in manure, with sources equally contributed by urine and feces (Nennich et al., 2005).

Increased fecal N may also be due to increased DMI stimulated by RUP rather than attributed to excretion of undigested feed protein (Flis and Wattiaux, 2005). Huhtanen et al. (2008) reviewed fecal N output from 207 trials and found fecal N was better associated with DMI than N intake, while the prediction was improved when both factors were included in the model. Including both DMI and N intake in prediction equations for fecal N excretion is important, as metabolic and endogenous N, which are major contributors to fecal N, are related to DMI (Van Soest, 1994). Huhtanen et al. (2008) estimated that every 1-kg increase in DMI corresponded to 6.7-g increase in fecal N output.

In contrast, Groff and Wu (2005) fed rations with increasing CP concentrations from 15.0 to 18.8% DM and observed increased fecal, urinary, and milk urea N excretion with increasing CP concentration, with small and varying differences in DMI and subsequent milk protein yield.. Olmos-Colmenero and Broderick (2006b) observed similar responses to rations ranging in CP concentration from 13.5 to 19.4% DM, resulting in increased fecal, urinary, and milk urea N (**MUN)** excretion and a quadratic milk protein yield response, peaking at 16.5% CP. In both studies, DMI was not affected by CP concentration, but N digestibility and intake increased linearly with increased CP, indicating N intake and absorption drove N excretion.

### ***Milk Urea Nitrogen***

Milk urea N represents a waste product of incomplete capture of ammonia in the rumen. Increased MUN is associated with rations with elevated CP, specifically increased proportion of RDP, which may be related to increased urinary and total N excretion. Greater MUN values are associated with increased blood concentration of circulating urea (DePeters and Ferguson, 1992). While MUN is directly influenced by nutritional factors, those most commonly associated with MUN are dietary carbohydrate source and extent of digestion in relation to solubility and degradability of protein sources in the rumen, and synchronization of carbohydrate and protein substrates to rumen microbes. Milk UN, though a waste product, is also considered a practical way to assess dietary balance and determine sufficient efficiency of nutrient utilization (Rotz, 2004). Kalscheur et al. (2006) fed cows diets differing only in percentage of RDP. Milk urea N was increased from 9.5 to 16.4 mg/ 100 mL as RDP increased from 6.8 to 11.0 % of DM, respectively. Incidentally, for the 11.0% RDP diet, milk production increased by 2.1 kg as CP of the diet linearly increased by 0.09 kg/d with increased diet RDP. Cows fed diets formulated with CP to be below NRC requirements for RDP had comparatively reduced milk production, milk fat, and milk protein due to reduced dietary RDP for rumen microbial growth. As RDP was increased in the diet of lactating dairy cows, MUN concentrations increased linearly and the efficiency of conversion of feed N to milk N decreased.

Researchers have also found a positive correlation between urinary N excretion and MUN (Kauffman and St-Pierre, 2001; Nennich et al., 2006). Nennich et al. (2006) compiled data from 16 individual feeding studies comprised of 372 data points for lactating Holstein cows and found MUN to be an excellent predictor of urinary N excretion. Factors related to urine excretion ($P < 0.01$) included DMI, N intake, BW, MUN, and DIM, with N intake as the best single predictor. The relationship of Urine N excretion, kg/d to MUN is predicted by the following equation:

$$UN = [BW \times 0.254] - [MILK \times 1.03] + [NI \times 0.2101] + [MUN \times 5.09] + [MTP \times 21.8] - [MF \times 6.5] - 138.8$$

where MILK is milk yield in kg/d, NI is N intake in kg/d, MUN is g/d, MTP is milk true protein in g/d, and MF is milkfat in g/d.

### ***Nitrogen Utilization Efficiency***

One way to decrease negative impacts of N on the environment is to increase the efficiency of protein utilization in the cow, which results in less N excreted per unit of milk produced. Increased nutrient utilization can lead to increased profitability on the farm as well as less excreted waste (Huhtanen et al., 2008). Jonker et al. (2002) reported that N utilization efficiency was decreased by 0.05 percentage units for every additional gram of N in the diet over the recommended intake of N. Due to extensive alteration of N from feed in the rumen, dietary CP concentration cannot accurately predict the amount or composition of protein ultimately supplied to the animal. Increased dietary CP concentrations generally corresponds with increased N intake by increasing DMI and increasing N excretion from feces, urine, and milk, with very little gain in N conversion to usable product (a measure of N utilization efficiency).

A recent study investigated diets with reduced CP fed to dairy cows and found dietary CP as low as 12% did not reduce milk production in dairy cows, although nutrient digestibility and MCP synthesis in the rumen were depressed (Aschemann et al., 2012). In that study, however, cows had relatively low production capability (approximately 29 kg/d), and intake was restricted, so the effect of protein on feed intake could not be demonstrated. Trials with high producing dairy cows have shown variable effects of decreasing dietary CP or MP on DMI. In trials where DMI was decreased when feeding the MP-deficient diets, milk production also decreased, reducing N efficiency (Lee et al., 2011a,2012a). In contrast, when DMI did not decrease, milk production was also not different from diets with adequate MP (Lee et al., 2012b; Giallongo et al., 2014).

In addition to altering protein in the diet, supplementation of concentrates to increase energy available to the cow can improve efficiency of N utilization. The increase in milk production observed in several studies as a result of increased proportion of concentrate of the diet was likely due to greater energy supply. For example, Benchaar et al. (2014) compared high forage (HF) diets to high concentrate (HC) diets supplemented with flax seed or flaxseed oil, and found feeding HC diets to cows increased ($P<0.01$) apparent total-tract digestibility of DM (8%) and OM (9%) , but had no effect on CP and ADF digestibility. Productivity increased by 100 g/kg N intake ($P<0.01$) from improved efficiency of energy utilization by the animal or increased digestibility and absorption of energy from the diet. In most cases, increasing energy in the diet leads to better efficiency (Benchaar et al., 2014). Their findings suggest that increasing the proportion of concentrate in the diet improved N utilization as illustrated by reduced concentrations of ruminal $NH_3$-N and MUN and the greater use of dietary N for milk protein synthesis (i.e., milk N:N intake). The improved N utilization is likely due to a greater starch supply from high-concentrate diets. Indeed, $NH_3$-N utilization in the rumen is strongly influenced by carbohydrate availability (Russell et al., 1992). Hristov and Giallongo (2014) suggested that supply of fermentable carbohydrates (*i.e.*, *via* increasing concentrate proportion in the diet) decreases $NH_3$-N production in the rumen (by reducing the deamination process or enhancing the capture of released AA by rumen microbes) or increases microbial capture of released $NH_3$-N in the rumen. This study confirmed an earlier one by Agle et al. (2010), who showed that increased concentrate proportion in the diet of dairy cows reduced ruminal $NH_3$-N concentration.

Dietary protein intake is the most important factor determining milk nitrogen efficiency, reduction of urinary nitrogen losses, and consequently, ammonia emissions from dairy cow manure. Dairy cows producing up to 44 kg/ d can be fed balanced diets with 16% CP without affecting milk production or composition (Olmos-Colmenero and Broderick, 2006a). Diets with CP < 15% (MP deficiency of < -12%) will likely result in decreased milk yield, partially through decreased DMI. Low CP diets (i.e., deficient in metabolizable protein) may benefit from supplementation with EAA, partially through an effect on increased DMI (Kalschuer et. al., 1999).

The loss in milk production from reducing diet CP or altering RDP ratio in the diet currently far outweighs the benefits of reduced N excretion. However, further research into balancing rations for groups of cows may find loss in milk production of the cows acceptable if the N excretion of an entire group is reduced. Milk losses from underfeeding CP need to be quantified and the risks and benefits of balanced protein feeding, particularly for groups of animals, before diets are drastically altered. Kalscheur et al. (1999) demonstrated that when feeding below CP requirements, as dietary CP increases, the efficiency of N use declines and the amount of urinary N loss increases. Thus, diets formulated for maximal milk production may not be optimal to minimize N excretion per unit of milk produced.

### *Nutrient Management*

Nutrient management is a complex issue because relationships among manure management, soil conservation, crop production, animal nutrition, and economic consequences must be considered. Outweighing all of these considerations is the economic viability of farms when approaching nutrient management issues (VandeHaar and St. Pierre, 2006). If economic viability cannot be achieved, then sustainability cannot be achieved.

Kohn et al., (1997) proposed a model to analyze the relative importance of altering various components of dairy farm nutrient management (manure management, soil, crop, and animal nutrition) on N reduction and concluded that optimizing the feeding strategy played the most important role in reducing N losses. Nutrient management should focus on maximizing utilization of farm-raised feeds to minimize purchased nutrient imports. Purchased feeds should be used only as needed to support animals’ nutrient requirements to meet the farm’s production goal (VandeHaar and St. Pierre, 2006; Rotz, 2004; VandeHaar, 1998). Where home-grown forages, both as harvested hay and pasture, comprise the largest portion of dairy cow diets, improving the efficiency of product conversion from consumed forages is the best way of optimizing feed usage on individual farms, that can cycle waste nutrients back into the crops for concentrate or forages fed to the cows, which reduces nutrient loss to the environment.

### *Effect of Forages on N Excretion*

Forage type may also affect N partitioning and excretion. Corn and legume silages are commonly fed together in rations for dairy cattle. The fermentable starch found in corn silage may complement the RDP found in legume silage in providing a fermentable source of carbohydrate to the rumen microbes, which may decrease ruminal N losses. Overall intake of N affects the amount of N excreted via manure, whereas types of carbohydrate (starch in corn silage vs. sugars in grass silage) and forage species (red clover and BFT legume vs. grasses) have greater impacts on the route (fecal or urinary) of excretion. In a recent study by Halmemies-Beauchet-Filleau et al. (2014) comparing different ratios of grass silage (**GS**) to red clover silage (**RCS**) in TMR, intake of DM and milk yield tended to be higher when RCS and GS were offered as a mixture than when fed alone. Red clover contains polyphenol oxidase, which binds protein and, as inclusion of RCS in the diet increased, it tended to reduce whole-body N balance, increased linearly the proportion of dietary N excreted in feces and urine, and decreased the utilization of dietary N for milk protein synthesis linearly. A reduction in recovery of N in milk was accompanied by increased partitioning of N into urine and feces (Halmemies-Beauchet-Filleau et al., 2014).

Legumes that contain CT, such as BFT, also have the potential to reduce the degradation of plant protein to $NH_3$-N in the rumen, but unlike polyphenol oxidase from clover, the CT in BFT release proteins in the abomasum, leading to improvements in feed efficiency and reduction of N waste excretion. Results of studies suggest that feeding

BFT may decrease excretion of N, especially via urine (Patra and Saxena, 2011). Improvements in N efficiency were seen in some trials utilizing grazed BFT (Waghorn and Shelton, 1997; Woodward et al., 2009), but some studies show that CT in the diet did not affect excretion of fecal or urinary N but improved milk protein efficiency (Hymes-Fecht et al., 2013). More research is needed to explore the interactions of CT-containing legume feeds such as BFT with other dietary components, BFT fiber digestion, and the consequential N partitioning effects these diets may have in reducing waste excretion from dairy cows and improving efficiency and environmental quality.

### ***Methane in Ruminant Livestock***

Enteric $CH_4$ emission from ruminant livestock and $CH_4$ emission from stored manure are major contributors to anthropogenic emission of greenhouse gases in many countries (EPA, 2012; NRC 2003). Enteric $CH_4$ production in ruminants is a well-understood process that is closely related to the production of VFA in the rumen (Hungate, 1967; Johnson and Johnson, 1995). Feeding and nutrition have modest (2.5 to 15%) potential to mitigate enteric $CH_4$ per unit of energy-corrected milk (**ECM**) in intensive dairy operations in developed countries, with significantly more potential when combined with methane-mitigating crop and forage production improvements in developing countries (FAO, 2010; Knapp et al., 2014). Methane emissions can be reduced by improving feed efficiency. Feed additives, chemical inhibitors, and biological approaches to altering methanogen populations, activities, and fermentation cannot compromise rumen digestibility and fiber fermentation if they are to be viable $CH_4$-reduction approaches. Whereas the primary substrate for methanogenesis is $H_2$, which is generated mostly during fermentation of plant cell wall carbohydrates to acetate and butyrate (Moss et al., 2000), alterations of rumen fermentation to favor propionate production are the most studied nutritional strategy to reduce $CH_4$ production.

A recent review (Knapp et al., 2014) examined management factors that could have an effect on $CH_4$ reduction (Figure 2.2). Approaches recently studied in genetics, physiology, and health to improve herd productivity can have a greater impact than diet manipulation on environmental sustainability. Integrating all the factors contributing to methane mitigation is the best approach in order to see quantifiable changes in methane output from dairy farms.

Methane generated in the rumen is formed primarily from hydrogen produced during the fermentation of feed, particularly of high fiber diets. The amount of $CH_4$ produced is therefore dependent upon the amount of hexose fermented and the amount of individual VFA produced during the fermentation of forage and diet components in the rumen. Changing the fermentation stoichiometry to produce more propionate at the expense of acetate and butyrate typically results in less $CH_4$ from fermentation. Forages that have greater concentrations of non-fiber carbohydrates, such as corn silage and BFT (Noviandi et al., 2014), generally promote fermentation that leads to greater propionate production, which competes for hydrogen at the expense of $CH_4$ production. Forages that are more digestible, due to less NDF, reduced maturity, increased digestibility or other agronomic differences, are also apt to produce less $CH_4$.

## GRAZING DAIRY SYSTEMS IN THE UNITED STATES

Significant variability in commodity prices, competition between animals and humans for feedstuffs like grains and concentrates, and perceived animal welfare concerns around permanent housing of livestock have led to increased global interest in grazing systems for dairy cows. Pastures, including temperate and tropical grasses and legumes, are, under most circumstances, the most cost-effective sources of nutrients (Peyraud and Delagarde, 2013), with the cost of milk production declining quadratically with increased utilization of grazed pasture (Dillon et al., 2008). However, one of the challenges of a pasture-based system is the seasonal variation in pasture availability and nutritive value resulting in the need to provide supplementary feed during periods of pasture or nutrient deficit in order to maintain the greatest milk production per unit of feed.

Consumers are increasingly aware of “functional food” components that can have positive effects on health maintenance and disease prevention. A number of specific FA are now recognized as having beneficial effects on human health, and these include the omega-3 FA and cis-9,trans-11 CLA that are present in milk fat (Lock and Bauman, 2004). Consumer demand for dairy products from grazed cows is increasing: studies have revealed that high consumption of dairy products may help to prevent heart disease, different types of cancer, and other chronic diseases, although the mechanisms are not understood (WHO, 2003; Leaf et al., 2003; Dewhurst, 2005), and grazing cows tend to have increased concentration of these desirable omega-3 fatty acids (Lock and Shingfield, 2004). Fat-soluble vitamins are important in human nutrition and they may also improve the oxidative stability of milk fat with increased proportions of polyunsaturated FA.

Small to medium sized dairies are decreasing in number and are interested in alternative strategies to remain profitable. There are 39% fewer small farms than a decade ago (von Keyserlingk, 2013), but consumer interest in organic and pasture-based dairy products is growing, creating a market for their products. Although conventionally managed farms with more than 500 milking cows now account for 63% of the milk supply in the U.S. (USDA-NASS, 2012), the other 37% present a potential pool of farms that could turn to pasture systems as a resource for a low cost feed and marketing strategy to increase profitability of dairy products (Paine, 2009). Although most of the milk supply in the U.S. comes from these large farms, most of the dairy operations (90%) in the U.S. have fewer than 200 cows (USDA-NASS, 2012). In addition, as farms focus on utilizing homegrown feeds, particularly pasture forage, theoretically the carbon foot print and need to import nutrients to the farm can be reduced, which could make pasture-based farms more sustainable than conventionally managed counterparts.

### ***Use of Pasture as a Forage Source***

Since the inception of the USDA National Organic Program pasture rule in 2010, which requires ruminants in organic systems to graze pasture for 120 days and receive a minimum of 30 percent dry matter intake from pasture during the grazing season (Rinehart and Baier, 2011), increased establishment of grazing systems to develop high quality forage sources has been a focus for grazing-based dairy farms.

Grazing systems can have lower operating expenses, lower feed costs, and higher net incomes per cow (Dartt et al., 1999). But because milk production is lower from dairy cows on pasture-only diets than from those fed a total mixed ration (TMR), this reduction in milk production can decrease the overall productivity and sustainability of grazing-based systems as it can have greater land requirements and waste generation per unit of milk produced, depending on the model used to estimate the nutrient parameters (Capper et al., 2009). When pasture plus concentrate is fed to high genetic merit cows, milk production is less than that of cows fed nutritionally balanced TMR in confinement feeding systems (Auldist et al., 2013; Bargo et al., 2003). Studies with high producing dairy cows on pasture (Reis and Combs, 2000; Soriano et al., 2000; Bargo et al., 2002) reported that energy supplementation with 8 to 9 kg/d of corn-based concentrates resulted in total DMI of 22 kg/d and milk production of 30 kg/d. In a comprehensive review of the literature investigating the supplementation of grazing dairy cows, Bargo et al. (2003) concluded that, on average, supplementation increases milk yield (1 kg/kg of DM concentrate DMI), milk protein percentage (+0.01%/kg of DM concentrate DMI) and yield (+0.01 kg/kg of DM concentrate DMI), and milk fat yield (+0.02 kg/kg of DM concentrate DMI), but reduces milk fat percentage (−0.13%/ kg of DM concentrate DMI; Bargo et al., 2003). This represents a reduction in animal performance compared with non-grazing systems and may be related to an alteration of ruminal fermentation and digestion when cows grazed good quality pasture only, or a reduction in DMI (reviewed in Hills et al., 2015). VandeHaar (1998) compared a grass-based grazing system and the expected milk outcome to 2 different conventional systems; one that used by-product feeds like cottonseed, bran, and distiller grains, and one that used no byproducts (Table 2.1). Within conventional systems, 3 different levels of milk production were analyzed. VandeHaar reported that even with lower feed costs using pasture, the decrease in cow performance reduced the land-use efficiency compared to confinement systems by 34%. In addition, the reduction in protein production per ha reduced the efficiency of land use even more. The protein production is reduced because the conventionally fed cows produced more milk, with increased protein concentration. Unspecified in the study is land use for grain and by-product production fed in the conventional system, or the increased contribution of longer cow productive life due to the grass pasture. Phelan et al. (2013) reported performance data of cows grazing in a legume- grass system that, when used in VandeHaar's scenario and provided equations, show land use that is 78% efficient, which was equal to or an improvement over some of the example confinement systems, even with reduced milk yield from grazing cows (Table 2.1). The model used to describe the scenario is conclusive only if the data inputs truly represent the scenario described. Advantages and disadvantages of grazing vs. confinement systems are best determined in a case-to-case basis, depend significantly on quality and source of forages and feeds, how they are managed and how effectively supplementation is used in the system, besides modeling details and accuracy of inputs used in the model.

Hills et al. (2015) report many factors that can affect DMI for grazing dairy cows and concluded that individualized supplementation based on cow needs, and not same rate of supplementation based only on pasture nutrient status was a more effective and productive strategy, which would lead to increased sustainability of pasture-based systems.

Pasture-based diets with high-quality pastures (<50% NDF) and concentrate supplementation are often characterized by reduced rumen pH (<6.0), low acetate:propionate ratio, high ruminal $NH_3$-N concentration, and high rate of ruminal passage of feed (Holden et al., 1994; Bargo et al., 2003; Perez-Ramirez et al., 2009). Supplementing a pasture-based feeding system with a TMR may improve performance through improvement in rumen digestion and fermentation. This system is called partial TMR (**pTMR**) because the pasture grazed by the cows is separate from the TMR. Feeding a pTMR results in increased performance because of an increased DMI and milk production with increased fat and protein concentrations (Alvarez et al., 2001; Bargo et al., 2001; Audlist et al., 2013). Potential positive effects on rumen fermentation could include increased or more stable rumen pH from the forage portion and a reduction in rumen $NH_3$-N concentration from forage sources when supplements are provided that are greater in effective fiber and reduced in CP content than pasture.

As grazing forages mature across a grazing season and from year to year, they produce a greater quantity of feed and are better able to recover after grazing, but fiber concentration increases, concurrently decreasing forage digestibility, energy, and protein concentration (Roche et al., 2013; Hills et al., 2015). Other approaches may be required to increase or maintain milk production as forages decrease in quality. Altering the time of cow access to pasture is one way to manage both the pasture quality and intake, as demonstrated in a study by Kennedy et al. (2009) where cows that were allotted less time to graze still maintained milk production and pasture quality was actually improved. Such a strategy would allow farms with limited pasture due to season or land area to still graze effectively. Pérez-Ramírez et al. (2009) looked at varying pasture allowance (low and high; 13 vs. 24 kg DM /d per cow, respectively) and time restriction (unrestricted, 5 hours and 9 hours) but report reduction in intake when pasture time is restricted. However, milk production was only decreased by 1.4 kg/d for both 5 and 9 h at pasture from the unrestricted treatment, which suggests that reducing time on pasture is a viable management scheme. Another strategy is using starch-containing forages as a supplement. Researchers in Austria supplemented cows grazing grass or consuming grass silage with corn silage for 15 wk and found the increased energy content due to corn-silage supplementation increased the efficiency of N utilization (30%), compared to the ryegrass control (26%, Baldinger et al., 2014).

Grazing systems generally focus on maximizing herbage DMI while maintaining a high quality and quantity of the grazed herbage over the grazing season (Tas et al., 2006; Peyraud and Delagarde, 2013). Dairy cows with a high proportion of forages in their diet, or those grazing intensively managed grassland (Kolver and Muller, 1998; Peyraud and Delagarde, 2013), had a lower MY than cows fed concentrate-supplemented diets. The lower MY could be attributed partly to a lower DMI due to reduction of herbage quality over the grazing season and partly to an imbalance among absorbed nutrients (Kolver and Muller, 1998). To determine the reasons for the differences in production, Kolver and Muller (1998) compared cows grazing high-quality pastures (*Dactylus glomerata*) with cows being fed TMR. They then simulated the pasture diets in the Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 1995) to partition energy expenditure attributed to various physiological and physical functions due to grazing. Grazing cows produced 15.4 kg/d less milk than cows on TMR; the results of the model simulation indicated that 61% of this effect was due to lower DMI, 24% of the difference was due to energy expenditure in grazing and walking, 12% was estimated to be due to the excretion of surplus N, 7% reflected the greater energy content of the milk from grazing cows, and 5% due to differences in the partitioning of energy between milk production and body condition score (Kolver and Muller, 1998). The authors concluded that overall intake of nutrients, rather than a limitation in any one nutrient in pasture, was the primary factor that limited milk production from high-quality pasture, with the remaining factors reflecting differences in energy partitioning to activity, milk composition, or urea synthesis. These data confirmed the difference in milk production between TMR-fed cows and cows grazing high-quality pasture was related to the system of farming and not to the nutritional profile of the feed, per se.

### *Sustainability of Grazing Dairies*

Although the idea of grazing forage and high forage rations seems to imply that a reduction in carbon use and an increase in sustainability should accompany grazing dairies, some studies of dairy systems (Capper et al., 2009; Lizarralde et al., 2014) in South America and the U.S. have shown grazing to reduce the efficiency of nutrient use and land use and increase in the carbon footprint. Improving the productivity of a dairy system through increased milk output with supplements to provide the nutrients needed to match pasture inadequacies was shown in these studies to have a greater effect on the carbon footprint of milk than converting from confinement systems to intensive grass-based systems or vice versa (Capper et al., 2009). However, the approach used by the authors to quantify sustainability in grazing and conventional systems is biased to support conventional systems, and it is arguable if the methods correctly represent the contribution of all inputs in conventional systems.

Hills et al. (2015) determined that maintaining greater amounts of milk production while minimizing grain supplementation of pasture-fed dairy cows can improve the sustainability of grazing-based dairy production in some cases, but more research is needed to refine the application of this theory to the farm. Some studies (Auldist et al., 2013; Phelan et al., 2013; Baldinger et al., 2014) have shown that conscientious management of pastures and utilization of high quality forage species and combinations of pasture species and the proper amount of supplementation increases the productivity of dairy cows on pasture compared to unsupplemented pasture treatments but production efficiency in some cases still lags behind performance in confinement settings.

Other studies investigating alternative types of grazing (i.e. rotatinous grazing; Carvahlo, 2013) offer additional methods of increasing sustainable use of forges without the need for supplementation, implying that many alternatives exist that can increase sustainability with use of grazed pastures as the major forage source. There is evidence that grazing management (e.g. moderate grazing) which promotes higher individual animal production fosters both parameters of efficient animal production and environmental or ecosystem balances, and described in a review by Carvalho (2013) as well as a recent study by Lemaire et al. (2014) Both studies emphasize that the creation of innovative practices and new areas of research are needed to preserve ecosystem balance while maintaining animal productivity, but that it is possible to do so.

### *Biohydrogenation of Dietary FA and Effects on Milk of Grazing Dairy Cows*

Milk from grazed dairy cows has been purported among researches and consumers to be more beneficial for human health than the milk of conventionally fed cows. Reduction in the overall consumption of saturated fatty acids (**SFA**), trans-fatty acids (**TFA**), and cholesterol has been encouraged by health professionals worldwide while an increased intake of n-3 polyunsaturated (**PUFA**) is said to improve health (Griel and Kris-Etherton, 2006; Kris-Etherton et al.,, 2007).

Unsaturated fats, while desirable in human diets, are toxic to rumen microbes, and they have evolved mechanisms to desaturate fats, though they do not metabolize fats for energy themselves. This ability to desaturate and alter fats from their native chemistry while in the rumen is known as rumen biohydrogenation (**BH**), and has complicated the study of fats in dairy cow diets.

Both the total concentration in the diet, and the composition of FA profiles in the diet are of concern. If total dietary fat concentration exceeds about 7%, studies have shown that rumen digestibility of other feeds is impaired, reducing energy efficiency through reduced fiber digestion and decreased milk fat secretion (Bauman and Griinari, 2001). In regards to fatty acid profiles, the greater the percentage of saturated fats in the diet, the less likely there will be negative effects on milk fat concentration and milk production, and some studies have shown that specific fatty acids act as bioactive mediators to alter milk fat due to a shift in the biohydrogenation pathway of *cis*-9, *cis*-12 18:2 to *trans*-10 18:1 rather than to *trans*-11 18:1 due to disturbance in rumen fermentation processes (Griinari and Bauman, 1999). Additionally, Dhiman et al. (2000) showed that specific fatty acids, such as linolenic and linoleic, when supplemented to cows increased CLA content of milk by an average of 250 %. Sources of linolenic and linoleic acids were roasted soybean, soy oil and linseed oil treatments compared with control which had no oil supplement. The average increase of CLA was 97, 438, 305, and 318% for roasted soybean, soy oil and linseed oil treatments, respectively. A recent study by Benchaar et al. (2014) investigating effect of forage-to-concentrate ratio (**F:C**) in the ration, grass silage, and supplementation with flaxseed (**FS**) or flaxseed oil (**FO**) showed that increasing the concentrate proportion in the diet by 10% reduced ruminal pH by 0.6 units, shifted volatile fatty acid (**VFA**) pattern toward more propionate (increase of 15 mol/ 100 mol) and less acetate, and decreased protozoal numbers by 32%. These changes in ruminal fermentation resulted in a decrease of *in sacco* effective ruminal degradability of acid detergent fiber and neutral detergent fiber of grass silage. Lower acetate: propionate and *trans*-11: *trans*-10 ratios were observed when feeding concentrate diets versus forage diets (Benchaar et al., 2014). However, the lack of changes in milk fat yield may suggest that the decrease in milk fat content was a result of a dilution effect due to increased milk yield when cows were fed higher proportion of concentrate in the diet. Clearly, diet F:C as well as fat sources used as supplements can alter rumen fermentation and lead to differences in FA profiles of milk fat.

Consumer interest in CLA has focused efforts of the dairy industry to improve CLA concentration in milk. Dairy products are one of the major sources of CLA in the human diet, and Kepler and Tove (1967) identified the cis-9, trans-11 isomer of C18:2 fatty acid as an intermediate and contributor in the BH of linoleic acid by the rumen bacterium *Butyrivibrio fibrisolvens*. In the rumen, dietary lipids are hydrolyzed, and resulting unsaturated FA are converted to SFA by the rumen microorganisms (Harfoot and Hazelwood, 1988). Much attention has been given to CLA because of its anticarcinogenic properties (Dhiman et al., 1999; Lock and Shingfield, 2004). Dietary management of dairy cows to increase CLA concentration in milk may be beneficial for human health and for the dairy industry. Conjugated linoleic acid in milk originates from CLA produced during ruminal BH of linoleic acid and desaturation of vaccenic acid (*trans*-11 18:1; **VA**) in the mammary gland (Harfoot and Hazelwood, 1988). Factors affecting the flow of CLA isomers and *trans*-18:1 FA to the duodenum as a result of ruminal BH need to be elucidated to increase the CLA concentration in milk and meat of ruminants used for human consumption.

There are limited data addressing the basis for increased concentration of CLA in milk from grazing cows. The increased concentration of CLA in milk fat from grazing animals could be a result of the increased concentration of octadecatrienoic acid (18:3) in fresh forage or specific plant chemicals (Lee and Jenkins, 2011) or increased concentration of rapidly degradable carbohydrates induces a microbial change resulting in a profound modification of ruminal BH and isomerization of C18:2 (Lechartier and Peyraud, 2010) from the soluble sugars in fresh plants (Kelly et al., 1998a). Concentration of CLA in milk fat is affected by the intake of unsaturated FA (Chouinard et al., 1999; Kelly et al., 1998b). In cows grazing pasture, the major dietary unsaturated FA is linolenic acid. A study of BH of linolenic acid, *cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid, showed that it was converted to *cis*-9, *trans*-11, *cis*-15 conjugated triene, then to *trans*-11, *cis*-15 C18:2, and finally to *trans*-11 C18:1, *trans*-15 C18:1, or *cis*-15 C18:1 (Harfoot and Hazelwood, 1988). Therefore, BH pathways of linolenic acid did not involve CLA as an intermediate. However, *cis*-9, *trans*-11 CLA may be produced endogenously from *trans*-11 octadenoic acid by $\Delta^9$-desaturase in the body tissues (Griinari et al., 2000).

Other factors may affect CLA production in the rumen when cows are switched to pasture. The type and source of dietary carbohydrate may influence rates of microbial fermentation in a way that alters the rate of CLA production or utilization by rumen microbes and ultimately the concentration of CLA in milk fat. Such an effect could help explain the reported differences in the CLA content of milk fat observed between cows fed fresh forage (pasture) and cows fed preserved forages (Dhiman et al., 1999; Roche et al., 2013). Replacing corn with wheat and feeding a rapidly fermentable carbohydrate source in diets increased the yield of *trans*-10 C18:1 and reduced the yield of *tran*s-11 C18:1 in milk (Lechartier and Peyraud, 2010). The linear increase in the yield of *trans*-10 C18:1 in milk as forage level decreased also suggests a shift in the BH pathways of C18:2 that increased the rumen ratio of *trans*-10 C18:1 from *trans*-11 C18:1 (Chouinard et al., 1999). One study that examined milk produced in different regions of the world and under different feeding systems found that milk from cows fed fresh green forage, especially those grazing grass, had a much higher unsaturated: saturated FA proportion, with more polyunsaturated FA and more CLA (in particular C18:2 *cis*-9, *trans*-11), than milk from silage-fed cows (Elgersma et al., 2006). Sugars, such as fructosans, starch, pectins, and soluble fiber content, greatly decline during the fermentation process used to preserve forage as silages (Van Soest, 1994) and during the drying process necessary to obtain hay, the forage is subject to respiration, which can lead to a 10 to 20% decrease in the carbohydrate fraction of the plant cell contents. Thus, the high concentrations of rapidly fermentable starch, sugars, and soluble fiber that are found in high quality pastures may create a rumen environment and conditions that favor a greater production of CLA by rumen bacteria over silage- and hay-fed cows (Holden et al., 1994). The dynamics of the rumen environment and microbial population could differ in the grazing animal from cows fed conserved feeds. Passage rate and fluid dilution rate increase because of the high water intake associated with grazing pasture. Meal size, feeding frequency, bite size, and time spent ruminating differ in cows grazing pasture, and these factors could be important in the alteration of rumen fermentation and the influence on rumen production and utilization of CLA (Kelly et al., 1998b). More in vivo research is needed to elucidate effects of differing forages and forage conservation methods and the resulting FA profiles in milk and rumen fluid. More recent studies have found that CT from forages inhibited ruminal BH, thus leading to the accumulation of C18:1 *trans*-11 (*trans* vaccenic acids; **TVA**) at the cost of C18:0 production (Khiaosa-Ard et al., 2009; Lee et al., 2011b), suggesting that bioactive compounds such as CT in BFT could alter both rumen and milk FA profiles.

### *Forage Legumes in Pasture*

The forage quality of legumes (CP and NDF content compared to grasses) does not decrease as rapidly with advancing maturity as does grasses (Waghorn and Clark, 2004). It has been shown that incorporating forage legumes into grass pastures offers advantages over all-grass pastures. Legumes fix N from the air into the soil via legume root nodules, where some of this N is transferred to companion grown grasses to improve their overall growth as well (Giller and Cadisch, 1995; Høgh-Jensen et al., 2004). Legumes continue growth in mid-summer when growth of cool-season grasses becomes reduced, improving the year round yield of forage (Sleugh et al., 2000; Berdahl et al., 2001; Lauriault et al., 2003). Legumes such as white clover, lucerne and BFT have higher nutritional value compared with perennial ryegrass due to their higher metabolizable energy content and lower levels of structural carbohydrate (Ulyatt, 1981). *In sacco* and *in vitro* incubation studies of digestion kinetics have shown legumes have higher digestible protein levels and faster rate of passage through the rumen than most grass species (Burke et al., 2000). Legumes maintain a higher nutritive value through late summer-autumn in contrast to perennial pastures containing grasses due to increased digestibility and CP concentrations and reduced NDF than pastures that are exclusively based on grasses (Van Soest, 1965; Kilcher, 1981). However, commonly grown legumes like alfalfa and clover (*Trifolium* sp.) can cause bloat; hence, they are not often used as sole pasture species and should not constitute more than 50% of a pasture mixture (Undersander et al., 2002). Indoor feeding experiments and short-term grazing studies have demonstrated various benefits as a result of feeding legumes including increased milk and milk solids production, reduced $CH_4$ emissions, and improved N use efficiency (Woodward et al., 2009). While the magnitude of benefits differs among legume species, the responses are usually due to the greater DMI of cows fed legumes, the greater nutritive value of the legumes, and the presence of CT in some legumes such as BFT (Woodward et al., 2002). Bryant et al. (2014) found that seasonal variations in forage nutrient content, particularly CP, affected the N use efficiency of cows on pasture. Longer-term and multi-season studies to examine these theories are few in the literature.

The ultimate goal of proper rumen function is to maximize microbial growth through the amount of RDP that is captured into rumen microbial cells. Low ruminal pH increases maintenance requirements of microbes to maintain ion balance across the cell membrane (Russell, 1998). On the other hand, increasing rate of passage from the rumen probably has dramatic effects on increasing microbial growth efficiency by decreasing microbial turnover (Wells and Russell, 1996). Improving nutrient utilization efficiency in pastured cows can be achieved by utilizing forages with increased energy content or increased digestibility, which can be the case with legume forages.

## BIRDSFOOT TREFOIL

Birdsfoot trefoil is a non-bloating, productive legume that is well suited to pasture-based dairy and beef production. Birdsfoot trefoil is a species distributed throughout the world, and grows under a wide range of environmental conditions. It is considered one of the major forage legumes after alfalfa (*Medicago sativa*) and white clover (*Trifolium repens*) (Singh et al., 2007). Yields reported for BFT cropped in agricultural lands range between 8000 and 10,000 kg DM $ha^{-1}$ $year^{-1}$ (Cassida et al., 2000), which represents around 50–80% of alfalfa performance (Blumenthal and McGraw, 1999). Cultivation of BFT in the U.S. is estimated to be 1 million ha with greatest use in the Northeast, Midwest, and along the Pacific coast (Blumenthal and McGraw, 1999). In comparison, 10 to 11 million ha of alfalfa are cultivated in the US annually (Barnes and Sheaffer, 1995).

Birdsfoot trefoil can be cropped as a stand-alone forage or a mixed species for both beef and dairy grazing applications. For example, inter-seeding BFT into tall fescue (*Festuca arundinacea* Schreb) pastures increased CP concentration and total yield of pasture. However, ADG of steers grazing rhizomatus BFT (**RBFT**) pastures was greater ($P < 0.10$) than that of steers on mixed BFT- tall fescue treatments in that study. Steers grazing normal phenotype BFT had about 12% lower ($P < 0.10$) ADG than those grazing RBFT but gained faster ($P < 0.10$) than those grazing BFT+TF, RBFT+TF or TF. Steers increased to a total weight of 445 kg/ha for RBFT pasture compared to 226 kg/ha for the TF treatment (Wen et al., 2002). Early experiments showed that dairy cows grazing BFT-dominant pastures produced more milk (+ 3.2 kg/d) and had a greater milk protein concentration (+ 0.15 %) than those grazing perennial ryegrass or white clover-dominant pastures (Harris et al., 1998). The greater milk yield of cows grazing BFT was due, in part, to improved pasture quality and greater DMI, and possibly the presence of CT in the BFT pasture (Harris et al., 1998). Woodward et al. (2000) fed cows either perennial ryegrass or BFT, and the cows were also drenched with either polyethylene glycol (**PEG**) to block the action of CT or water (as a control). Milk yields were greater on BFT (21.2 kg/d) than on BFT + PEG (18.6 kg/d), ryegrass (15.5 kg/d), or ryegrass + PEG (15.5 kg/d), indicating that CT contributed 46% to the increased milk yield that resulted from feeding BFT rather than ryegrass. In addition, an in vitro study reported by our group also showed that tall fescue and BFT mixed diets improved nutrient utilization by decreasing $NH_3$-N concentration and $CH_4$ production compared to alfalfa and tall fescue mixes (Noviandi et al., 2014).

### *Forage Quality of BFT*

The nutritive value of *Lotus* species, including BFT, is considered to be similar or even superior to that of AF and WC. Digestibility of DM for *Lotus* species ranges between 72% and 78%, whereas CP ranges between 16% and 24%, and ADF varied between 24% and 30% (Blumenthal and McGraw, 1999). In the U.S., BFT has replaced much of the clover previously grown with grasses in the Northeast U.S. (Blumenthal and McGraw, 1999).

One of the most important features of some *Lotus* species is their content of condensed tannins (CT), also known as proanthocyanidins, in vacuoles of leaves and stems. Tannins are a secondary compound produced by many families of dicotyledonous and other higher-order plants. They are water soluble, polyphenolic compounds that vary widely in chemical structure but exhibit the common characteristic of binding proteins. Tannins thought to be produced by plants to deter herbivory (Feeney, 1976; Rhoades and Cates, 1976) and provide a sink for excess carbon (Bryant et al., 1983; Hernandez and Van Breusegem, 2010). When ingested by animals, they increase the efficiency of protein metabolism when present in low to moderate concentrations (Aerts et al., 1999; Mueller-Harvey, 2006; Waghorn, 2008), and have been shown to have antihelmintic, antimicrobial and antifungal properties (Scalbert, 1991; Schultz et al., 1992). There are two main types of tannins in plants, hydrolysable tannins, and condensed tannins (CT). Ruminants consuming forages containing moderate amounts of CT (around 5 mg CT/g DM) show reduced ruminal protein degradation to $NH_3$ which can increase the quantity of protein that appears in the small intestine. Condensed tannins prevent the formation of protein films in the rumen which allows control of rumen bloat without using additional chemicals (Aerts et al., 1999; Patra and Saxena, 2009). However, forages with excess CT concentrations have been reported to cause decreased palatability and feed intake and reductions in nutrient utilization (Aerts et al., 1999; Makkar, 2003). The CT concentration of BFT is moderate, yet has been shown to be effective at improving protein use in ruminants.

Condensed tannins in BFT bind to proteins at the near-neutral pH of the rumen, reducing ruminal protein degradation and ammonia production, and then dissociate at the low pH of the abomasum, releasing protein for digestion and absorption in the lower digestive tract of the ruminant (Waghorn et al., 1987). This mechanism has been the explanation for increases in milk yield in dairy cows consuming BFT (Woodward et al., 2000, 2009; Turner et al., 2005), and the diversion of excreted nitrogen from urine to feces, contributing to the reduction of the potential for volatilization of ammonia from dairy manure (Misselbrook et al., 2005; Woodward et al., 2009).

### *Use of BFT in Lactation Dairy Diets*

There is increasing interest in the use of BFT both alone in grazing situations or as a TMR ingredient to support the efficient production of ruminants on pasture and in confined operations while reducing negative environmental impacts from excessive N waste. In grazing-based dairy production, BFT provides diets with potential for increased feed intake due to its increased rate of digestibility and moderate CP concentration. In one study (Woodward et al., 2000), milk production of cows fed BFT was 21.2 kg/d compared to 15.5 kg/d for the ryegrass treatment, due to greater DMI, improved forage quality due to increases in CP (21.7 % vs. 16.6% for ryegrass) and decreased NDF (40.8 % vs. 54.8% for ryegrass) and the action of CT. The CT not only contributed to around 40-50% of the increase in milk production (Woodward et al., 2000) but also improved energy use efficiency by the cows (an increase of 34 ml FCM/MJ ME vs ryegrass).

Increases in milk components are also reported in studies feeding BFT to dairy cows. Woodward et al. (2000) attribute the action of CT in BFT pasture to explain the increase in milk protein concentration. Cows were fed BFT or BFT+PEG treatments to separate legume effect from CT effect. Both BFT and BFT+PEG treated cows had increases ($P<0.001$) in milk protein concentrations compared to ryegrass or ryegrass+PEG fed cows (3.24 vs 3.10%). This difference in protein concentration may have been associated with the increased protein in the BFT compared with the ryegrass (21.7 vs. 16.5%, respectively), along with increased ME intakes, promoting increased availability and absorption of protein. However, the increased ($P<0.001$) milk protein concentration of cows fed BFT compared with BFT+PEG (3.34 vs. 3.16%, respectively) also suggests a specific role for CT in the change, which in this case accounted for 75% of the increase above ryegrass-fed cows (3.10% milk protein).

Very few studies have been conducted to determine the use of BFT hay in dairy rations and the effects of these rations on milk production and nutrient utilization. In vitro studies have been conducted (Williams et al., 2010, 2011) and reported increases in VFA production and decreases in ruminal $NH_3$-N concentration for diets that contained BFT hay alone or as a TMR ingredient, indicating that BFT hay in dairy rations has promise to improve lactational performance, but few data exist to show if this is really the case in vivo. Birdsfoot trefoil conserved as a hay crop is cheaper to store and easier to transport than silage, so it would provide a way to manage excess spring forage production that could be used later in the year or as a cash crop.

### *Digestibility of BFT by Ruminants*

Birdsfoot trefoil digestibility can exceed that of other legumes. Digestibility is closely related with NDF concentration (Van Soest, 1994). Based on a 3-year study, Cassida et al. (2000) reported that concentration of CP, NDF, and acid detergent fiber (ADF) in BFT averaged 20.9, 35.9, and 29.5% DM, respectively, while alfalfa averaged 19.7, 39.0, and 28.4% DM, respectively. Williams et al. (2010) reported similar results, with 20.0 and 18.5% CP for alfalfa and BFT, respectively, while NDF for alfalfa and BFT were 40.8 and 35.8%, respectively. Digestibility of forages can be affected by many factors, primarily by forage species, as cellulose, lignin, and relative ratios of these chemical constituents vary greatly among forage species. Tomlin et al. (1965) showed that BFT cellulose at the early bloom stage was digested more rapidly than ALF cellulose, despite similar lignin concentrations (11.2 vs. 11.4 % DM, respectively).

Mowat et al. (1969) found a negative correlation between cell wall lignin and digestibility in alfalfa, though BFT cell wall lignin content was not correlated with overall digestibility. Increased cell wall digestibility in BFT may lead to increased DMI in ruminants.

Fiber digestibility of forages is determined by content and extent of NDF digestion. Digestibility of NDF is complex and influenced by the combination of several factors. The fraction of NDF that is potentially digestible determines digestibility, the rate of NDF degradation in the rumen, and the rate of passage from the rumen (Allen, 2000). Potentially digestible NDF **(pdNDF)** is a laboratory measure of the absolute extent of NDF digestion by ruminal microorganisms. Increasing proportion of pdNDF and decreasing the indigestible NDF (**iNDF**) fraction could result in greater fiber digestibility. Birdsfoot trefoil could potentially have increased pdNDF and reduced iNDF fractions, due to the unique structural features mentioned above. Grasses often have a greater proportion of pdNDF to iNDF and higher in vitro NDF digestibility than legume forages, but digestion of legume NDF occurs faster (Smith et al., 1972) and could increase total amount of NDF digested *in vivo*.

### ***Effects of BFT on $CH_4$ Production***

There is a body of evidence to demonstrate that feeding CT-containing forages or supplementing with CT extracts decreases $CH_4$ production *in vitro* (Bhatta et al., 2009; Tan et al., 2011; Williams et al., 2011) and *in vivo* (Animut et al., 2008; Woodward et al., 2009; Sun et al., 2012). The inhibitory effects of CT on rumen methanogenesis have been attributed to direct effects of defaunation on methanogenic archaea (Finlay et al., 1994; Patra and Saxena 2009, 2011) and indirect effects through a depression of fiber digestion in the rumen (Patra and Saxena, 2011). Other rumen factors likely contribute to overall fermentation patterns, and more studies in dairy cows are needed to understand the mechanisms that drive methane production and possible effects CT may have *in vivo*.

Condensed tannins may also reduce $CH_4$ possibly by directly inhibiting the activity of methanogenic bacteria (Tavendale et al., 2005) and is dependent on source, chemical structure and concentration in the diet. Birdsfoot trefoil tannin concentrations from 2.5 to 8.5% DM have been found to reduce enteric $CH_4$ production without reducing digestibility (Carulla et al., 2005; Animut et al., 2008). As the BFT proportion in the diets utilized in one *in vitro* study increased, $CH_4$ production decreased (Noviandi et al., 2014), with less $CH_4$ produced in diets containing greater concentration of BFT forage and increased overall CT concentration. Cultures that were fed with diets that contained a greater concentration of CT (2.43%) showed reduced $CH_4$; however, the treatment that had 1.65% CT exhibited no measurable effect on $CH_4$ production. Also in that study (Noviandi et al., 2014), VFA concentration was similar across dietary treatments, suggesting no detrimental effects on ruminal fermentation. Therefore, the decreased $CH_4$ production may have resulted from direct toxicity of CT on methanogens resulting in decreased methanogenesis rather than indirect effects of depression of ruminal fiber digestion in cultures (Noviandi et al., 2014). Eun and Min (2012) stated that reliable and distinguishable effects of CT on $CH_4$ reduction can be expected only from CT concentrations greater than 2.0% DM.

In several studies, the reduction in $CH_4$ due to feeding forages containing condensed tannins was confounded with changes in forage quality, such as lower NDF content. For example, in the study by Woodward et al. (2002), $CH_4$ emissions from lactating dairy cows were lower for cows grazing sulla pastures than perennial ryegrass pastures (6.1 vs. 7.2% of GE intake), but the NDF content of sulla was much less than that of the ryegrass (14.7 vs. 48.3% of DM). Because lower-fiber diets are associated with lower $CH_4$ emissions (Johnson and Johnson, 1995), the methane reduction in that study may have been due to a change in nutrient composition. Thus, it is uncertain that condensed tannin extracts and condensed tannin-containing forages are effective at reducing enteric $CH_4$ emissions from cattle. In a study by Beauchemin et al. (2007), the lack of effect of quebracho tannin extract supplementation on $CH_4$ production was consistent with the lack of effect on total tract DM and fiber digestibility. Despite no effects of quebracho tannin extract on total tract digestibility, the linear reduction in total VFA concentration with increasing concentration of quebracho tannin extract supplementation (0, 1%, 2% of DM supplementation) together with linear reduction in acetate:propionate ratio, suggests some changes occurred in ruminal fermentation as a result of dietary inclusion of tannins. More research is needed to elucidate effects of tannins on interactions of fermentation products and rumen methanogenic archaea.

### ***Effects of CT on Fiber Digestion***

The tannin-protein reaction has been widely exploited to improve efficiency of N utilization in ruminants (Aerts et al., 1999). However, the effects of CT on fiber, carbohydrates and VFA is less understood and the extent to which they complex with other nutrients in the rumen and consequential effects on energy and N metabolism in ruminants have been variable.

The major fiber-degrading bacteria in the rumen such as *Fibrobacter succinogenes, Ruminococcus albus,* and *Ruminococcus flavefaciens* have been found to be inhibited by tannins, although the degree of inhibition varied among studies depending upon the concentration and type of tannins (Patra and Saxena, 2011). The rate of fiber digestion is slowed due to complexing of tannins with fiber digesting enzymes or selective reduction of fiber-digesting bacteria due to specific tannin toxicity. The exposure of *F. succinogenes* to CT appears to cause the formation of tannin-protein complexes on the cell surface, which is suggested to interfere with the adhesion process of bacterial cells to cellulose (Patra and Saxena, 2011). This interference explains the reduction of the rate and extent of fiber digestion. As fiber digestion slows, subsequent release of energy metabolites (specifically propionate) would be reduced, but at a rate that achieves better synchrony with rumen utilization of N compounds for protein production. Similarly, CT in BFT inhibited the growth of *Clostridium proteoclasticum*, *B. fibrisolvens*, *Eubacterium spp. R. albus, F. succinogenes* and *S. bovis*, and the rate of proteolysis *in vitro* (Min et al., 2005). Jones et al. (1994) demonstrated that the growth of proteolytic bacteria i.e., *B. fibrisolvens, R. amylophilus* and *S. bovis* was reduced by sainfoin (*Onobrychris viciifolia*) CT, but a strain of *P. ruminicola* was tolerant to these CT. However, an inclusion of 30% Calliandra leaves containing tannins in the diet significantly reduced total cellulolytic bacteria including primary fiber degrading *F. succinogenes* and *Ruminococcus spp.* without affecting the total proteolytic bacteria and fungi, or efficiency of microbial protein synthesis (McSweeney et al., 2001).

## SUMMARY

To date, the majority of agricultural research in the United States has been focused on increasing productivity and efficiency, particularly on utilizing technologies that complement existing production systems and lead to benefits for the private sector (NRC, 2010). This mindset leaves several gaps in knowledge, so a need exists in current research to provide education and awareness of options producers can use to increase sustainability. It is possible to increase efficiency using low-input options like alternative forages. The same system that caused the problems cannot be expected to solve the problems without significant alteration in many aspects. Research that looks at alternative systems or technology, such as pasture-based dairy production, using new forages including BFT, looking at bioactive compounds such as CT to help solve ruminant waste issues, and allowing for whole system redesign (Reganold et al., 2011) may have to be the approach the industry uses to meet the challenges of sustainability. However, this approach will require a shifting of resources to fund new transdisciplinary research and to eliminate non-sustainable practices. This research must also address the significant gaps regarding the growing public concerns about dairy cattle production, including sustainability, environmental impact, and the welfare of food-production animals.

Three separate experiments were designed to address these principles of sustainability by incorporating new forage, bioactive compounds, and nutrient management strategies mentioned above by use of birdsfoot trefoil in dairy cow diets. The specific aim of these projects is to help answer the following questions: 1) Is birdsfoot trefoil a feasible option to feed to confined and grazing dairy cows to help achieve the desired increase in nutrient utilization by affecting an increase in N production and reduction in N waste? and, 2) Can birdsfoot trefoil be effectively used and managed in practical settings and in diets of dairy farms in the Western regions of the United States? Increasing nutrient utilization will lead to improved sustainability and efficient resource use, which will help to support long-term sustainability in the dairy industry.

Engagement in sustainable practices means more than public awareness of an established position (von Keyserlingk, et al., 2013); it will involve the opportunity to meet social needs in the broader society, and still meet the three core facets of sustainability. The dairy industry will need to be prepared to make changes to everyday production practices. It may require the acceptance that, in some cases, less milk production per cow may be desirable and necessary in order to support long term sustainability, as less milk may require less input, may lower the cost of production, and generate less waste, while still generating profit for dairy farms to be sustainable in the future.

## REFERENCES

Aerts, R. J., T .N. Barry, and W. C. McNabb. 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agric. Ecosyst. Env. 75:1–12.

Agle, M., A. N. Hristov, S. Zaman, C. Schneider, P. M. Ndegwa, and V. K. Vaddella. 2010. Effect of dietary concentrate on rumen fermentation, digestibility, and nitrogen losses in dairy cows J. Dairy Sci. 93:4211–4222.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle J. Dairy Sci. 83:1598–1624.

Alvarez, H. J., F. J. Santini, D. H. Rearte, and J. C. Rizzalde. 2001. Milk production and ruminal digestion in lactating dairy cows grazing temperate pastures and supplemented with dry cracked corn or high moisture corn. Anim. Feed Sci. Technol. 91:183-195

Animut, G., A. L. Goetsch, R. Puchala, A. K. Patra, T. Sahlu, and V. H. Varel. 2008. Methane emission by goats consuming diets with different levels of condensed tannins from lespedeza. Anim. Feed Sci. Technol. 144:212–227.

Aschemann, M., P. Lebzien, L. Hüther, S. Döll, K. H. Südekum, and S. Dänicke. 2012. Effect of niacin supplementation on digestibility, nitrogen utilization and milk and blood variables in lactating dairy cows fed a diet with a negative rumen nitrogen balance. Arch. Anim. Nutr. 66:200-214.

Auldist, M. J., L. C. Marett, J. S. Greenwood, M. Hannah, J. L. Jacobs, and W. J. Wales. 2013. Effects of different strategies for feeding supplements on milk production responses in cows grazing a restricted pasture allowance. J. Dairy Sci. 96: 1218-1231.

Baldinger, L., W. Zollitsch, and W. F. Knaus. 2014. Maize silage and Italian ryegrass silage as high-energy forages in organic dairy cow diets: Differences in feed intake, milk yield and quality, and nitrogen efficiency. Ren. Ag. Food Sys. 29:378-387.

Bargo, F., D. H. Rearte, F. J. Santini, and L. D. Muller. 2001. Ruminal digestion by dairy cows grazing winter oats pasture supplemented with different levels and sources of protein. J. Dairy Sci. 84:2260–2272.

Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. J. Dairy Sci. 85:1777–1792.

Bargo F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited Review: Production and digestion of supplemented dairy cows on pasture. J Dairy Sci. 86:1–42.

Barnes, D. K., and C. C. Sheaffer. 1995. Alfalfa. Pages 205–217 in Forages. An Introduction to Grassland Agriculture. Vol I, 5th ed. R. F. Barnes, D. A. Miller and J.C. Nelson, ed. Iowa State University Press, Ames.

Barry, T. N., and W. C. McNabb. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Brit. J. Nutr. 81:263–272.

Bauman, D. E., and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70:15–29.

Benchaar, C., T. A. McAllister, H. V. Petit, and P. Y. Chouinard. 2014. Whole flax seed and flax oil supplementation of dairy cows fed high-forage or high-concentrate diets: Effects on digestion, ruminal fermentation characteristics, protozoal populations and milk fatty acid profile. Anim. Feed Sci. Technol. 198:117–129.

Berdahl, J. D., J. F. Karn, and J. R. Hendrickson. 2001. Dry matter yields of cool-season grass monocultures and grass–alfalfa binary mixtures. Agron. J. 93:463–467.

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.

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.

Blumenthal M. J., and R. L. McGraw. 1999. Lotus adaptation, use and management. Pages 97–121 in Trefoil: The Science and Technology of Lotus. D. R. Beuselinck, ed. American Society of Agronomy, Crop Science Society of America, Madison, WI.

Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40: 357–368.

Bryant, R. H., D. E. Dalley, J. Gibbs, and G. R. Edwards. 2014. Effect of grazing management on herbage protein concentration, milk production and nitrogen excretion of dairy cows in mid-lactation. Grass Forage Sci. 69:1365-2494

Burke, J. L., G. C. Waghorn, I. M. Brookes, G. T. Attwood, E. S. Kolver, and S. W. Peterson. 2000. Formulating total mixed rations from forages-defining the digestion kinetics of contrasting species. NZ Soc. Anim. Prod. 60:9–14.

Capper, J. L., R. A. Cady, and D. E. Bauman. 2009. The environmental impact of dairy production: 1944 compared with 2007. J. Anim. Sci. 87:2160–2167.

Carvalho, P. C. de Faccio .2013. Harry Stobbs Memorial Lecture: Can grazing behavior support innovations in grassland management? Tropical Grasslands 1:137-155.

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

Cassida, K. A., T. S. Griffin, J. Rodriguez, S. C. Patching, O. B. Hesterman, and S. R. Rust. 2000. Protein degradability and forage quality in maturing alfalfa red clover, and birdsfoot trefoil. Crop Sci. 40:209–215.

Castillo, A. R., E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby, and J. France. 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. J. Anim. Sci. 79:247–253.

Chouinard, P. Y., L. Corneau, D. M. Barbano, L. E. Metzger, and D. E. Bauman. 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579–1584.

Dartt, B. A., J. W. Lloyd, B. R. Radke, J. R. Black, and J. B. Kaneene. 1999. A comparison of profitability and economic efficiencies between management-intensive grazing and conventionally managed dairies in Michigan. J. Dairy Sci. 82:2412–2420.

DePeters, E. J., and J. D. Ferguson. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. J. Dairy Sci. 75:3192–3209.

Dewhurst, R. J. 2005. Targets for milk fat research: nutrient, nuisance or nutraceutical? J. Agric. Sci., Camb. 143:359–367

Dhiman, T. R., G. R. Anand, L. D. Satter, and M. Pariza. 1999. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 79(Suppl. 1):137. (Abstr.)

Dhiman, T. R., L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright, and M. X. Tolosa. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 83:1016–1027.

Dillon, P, T. Hennessy, L. Shalloo, F. Thorne, and B. Horan. 2008. Future outlook for the Irish dairy industry: A study of international competitiveness, influence of international trade reform and requirement for change. Int. J. Dairy Technol. 61:16–29.

Elgersma, A., S. Tamminga, and G. Ellen. 2006. Modifying milk composition through forage. Anim. Feed Sci. Technol. 131: 207-225.

EPA. 2010. What is Sustainability? Accessed 15 March 2014. http://ww.epa.gov/sustainability/basicinfo.htm.

EPA. 2012. Science for a Sustainable Future: EPA Research Program Overview 2012-2016. P.3. Accessed Jan. 9, 2015. http://www.epa.gov/ord/docs/strap-overview2012.pdf.

Eun, J.-S., and B. R. Min. 2012. Emerging opportunities and challenges on exploitation of bioactive plant secondary compounds to mitigate environmental impacts by ruminants. J. Anim. Sci. 90 (Suppl. 3):491. (Abstr.)

FAO (Food and Agriculture Organization of the United Nations). 2009. How to Feed the World in 2050. Rome, Italy.

FAO (Food and Agriculture Organization of the United Nations). 2010. Greenhouse Gas Emissions from the Dairy Sector: A Life Cycle Assessment. Prepared by P. Gerber, T. Vellinga, C. Opio, B. Henderson, and H. Steinfeld. FAO, Rome, Italy.

Finlay, B. J., G. Esteban, K. J. Clarke, A. G. Williams, T. M. Embley, and R. P. Hirt. 1994. Some rumen ciliates have endosymbiotic methanogens. FEMS Microbiol. Lett. 117: 157–162.

Feeney, P. 1976. Plant apparency and chemical defense. Pages 168-214 in Recent Advances in Phytochemistry Vol. 10. Biochemical Interactions between plants and Insects. J. W. Wallace and R. L. Mansell, ed. Plenum Press, New York, NY.

Flis, S. A., and M. A. Wattiaux. 2005. Effects of parity and supply of rumen-degraded and undegraded protein on production and nitrogen balance in Holsteins. J. Dairy Sci. 88:2096–2106.

Fox, D. G., M. C. Barry, R. E. Pitt, D. K. Roseler, and W. C. Stone. 1995. Application of the Cornell Net Carbohydrate and Protein model for cattle consuming forages. J. Anim. Sci. 73:267-277.

Giallongo, F., J. Oh, T. Frederick, H. Weeks, A. N. Hristov, H. Lapierre, R. A. Patton, A. Gehman, and C. Parys. 2014. Effects of slow-release urea, rumen-protected methionine, and histidine on performance of dairy cows fed metabolizable protein-deficient diets. J. Dairy Sci. 98:3292–3308.

Gill, M., P. Smith, and J. M. Wilkinson. 2010. Mitigating climate change: the role of domestic livestock. Animal 4:323–333.

Giller, K. E., and G. Cadisch, G. 1995. Future benefits from biological nitrogen fixation: an ecological approach to agriculture. Pages 255-277 in Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems. Springer, Netherlands.

Griel, A. E., and P. M. Kris-Etherton. 2006. Beyond saturated fat: The importance of the dietary fatty acid profile on cardiovascular disease. Nutrition rev. 64:257–262.

Griinari, J. M., and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Pages 180–200 in Advances in Conjugated Linoleic Acid Research, vol 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M.W. Pariza, G. J. Nelson, ed. AOCS Press, Champaign, IL.

Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. J. Nutr. 130:2285-2291.

Groff, E. B., and Z. Wu. 2005. Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying proportion of alfalfa and corn silage. J. Dairy Sci. 88:3619–3632.

Halmemies-Beauchet-Filleau, A., A. Vanhatalo, V. Toivonen, T. Heikkilä, M. R. F. Lee, and K. J. Shingfield. 2014. Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60: 40 forage-to-concentrate ratio. J. Dairy Sci. 97:3761–3776.

Harfoot, C. G., and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. Pages 285–322 in The Rumen Microbial Ecosystem, P. N. Hobson and C. S. Stewart, ed. Elsevier Sci. Publ., New York, NY.

Harris, S. L., D. A. Clark, and P. J. Laboyrie. 1998. Birdsfoot trefoil-an alternative legume for New Zealand dairy pastures. Pages 99-104 in Proc. NZ Grassl. Assoc. B. V., Amsterdam, The Netherlands.

Hernandez I., and F. Van Breusegem. 2010. Opinion on the possible role of flavonoids as energy escape valves: Novel tools for nature's Swiss army knife? Plant Sci. 179:297–301.

Hills, J. L., W. J. Wales, F. R. Dunshea, S. C. Garcia, and J. R. Roche. 2015. Invited Review: an evaluation of the likely effects of individualized feeding of concentrate supplements to pasture-based dairy cows. J. Dairy Sci. 98:1–39.

Høgh-Jensen, H., R. Loges, F. V. Jørgensen, F. P. Vinther, and E. S. Jensen. 2004. An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. Ag. Sys. 82:181–194.

Holden, L. A., L. D. Muller, G. A. Varga, and P. J. Hillard. 1994. Ruminal digestion and duodenal nutrient flows in dairy cows consuming grass as pasture, hay, or silage. J. Dairy Sci. 77:3034–3042.

Hristov, A. N., and F. Giallongo. 2014. Feeding low protein diets to dairy cows. Fact Sheet. Dept of Animal Science, Pennsylvania State University. Accessed 22 Jan. 2015. http://redrockcorp.com.au/wp-content/uploads/2014/09/Feeding-Low-Protein-Diets-to-Dairy-Cows.pdf.

Hungate, R. E. 1967. Hydrogen as an intermediate in the rumen fermentation. Archiv für Mikrobiologie 59:158–164.

Huhtanen, P., J. I. Nousiainen, M. Rinne, K. Kytola, and H. Khalili. 2008. Utilization and partition of dietary nitrogen in dairy cows fed grass silage-based diets. J. Dairy Sci. 91:3589–3599.

Hymes-Fecht, U. C., G. A. Broderick, R. E Muck, and J. H Grabber. 2013. Replacing alfalfa or red clover silage with birdsfoot trefoil silage in total mixed rations increases production of lactating dairy cows. J. Dairy Sci. 96:460–469.

IPCC (Intergovernmental panel on Climate Change). 2007. Climate change 2007; the physical science basis. Pages 3-15 in Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. S. Solomon,

D. Qin, M. Manning M. and Z. Chen, ed. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

John, A. and J. A. Lancashire. 1981. Aspects of feeding and nutritive value of lotus species Proc. NZ Grassl. Assoc. 42:152–159.

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

Jones, G. A, T. A. McAllister, A. D. Muier, and K.-J. Cheng. 1994. Effects of sainfoin (*Orobrychis viciifolia Scop*.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol. 60:1374–1378

Jonker, J. S., R. A. Kohn, and J. High. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. J. Dairy Sci. 85:1218–1226.

Kalscheur, K. F., J. H. Vandersall, R. A. Erdman, R. A. Kohn, and E. Russek-Cohen. 1999. Effects of dietary crude protein concentration and degradability on milk production responses of early, mid, and late lactation dairy cows. J. Dairy Sci. 82:545–554.

Kalscheur, K. F., R. L. Baldwin, B. P. Glenn, and R. A. Kohn. 2006. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. J. Dairy Sci. 89: 249–259.

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

Kilcher, M. R. 1981. Plant development, stage of maturity and nutrient composition. J. Range Manag. Archives 34:363-364

Kebreab, E., J. France, D. E. Beever, and A. R. Castillo. 2001. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. Nutr. Cycl. Agroecosys. 60:275–285.

Khiaosa-Ard, R., S. F. Bryner, M. R. L. Scheeder, H. R. Wettstein, F. Leiber, M. Kreuzer, and C. R. Soliva. 2009. Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. J. Dairy Sci. 92:177–188.

Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. Van Amburgh, and D. E. Bauman. 1998a. Dietary fatty acid sources effect conjugated linoleic acid (CLA) concentrations in milk from lactating dairy cows. J. Nutr. 128: 881–885.

Kelly, M. L., E. S. Kolver, D. E. Bauman, M. E. Van Amburgh, and L.D. Muller. 1998b. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. J. Dairy Sci. 81:1630–1636.

Kennedy, E., M. McEvoy, J. P. Murphy, and M. O’Donovan. 2009. Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. J. Dairy Sci. 92:168–176.

Kepler, C. R., and S. B. Tove. 1967. Biohydrogenation of unsaturated fatty acids III. Purification and properties of a linoleate $\Delta^{12-}$cis, $\Delta^{11}$-trans-isomerase from Butyrivibrio fibrisolvens. J. Biol. Chem. 242: 5686–5692.

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

Kohn, R. A., Z. Dou, J. D. Ferguson, and R. C. Boston. 1997. A sensitivity analysis of nitrogen losses from dairy farms. J. Environ. Manag. 50:417–428.

Kolver, E. S., and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 81:1403–1411.

Kris-Etherton, P. M., S. Innis, and D. A. Ammerican. 2007. Position of the American Dietetic Association and Dietitians of Canada: Dietary fatty acids. J. Am. Dietetic Assoc. 107:1599–1611.

Lauriault, L. M., S. J. Guldan and C. A. Martin. 2003. Irrigated tall fescue–legume communities in the southern Rocky Mountains: Years five to eight. Agron J. 95:1497–1503.

Leaf, A., Y. F. Xiao, J. X. Kang, and G. E. Billman. 2003. Prevention of sudden cardiac death by n − 3 polyunsaturated fatty acids. Pharm. Therapeut. 98, 355–377.

Lechartier, C., and J.-L. Peyraud. 2010. The effects of forage proportion and rapidly degradable dry matter from concentrate on ruminal digestion in dairy cows fed corn silage-based diets with fixed neutral detergent fiber and starch contents. J. Dairy Sci. 93:666–681.

Lee, C., A. N. Hristov, K. S. Heyler, T. W. Cassidy, M. Long, B. A. Corl, and S. K. R. Karnati. 2011a. Effects of dietary protein concentration and coconut oil supplementation on nitrogen utilization and production in dairy cows. J. Dairy Sci. 94:5544–5557.

Lee, C., A. N. Hristov, H. Lapierre, T. Cassidy, K. Heyler, G. A. Varga, and C. Parys. 2011b. Effect of dietary protein level and rumen protected amino acid supplementation on dietary amino acid apparent digestibility and recovery in milk in lactating dairy cows. J. Dairy Sci. 94(Suppl. 1):689(Abstr.)

Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012a. Rumen protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed metabolizable protein-deficient diet. J. Dairy Sci. 95:6042–6060.

Lee, C., A. N. Hristov, K. S. Heyler, T. W. Cassidy, H. Lapierre, G. A. Varga, and C. Parys. 2012b. Effects of metabolizable protein supply and amino acids supplementation on nitrogen utilization, production and ammonia emissions from manure in dairy cows. J. Dairy Sci. 95:5253–5268.

Lee, C., G. W. Feyereisen, A. N. Hristov, C. J. Dell, J. Kaye, and D. Beegle. 2014. Effects of dietary protein concentration on ammonia volatilization, nitrate leaching and plant nitrogen uptake form dairy manure applied to lysimeters. J. Environ. Qual. 43:398–408.

Lee, Y. J., and T. C. Jenkins. 2011. Biohydrogenation of linolenic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. J. Nutr. 141:1445–1450.

Lemaire, G., A. Franzluebbers, P. C. de Faccio Carvalho, and B. Dedieu. 2014. Integrated crop–livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. Agric. Ecosystems and Environ. 190:4-8.

Lizarralde, C., V. Picasso, C. A. Rotz, M. Cadenazzi, and L. Astigarraga. 2014. Practices to reduce milk carbon footprint on grazing dairy farms in Southern Uruguay: Case studies. Sus. Ag. Res, 3:1.

Lock, A. L., and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39: 1197-1206.

Lock, A. L., and K. J. Shingfield. 2004. Optimizing milk composition Pages 107–188 in UK Dairying: Using Science to Meet Consumers' Needs. E. Kebreab, J. Mills, and D. E. Beever, ed. Nottingham University Press, Nottingham, UK.

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 Rumin. Res. 49:241–256.

Mandok, K. S., J. K. Kay, S. L. Greenwood, G. R. Edwards, and J. R. Roche. 2013. Requirements for zero energy balance of nonlactating, pregnant dairy cows fed fresh autumn pasture are greater than currently estimated. J. Dairy Sci. 96:4070–4076.

McSweeney, C. S., B. Palmer, R. Bunch, and D. O. Krause. 2001. Effect of the tropical forage calliandra on microbial protein synthesis and ecology in the rumen. J Appl. Microbiol. 90:78–88.

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 temperate forages: A review. Anim. Feed Sci. Technol. 106:3–19.

Min, B. R., W. E. Pinchak, J. D. Fulford, and R. Puchala. 2005. Wheat pasture bloat dynamics, *in vitro* ruminal gas production and potential bloat mitigation with condensed tannins. J. Anim. Sci. 83:1322–1331.

Misselbrook, T. H., J. M. Powell, G. A. Broderick, and J. H. Grabber. 2005. Dietary manipulation in dairy cattle: Laboratory experiments to assess the influence on ammonia emissions. J. Dairy Sci. 88:1765–1777.

Moss, A. R., J. P. Jouany, and J. Newbold. 2000. Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49:231–253.

Mowat, D. N., M. L. Kwain, and J. E. Winch. 1969. Lignification and *in vitro* cell wall digestibility of plant parts. Can. J. Plant Sci. 49:499–504.

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

Nennich, T. D., J. H. Harrison, L. M. VanWieringen, D. Meyer, A. J. Heinrichs, W. P. Weiss, N. R. St-Pierre, R. L. Kincaid, D. L. Davidson, and E. Block. 2005. Prediction of manure and nutrient excretion from dairy cattle. J. Dairy Sci. 88:3721–3733.

Nennich, T. D., J. H. Harrison, L. M. VanWieringen, N. R. St-Pierre, R. L. Kincaid, M. A. Wattiaux, D. L. Davidson, and E. Block. 2006. Prediction and evaluation of urine and urinary nitrogen and mineral excretion from dairy cattle. J. Dairy Sci. 89:353–364.

Nocek, J. E., and S. Tamminga. 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74:3598–3629.

Noviandi, C. T., K. Neal, J-S. Eun, M. D. Peel, B. L. Waldron, D. R. Zobell, and B. R. Min. 2014. Comparison of alfalfa, birdsfoot trefoil, and cicer milkvetch in combination with 25, 50, or 75% tall fescue in a continuous-culture system. Prof. Anim. Sci. 30:23–32.

NRC (National Research Council). 2003. Air Emissions from Animal Feeding Operations: Current knowledge, future needs. Natl. Acad. Sci., Washington, DC.

NRC (National Research Council). 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

NRC (National Research Council). 2010. Toward sustainable agricultural systems in the 21st Century. Natl. Acad. Sci., Washington, DC.

Olmos-Colmenero, J. J., and G. A. Broderick. 2006a. Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. J. Dairy Sci. 89:1635–1643.

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

Paine, L. 2009. Grass-based dairy products: challenges and opportunities. UW-Madison Center for Integrated Agricultural Systems. Accessed online Jan. 2015. http://www.cias.wisc.edu.

Patra, A. K., and J Saxena. 2009. Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. Anton van Leeuwen 96:363–375.

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.

Pérez-Ramírez, E., J. L. Peyraud, and R. Delagarde. 2009. Restricting daily time at pasture at low and high pasture allowance: Effects on pasture intake and behavioral adaptation of lactating dairy cows. J. Dairy Sci. 92:3331–3340.

Peyraud, J. L., and R. Delagarde. 2013. Managing variations in dairy cow nutrient supply under grazing. Animal 7:57–67.

Phelan, P., I. A. Casey, and J. Humphreys. 2013. The effect of target postgrazing height on sward clover content, herbage yield, and dairy production from grass-white clover pasture. J. Dairy Sci. 96:1598–1611.

Reganold, J. P., D. Jackson-Smith, S. S. Batie, R. R. Harwood, J. L. Kornegay, D. Bucks, C. B. Flora, J. C. Hanson, W. A. Jury, D. Meyer, A. Schumacher Jr., H. Sehmsdorf, C. Shennan, L. A. Thrupp, and P. Willis. 2011. Transforming U.S. agriculture. Science 332:670–671.

Reis, R. B., and D. K. Combs. 2000. Effects of increasing levels of grain supplementation on rumen environment and lactation performance of dairy cows grazing grass legume pasture. J. Dairy Sci. 83:2888–2898.

Rhoades, D. F., and R. G. Cates. 1976. A general theory of plant herbivore chemistry. Pages 168–214 in Recent Advances in Phytochemistry Vol. 10. Biochemical Interactions between Plants and Insects. J. W. Wallace, and R. L. Mansell, ed. Plenum Press, New York, NY.

Rinehart, L., and A. Baier. 2011. Pasture for organic ruminant livestock: Understanding and implementing the National Organic Program (NOP) pasture rule. USDA National Center for Appropriate Technology. Accessed 5 Nov 2014. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5091036

Roche, J. R., J. K. Kay, A. G. Ruis, T. M. Grala, A. J. Sheahan, H. M. White, and C.V.C. Phyn. 2013. Short communication: Immediate and deferred milk production responses to concentrate supplements in cows grazing fresh pasture. J. Dairy Sci., 96:2544–2550.

Rotz, C. A. 2004. Management to reduce nitrogen losses in animal production. J. Anim. Sci. 82: E119–E137(Suppl.)

Russell, J. B. 1998. Strategies that ruminal bacteria use to handle excess carbohydrate. J. Anim. Sci. 76:1955–1963.

Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A Net Carbohydrate and Protein System for evaluating diets: I. Ruminal fermentation. J. Anim. Sci. 70:3551–3561.

Scalbert, A. 1991. Antimicrobial properties of tannins. Review article number 63. Phytochem 30:3875–3883.

Schultz, J. C., M. D. Hunter, and H. M. Appel. 1992. Antimicrobial activity of polyphenols mediates plant-herbivore interactions. Page 621 in Plant Polyphenols. Synthesis, Properties and Significance. R. W. Hemingway and P. E. Laks, ed. Plenum Press, New York, NY.

Singh, R. J., G. H. Chung, and R. L. Nelson. 2007. Landmark research in Legumes. Genome 50:525–537.

Sleugh, B., K. J. Moore, J. R. George, and E. C. Brummer. 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agron. J. 92:24–29.

Smith, L. W., H. K. Goering, and C. H. Gordon. 1972. Relationships of forage compositions with rates of cell wall digestion and indigestibility of cell walls. J. Dairy Sci. 55:1140–1147.

Soriano, F. D., C. E. Polan, and C. N. Miller. 2000. Milk production and composition, rumen fermentation parameters, and grazing behavior of dairy cows supplemented with different forms and amounts of corn grain. J. Dairy Sci. 83:1520–1529.

Sun, X. Z., G. C. Waghorn, S. O. Hoskin, S. J. Harrison, S. Muetzel, and D. Pacheco. 2012. Methane emissions from sheep fed fresh brassicas (*Brassica* spp.) compared to perennial ryegrass *Lolium perenne.* Anim. Feed Sci. Technol. 176:107–116.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75:345–357.

Tan, H. Y., C. C. Sieo, N. Abdullah, J. B. Liang, X. D. Huang, and Y. W. Ho. 2011. Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. Anim. Feed Sci. Technol. 169:185–193.

Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. J. Dairy Sci. 89:3494-3500.

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. 123:403–409.

Thompson, P. B. 2007. Agricultural sustainability: What it is and what it is not. Int. J. Agric. Sustain. 5:5–16

Tomlin, D. C., R. R. Johnson, and B. A. Dehority. 1965. Relationship of lignification to in vitro cellulose digestibility of grasses and legumes. J. Anim. Sci. 24:161–165.

Turner, S. A., G. C. Waghorn, S. L. Woodward, and N. A. Thomson. 2005. Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) affect the detailed composition of milk from dairy cows. Proc. NZ Soc. Anim. Prod. 65:283–289.

Ulyatt, M. J. 1981. The feeding value of herbage; can it be improved? NZ Agric. Sci. 15:200–205.

Undersander, D., B. Albert, D. Cosgrove, D. Johnson, and P. Peterson. 2002. Pastures for profit: A guide to rotational grazing, A3529. University of Wisconsin-Extension, Madison.

USDA-NASS (US Department of Agriculture National Agricultural Statistics Service). 2012. Farms, Land in Farms, and Livestock Operations. Accessed Jan. 18, 2015. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1259

Van Nevel, C. J., and D. I. Demeyer. 1996. Control of rumen methanogenesis. Environmental Monitoring and Assess. 42:73–97.

Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. J. Anim. Sci. 24:834–843.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. McGraw-Hill, New York, NY.

VandeHaar, M. E. 1998. Efficiency of nutrient use and relationship to profitability on Dairy Farms. J. Dairy Sci. 81:272–282.

VandeHaar, M. E., and N. R. St. Pierre. 2006. Major advances in nutrition: Relevance to the sustainability of the dairy industry. J. Dairy Sci. 89:1272–1291

von Keyserlingk, M. A. G., N. P. Martin, E. Kebreab, K. F. Knowlton, R. J. Grant, M. Stephenson, and S. I. Smith. 2013. *Invited review*: Sustainability of the US dairy industry. J. Dairy Sci. 96:5405–5425.

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. 2004. Feeding value of pastures for ruminants. NZ Vet. J. 52:320–331.

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. 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 corniculatus* L. Brit. J. Nutr. 57:115–126.

Wang, F., Z. Dou, L. Ma, W. Ma, J. T Sims, and F. Zhang. 2010. Nitrogen mass flow in China's animal production system and environmental implications. J. Environ. Qual. 35:1537–1544.

Weiss, W. P., L. B. Willett, N. R. St-Pierre, D. C. Borger, T. R. McKelvey, and D. J. Wyatt. 2009. Varying forage type, metabolizable protein concentration, and carbohydrate source affects manure excretion, manure ammonia, and nitrogen metabolism of dairy cows. J. Dairy Sci. 92:5607–5619.

Wells, J. E., and J. B. Russell. 1996. Why do many ruminal bacteria die and lyse so quickly? J. Dairy Sci. 79:1487–1495.

Wen, L., R. L. Kallenbach, J. E. Williams, C. A. Roberts, P. R. Beuselinck, R. L. McGraw, and H. R. Benedict. 2002. Performance of steers grazing rhizomatous and nonrhizomatous birdsfoot trefoil in pure stands and in tall fescue mixtures. J. Anim. Sci. 80:1970–1976.

WHO (World Health Organization). 2003. Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO Expert Consultation. WHO Technical Report Series 916, Geneva, Switzerland.

Williams, C. M., J-S. Eun , C. M. Dschaak, J. W. MacAdam, B. R. Min, and A. J. Young. 2010. Case study: in vitro ruminal fermentation characteristics of birdsfoot trefoil (Lotus corniculatus L.) hay in continuous cultures. Prof. Anim. Sci. 26:570-576.

Williams, C.W., J-S. Eun, J. W. MacAdam, A. J. Young, V. Fellner, and B. R. Min. 2011. Effects of forage legumes containing condensed tannins on methane and ammonia production in continuous cultures of mixed ruminal microorganism. Anim. Feed Sci. and Technol. 166:364–372.

Woodward, S. L., A. V. Chaves, G. C. Waghorn, and P. G. Laboyrie. 2002. Supplementing pasture-fed dairy cows with pasture silage, maize silage, Lotus silage or sulla silage in summer - does it increase production? Proc. NZ Grassl. Assoc. 64:85–89.

Woodward, S. L., P. J. Laboyrie and E. B. L. Jansen. 2000. *Lotus corniculatus* and condensed tannins - Effects on milk production by dairy cows. Asian-Aust. J. Anim. Sci. 13:521–525.

Woodward, S. L., G. C. Waghorn, K. A. Watkins, and M. A. Bryant. 2009. Feeding birdsfoot trefoil (*Lotus corniculatus*) reduces the environmental impacts of dairy farming. Proc NZ Soc Anim Prod 69:1–5.

**Table 2.1** Effect of annual milk production and type of diet on lifetime efficiency of land use by a dairy cow.

| | Grazing | | Confined Feeding[1] | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | Grass-legume system[2] | Grass only system[1] | Without by-product feeds | | | With by-product feeds | | |
| Milk production, kg/yr | 6400 [3] | 5000 | 5000 | 10,000 | 15,000 | 5000 | 10,000 | 15,000 |
| Feed,[4] kg/yr of DM/yr | 5140[5] | 6050[6] | 5870 | 8120[7] | 10,540 | 5950[8] | 8380 | 11,150 |
| Land required,[5] ha/yr | 0.47 | 0.54 | 0.66 | 0.97 | 1.34 | 0.30 | 0.49 | 0.68 |
| Protein production, kg/ha | 646 | 359 | 295 | 371 | 392 | 642 | 731 | 770 |
| Efficiency of land use,[8] % | 78 | 43 | 35 | 45 | 47 | 76 | 88 | 93 |

[1] Adapted from VandeHaar, 1998 J. Dairy Sci. 82:272-282.

[2] Estimated from data reported by Phelan, et al., 2013 J Dairy Sci. 96:1598-1611 using VandeHaar (1998) equations.

[3] The average lifetime of a cow is 4.83 yr and consists of 730 d as a heifer, three 305-d lactations, and two 60-d dry periods. Milk production is the 3.5% FCM production of a cow during its productive lifetime, divided by the 2.83 yr spent as a cow.

[4] The annual feed DMI for an animal is the lifetime DM consumption divided by the 2.83 yr spent as a lactating or dry cow. Thus, the annual feed DM required per cow is similar to the predicted annual feed DM needs per cow in a dairy enterprise (cows plus replacement heifers) of similar annual milk production. Digestibility is assumed to decrease as intake increases and is discounted for a cow or heifer at a given life stage as [1.0 – DF (MM – 1)0.8], where DF = discount factor, and MM = multiple of maintenance intake. The base discount factor is 4% for the grazing system and confined feeding system without by-product feeds and 6% for the confined feeding system with byproduct feeds.

[5] Feed DM consumed during the lifetime of cow in a legume-grass grazing system (Phelan et al., 2013) consists of 92% pasture and 8 % concentrate which consists of 26% barley grain, 25% corn gluten feed, 35% beet pulp, 12 % soybean meal and 1% minerals and vitamins. High quality pasture is considered to be available for the entire year so that the average diet contained 1.6 Mcal of NEL at 3× maintenance intake per kilogram of DM and 21 % CP

[6] Feed DM consumed during the lifetime of cow in a grazing system consists of 99% pasture and 1% minerals and vitamins. High quality pasture is considered to be available

for the entire year so that the average diet contained 1.6 Mcal of NEL at 3× maintenance intake per kilogram of DM and 16% CP.

[7] Feed DMI during the lifetime of a confined cow fed no by-product feeds and producing 10,000 kg/yr consists of 34% alfalfa, 26% corn silage, 26% corn grain, 12% soybeans, and 2% minerals and vitamins. The average diet during the lifetime of the cow contains 1.65 Mcal of 3× NEL/kg of DM and 16% CP. The amount of soybeans fed is decreased with lower milk production and increased with higher milk production.

[8] Feed DMI during the lifetime of a confined cow fed by-product feeds and producing 10,000 kg/yr consists of 25% alfalfa, 25% corn silage, 13% corn grain, 2% soybeans, 12% corn gluten feed, 10% cottonseeds, 10% wheat middlings, 1% blood meal, and 2% minerals and vitamins. The average diet during the cow's lifetime contains 1.65 Mcal of 3× NEL/kg of DM and 16% CP. The amount of soybeans fed is decreased with lower milk production and increased with higher milk production.

[9] Annual cropping yields are 11,120 kg/ha for alfalfa, 20,210 kg/ha for corn silage, 8150 kg/ha for corn grain, and 2690 kg/ha for soybeans.

[10] Protein production per hectare is 3.5% FCM yield times 3.3% protein plus accretion of body and conceptus protein during the lifetime of the cow divided by the 2.83 yr spent as a cow divided by the land required per year. A cow is considered to weigh 625 kg at maturity and calves to weigh 45 kg at birth. Body mass is considered to be 12% protein.

[11] Efficiency of land use is the protein production per hectare from dairy farming relative to the amount of protein that could be produced from soybeans and corn grown for direct human consumption. Equal cropping of corn and soybeans would provide 986 kg protein/ha with the same protein to calorie ratio as whole milk at 3.5% fat. This calculation of efficiency assumes that milk protein, because of its greater digestibility and better amino acid profile, is 20% more valuable on a weight basis than is the protein of the mix of corn and soybeans.

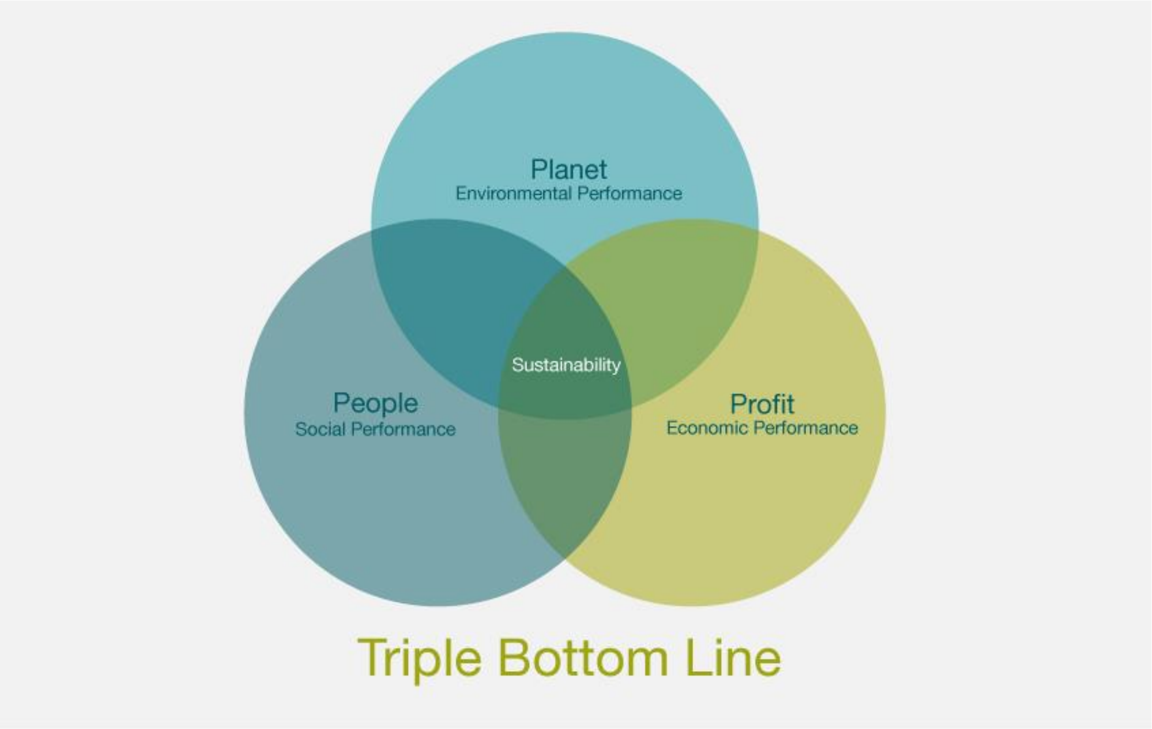

Figure **2.1** The 3 pillars of sustainability. Image courtesy of Triumvirate environmental (http://www.triumvirate.com)

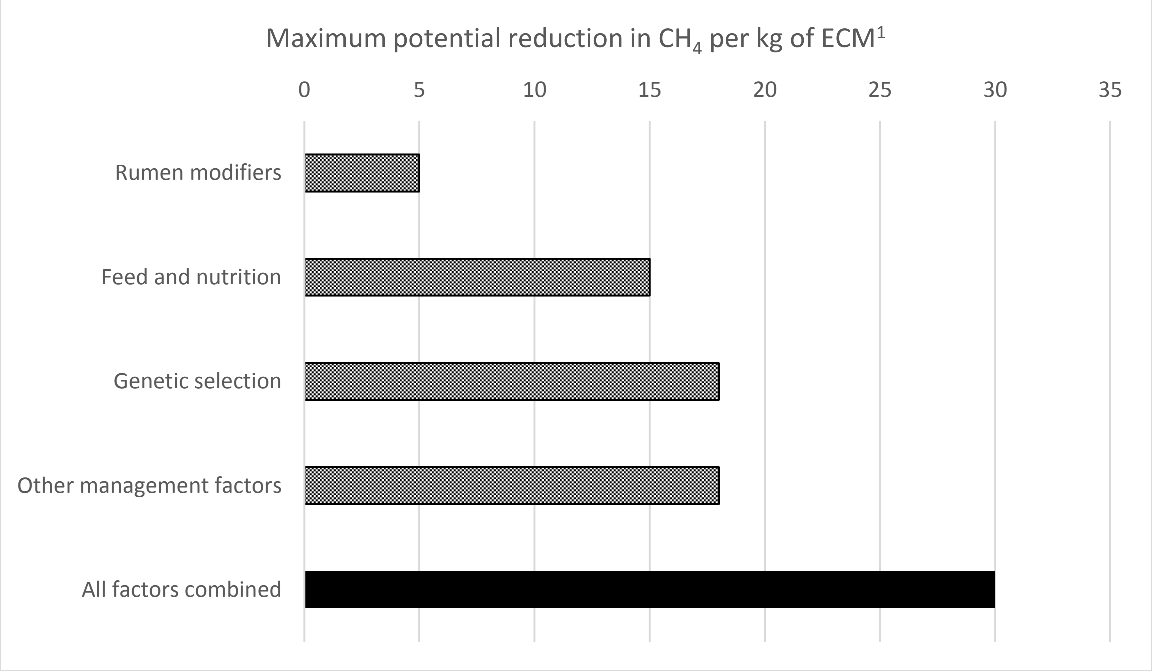

**Figure 2.2** Estimated maximum impact of factors influencing methane mitigation in intensive dairy production.[1]Various approaches to mitigating CH4 in that have been demonstrated to be effective on an in vivo basis. Combined factors are not completely additive due to overlap of metabolic functions affecting methane production. Adapted from Knapp, et al., 2014.

# CHAPTER 3

# FEEDING BIRDSFOOT TREFOIL HAY IN HIGH-FORAGE DIETS INCREASES FIBER DIGESTION, NITROGEN UTILIZATION EFFICIENCY, AND LACTATIONAL PERFORMANCE BY DAIRY COWS

## INTRODUCTION

Forage quality affects feed intake and dietary energy density, lactational performance, cow health, and feed cost. Dairy producers in the western US use alfalfa (*Medicago sativa* L.) to make up the majority of the forage portion in dairy diets. Alfalfa is a very palatable legume that is high in CP and low in fiber, and thus feeding alfalfa can maximize intake and production of dairy cattle (Martin and Mertens, 2005). However, the protein in alfalfa is extensively degraded to ammonia in the rumen, resulting in excess N waste excretion. Birdsfoot trefoil (*Lotus corniculatus* L.; **BFT**) is a non-bloating forage legume that is similar to alfalfa in feeding value, and has a potential to be fed as a main forage to dairy cows due to its unique cell wall digestion characteristics as well as condensed tannins (**CT**; Williams et al., 2011). Within the legume family, there is evidence that lignification has less effect on digestibility in BFT than in alfalfa. At the early bloom stage, Tomlin et al. (1965) demonstrated BFT cellulose to be digested more rapidly than alfalfa cellulose, despite similar lignin concentrations. McGraw and Marten (1986) reported greater digestibility of BFT stems than alfalfa stems. Mowat et al. (1969) found a highly negative correlation ($R^2$ = 0.78) between cell wall lignin concentration and in vitro cell wall digestibility in alfalfa, BFT cell wall digestibility was not correlated with lignin concentration ($R^2$ = 0.13). Greater cell wall digestibility in BFT may lead to an increase in DMI by lactating dairy cows (Allen, 2000). In addition, feeding BFT has been reported to improve N utilization by ruminants due to CT in BFT (Barry and McNabb, 1999; Min et al., 2003). Moderate concentrations of CT (2.0–4.0% DM) bind protein by hydrogen bonding at near neutral pH (i.e., pH 6.0–7.0) in the rumen to form CT–protein complexes, but dissociate and release bound protein at pH < 3.5 in the abomasum (Barry et al., 2001). Using continuous cultures, we previously reported that ammonia-N (**$NH_3$-N**) concentration and flow were reduced when replacing alfalfa hay (**AH**) with BFT hay (**BFTH**) in dairy TMR (Williams et al., 2011). This in vitro result suggests that incorporating BFTH as a CT-containing legume forage into dairy TMR can have a potential to improve environmental performance of dairy operations through a reduction in N excretion.

Several studies have shown that BFT fed to dairy cows as a preserved silage (Hymes-Fecht et al., 2013) or fresh forage (Woodward et al., 2000) increases N utilization, reduces N excretion in urine, and shifts N excretion to feces. However, no studies have been done to determine if BFT preserved as a dry hay have the same effects as fresh or wet-preserved forages. Thus, we sought to determine effects of feeding BFTH in high-forage dairy diets on nutrient intake and utilization, ruminal fermentation profiles, and lactational performance of dairy cows. It was hypothesized that feeding BFTH-based lactation diet would increase intake of DM and digested NDF by dairy cows compared with AH-based diet due to increased NDF digestibility, leading to improved lactational performance and N utilization efficiency.

## MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University. The experiment was conducted at the Caine Dairy Research Center (Wellsville, UT), Utah State University.

### *Cows, Experimental Design, and Diets*

Nine multiparous lactating Holstein cows, 3 of which were previously surgically fitted with rumen cannula, were used for this experiment. Cows began the experiment averaging 131 ± 22.6 DIM. Average BW were 778 ± 73.6 and 787 ± 65.7 kg at the beginning and the end of the experiment, respectively.

The design of the experiment was a triple 3 × 3 Latin square. Within each square, cows were randomly assigned to a sequence of 3 diets during each of the three 21-d periods (14 d of treatment adaptation and 7 d of data and sample collections). The 3 experimental diets included: 1) AH-based TMR (**AHT**); 2) AH and BFTH-based TMR (**ABT**); and BFTH-based TMR (**BT**; Table 1). As a forage source, AH was partially or completely replaced with BFTH in ABT or BT, respectively. Corn silage was included in the 3 diets at 15.4% DM. The diets included AH at 42% in AHT or 21% in ABT. Typical lactating dairy diets in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) contain more AH than corn silage, and baled AH is commonly fed to provide 50 to 75% of the dietary forage with total forage levels averaging 45 to 55% of the dietary DM. The BT contained BFTH at 42% of diet DM. Therefore, 3 experimental diets maintained forage-to-concentrate ratio of 58:42 on a DM basis. The BFTH used in this experiment was Norcen variety, planted in fall 2009 on a private ranch (Garland, UT), and was the second cutting harvested at early-bloom stage July 17, 2011 by mower-conditioner (model 830, John Deere, Moline, IL), and allowed to sun-cure for 5 d before baling. The chemical composition of the BFTH was 15.8, 39.0, and 33.8% DM for CP, NDF, and ADF, respectively. The AH used in our experiment was clean, bright green, fine-stemmed second cutting, and preserved as sun-cured hay with a chemical composition of 17.7, 34.6, and 30.0% DM for CP, NDF, and ADF, respectively.

Condensed tannin of AH and BFTH were isolated and purified from ground samples of the hay (0.5 mm) using the modified HCl-butanol-acetone assay (Grabber et al., 2013); a BioMate 3 spectrophotometer was used to quantify the CT. While CT concentration of AH was negligible, BFTH contained 1.55% CT, resulting in 0.38 and 0.51% CT concentration to ABT and BT, respectively (Table 3.1).

The TMR diets excluding corn silage were processed for approximately 15 min in a TMR wagon (model 455, Roto-Mix, Dodge City, KS). The corn silage used in this experiment had a chemical composition of 6.27, 39.4, and 25.8% DM for CP, NDF, and ADF, respectively, and was added just prior to feeding each diet, mixed and wieghed with a Rissler TMR mixer cart (I. H. Rissler Mfg, Curtiss, WI). Diets were formulated to be isonitrogenous across treatments averaging 15.6% CP, isocaloric (1.60 Mcal/kg), and to meet NRC (2001) recommendations for RDP, RUP, minerals, and vitamins of a mid-lactation dairy cow weighing 780 kg (BCS = 3.0) and producing 36.3 kg of milk/d containing 3.5% fat and 3.0% true protein with 24.9 kg/d of DMI. Concentration of NDF was slightly less for both BFTH diets (39.7 and 38.5% of DM for ABT and BT, respectively) compared with AHT (41.1% of DM; Table 3.1), whereas NFC concentration was 2.8% units greater in BT than AHT and ABT (34.3 vs. 31.5%), respectively.

Cows were housed in a tie-stall barn on a rubber mattress covered with straw and were individually fed. Cows had free access to water. Diets were mixed at 0400 h and fed twice daily as a TMR with 70% of allotted feed fed at 0500 h and 30% fed at 1400 h. Feed offered and refused was recorded daily, and samples taken during the sampling week to determine DMI with weekly adjustment to achieve between 5 and 10% refusals.

Cows were milked twice daily at 0300 and 1500 h. Milk weights were recorded using BouMatic automated meters (Madison, WI) at each milking throughout the experiment. Milk samples were collected from 6 consecutive milkings during d 15 through 17 in each sampling period. Individual milk samples were analyzed by the Rocky Mountain DHIA Laboratory (Nibley, UT) for fat, true protein, lactose, and MUN. Daily milk composition was calculated from the weighted a.m. and p.m. observations. Yields of milk fat, true protein, and lactose were calculated by multiplying milk yield from respective day by fat, true protein, and lactose concentrations of the milk from each individual cow. To convert milk true protein to milk N, 6.38 was used as the conversion factor (DePeters and Cant, 1992), and total milk N (kg/d) was calculated as milk true protein/6.38 + MUN (DHIA, 2013), where milk true protein and MUN were expressed as kg/d.

### *Feed Sampling and Analysis*

Samples of AH, BFTH, and corn silage were taken weekly to determine DM concentration, and dietary concentrations of forages and concentrates were adjusted weekly on an as-fed basis to reflect changes in the DM concentrations. Samples of feeds were collected weekly to determine DM concentration and composited by period for chemical analysis. Samples of TMR fed and orts for each cow were collected daily during the sampling week and stored frozen at -20°C until composited for analysis. Then, the composite samples were dried at 60°C for 48 h, ground to pass a 1 mm screen (Wiley Mill model 4; Thomas Scientific Co., Swedesboro, NJ), and retained for chemical analyses. The DM concentrations of samples were used to calculate DM and nutrient intake.

Analytical DM concentration of samples was determined by oven drying overnight at 105°C, and OM was determined by ashing at 550°C for 5 h (AOAC, 2000; method 942.05). Crude protein concentration was determined by automated N dry combustion (Flash 2000 Automatic Elemental Analyzer, ThermoFischer Scientific, The Netherlands; AOAC, 2000; method 968.06). Concentrations of NDF and ADF were determined sequentially using a fiber analyzer (200/220, Ankom Technology Corp., Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination with pre-treatment of heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Apparent total tract digestibility of CP and NDF were measured during the last week in each period using acid-insoluble ash (**AIA**) as an internal marker (Van Keulen and Young, 1977). Fecal samples (approximately 100 g, wet weight) were collected for each cow from the rectum twice daily (a.m. and p.m.) every 12 hours moving ahead 2 h each day for the 5 d sampling of feces beginning on d 15. This schedule provided 12 representative samples of feces for each cow. Samples were composited across sampling times for each cow, dried at 60°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Intake of digested NDF was calculated from NDF intake and concentrations of AIA and NDF in diets fed, orts, and feces using the following equation: intake of digested NDF (kg/d) = intake of NDF (kg/d) × 100 − [100 × $(AIA_d/AIA_f) \times (NDF_f/NDF_d)$], where $AIA_d$ = AIA concentration in the diet actually consumed, $AIA_f$ = AIA concentration in the feces, $NDF_f$ = concentration of the NDF in the feces, and $NDF_d$ = concentration of the NDF in the diet actually consumed. Intake of undigested NDF was calculated from the following equation: intake of undigested NDF (kg/d) = intake of NDF (kg/d) − intake of digested NDF (kg/d) (Eun and Beauchemin, 2005).

### ***Ruminal Fermentation Sampling and Analysis***

Ruminal pH was continuously measured on cannulated cows for 2 consecutive days starting on d 15 using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA) as described by Penner et al. (2006). Readings in pH buffers 4 and 7 were recorded prior to placing the LRCpH system in the rumen. Ruminal pH readings were taken every 30 s and stored by the data logger. After about 48 h of continuous pH measurement, the LRCpH was removed from the rumen, washed in 39°C water, and millivolt readings were recorded in pH buffers 4 and 7. The daily ruminal pH data was averaged for each minute and summarized as minimum pH, mean pH, and maximum pH. Also, when ruminal pH was less than 5.8, daily episodes, duration (h/d), and area (pH × min) were calculated. The threshold of 5.8 was chosen because it has been previously described by others (Nocek, 1997; Maekawa et al., 2002; Beauchemin and Yang, 2005) to cause ruminal acidosis.

Samples of whole ruminal contents were collected at 0, 2, 4, 6, 8, 10, and 12 h following the a.m. feeding on d 20 and 21 of each experimental period from cannulated cows. Rumen samples were collected from different locations in the reticulo-rumen (ventral sac, reticulum, and feed mat in the dorsal rumen; approximately 250 g each), composited, and filtered through a polyester screen (pore size 355μm; B & S H Thompson, Ville Mont-Royal, QC, Canada) and immediately analyzed for pH. Two separate 5-mL aliquots of the rumen samples were conserved and immediately frozen until processed for analyses of $NH_3$-N and VFA. Concentration of $NH_3$-N of rumen contents was determined using methods described by Rhine et al. (1998) after thawing samples and adding 1 mL of 1% sulfuric acid. For VFA determination, 5-mL samples taken at 0 and 6 h post a.m. feeding were added to 1 mL of 25% meta-phosphoric acid and quantified using a GLC (model 5890 series II; Hewlet Packard Co, Palo Alto, CA.) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium (Eun and Beauchemin, 2007).

### *Urine Sampling and Analysis*

Spot urine samples were collected at 0600 and 1800 on d 18 and 19 in each period from all cows. Urine samples were acidified during collection to a pH < 3.8 by addition of 4 *M* HCl. The acid solution was added to urine in the urine containers during collection, and then the urine was frozen in separate containers at −20°C. For analysis, samples were thawed and composited by cow and period, and aliquots were diluted at 1:39 with urine diluent. Diluent was 0.202% sodium 1-heptane sulfonic acid and 0.086% ammonium dihydrogen phosphate ($NH_4H_2PO_4$), acidified to pH 2.1 with 4 *M* HCl, and directly analyzed for the pyrimidine and purine derivatives (**PD**) allantoin, uric acid, xanthine, hypoxanthine, and pseudouridine, as well as creatinine by using an HPLC (Waters Corp., Milford, MA) according to the procedures of Shingfield and Offer (1999). Urinary creatinine was used as a marker to estimate urine volume (Valadares et al., 1999; Leonardi et al., 2003). In calculating urine volume, we assumed that creatinine output averaged 28 mg/kg of BW as estimated by Whittet (2004). Similar daily creatinine outputs, ranging from 25 to 30 mg/kg of BW have been reported (McCarthy et al., 1983; Jones et al., 1990). The ratio of the urinary PD allantoin and uric acid to creatinine was used to estimate the relative differences in MCP production (Shingfield and Offer, 1998). Supply of MCP to the small intestine was estimated based on estimates of urinary excretion of PD, according to the method of Chen and Gomes (1992). Pyrimidine and PD were calculated based on methods of Wattiaux and Karg, 2004 and Janicek et al., 2008.

### ***Statistical Analyses***

Data were summarized for each cow by measurement period. All data were statistically analyzed using the mixed model procedure of SAS (SAS Institute, 2012) with a model that included the fixed effect of dietary treatment using the repeated option. Cow and period were the terms of the random statement. Nutrient and DM intake, milk yield, milk composition data, VFA profiles, and N utilization were analyzed as repeated measures in sampling days. Simple, autoregressive one, and compound symmetry covariance structures were used in the analysis depending on low values for the Akaike’s information criteria and Schwartz's Bayesian criterion. Milk yield and milk composition data were subjected to analyses using unstructured covariance structures. Data for $NH_3$-N were analyzed by the heterogeneous compound symmetry structure. For all models used, degrees of freedom were estimated with the Kenward-Roger specification in the models. Means were compared using a protected ($P \leq 0.10$) LSD test. Unless otherwise stated, significance was declared at $P \leq 0.05$, and tendency towards significance at $0.05 < P \leq 0.10$. All results are reported as least squared means.

## RESULTS AND DISCUSSION

### *Intake of DM and Nutrients, Digestibility, and Milk Production*

Intake of DM averaged 26.2 kg/d and CP averaged 16.1 % DM and was similar across treatments, while feeding BFTH-containing diets resulted in 3 and 6% less NDF fiber intake ($P<0.01$) for ABT and BT diets, respectively, compared with AHT (Table 3.2). Intake of ADF was 4 and 5% less for ABT and BT, respectively, than AHT ($P<0.02$). Previously reported intake due to the effects of feeding CT-containing forages or CT extracts in ruminants have yielded inconsistent results, and focused only on effects of CT on N digestion (Woodward et al., 2001; Benchaar et al., 2008; Dschaak et al., 2011). Increased DMI was observed due to feeding BFT silage-based diets (2.59% CT; Woodward et al., 2001), whereas others found no effect of supplementing CT extract on DMI in either Jersey heifers (Baah et al., 2007 with 0.60% quebracho CT extract) or lactating dairy cows (Benchaar et al., 2008 with 0.45% CT). Conversely, decreases in DMI of diets containing CT have been reported either in dairy cows (Dschaak et al., 2011 with 2.25% CT from quebracho CT extract) or in sheep (Barry and McNabb, 1999 with 7.5 to 10.0% CT from BFT pasture; Priolo et al., 2000 with 2.5% CT from carob pulp). In the current study, the CT in BFTH did not lead to the increased DMI, because the CT concentration of ABT and BT was less (0.38 and 0.51%, respectively) than that reported in other studies. Meanwhile, the depressing effect on feed intake reported in the literature was attributed to the negative impact of palatability (Cooper and Owen-Smith, 1985) or to a short-term effect of astringency (Landau et al., 2000) in many of these cases where the CT concentration was greater than that used in the present study. These results suggest that effects of feeding CT-containing forages or CT extracts on feed intake in ruminants could be influenced by source and chemical nature of CT, as CT activity is variable, reactive in some cases binding to each other, and in other cases may have no activity whatsoever.

Feeding BT tended to increase ($P = 0.10$) NDF digestibility compared with AHT and ABT, leading to an increase of digested NDF intake but a 16% reduction in undigested NDF intake when cows were fed BT (Table 3.2). Ward (2012) reported that the indigestible fraction of NDF in legume hay averaged 54.2% of NDF for recently submitted forage samples. In the current study, 49.5% NDF of the AHT was undigested, but only 45.0% of NDF in the BT was undigested. Differences in digestibility of forages affect retention time in the rumen, and are related to differences in lignin concentration and lignification patterns in stems and leaves of the forage (Hoffman et al., 1993; Buxton, 1996). An increase in ruminal digestion of fibrous feed particles can increase particle fragility and makes particles more susceptible to breakdown during chewing (Chai et al., 1984). In an early study by Ingalls et al. (1966), an initial rate of disappearance of DM, cell wall constituents, fiber, and lignin were faster for BFT than for alfalfa. In addition, in a comparative study of commonly cultivated forages, voluntary intake relative to cell wall content substantially increased for BFTH compared with AH and other forages above the standard deviation of the other forages (Van Soest, 1965). Differences in lignin composition and structure between AH and BFTH can also influence the digestibility of NDF in the rumen. In a study by Buxton and Russell (1988), the ratio of the oxidation products of sinapyl: coniferyl alcohols, a measurement of differing subunits of lignin, were 0.79 in AH and 0.14 in BFTH, suggesting differences in the lignin composition of these 2 legume species. More recently, Hunt et al. (2014) reported that lignification of stems of BFT differed from alfalfa stems, as the plant elongated and matured, resulting in reduced liginification of fiber in plant cell walls that potentially could increase digestion rates of BFT NDF relative to AH NDF. In addition to cell wall chemical differences, MacAdam and Griggs (2013) reported greater NFC concentration in BFTH than AH when both species were harvested at 6-wk intervals. Because initiation of digestion of fiber by rumen microbes occurs in the cell lumen, a much more digestible layer in the stem fiber cells would enhance microbial colonization of fiber and increase digestion of NDF.

Milk and ECM yields tended to increase (P = 0.09) for cows consuming BFTH-containing diets (Table 3.2). This is in agreement with Hymes-Fecht et al., (2013) where BFT silage-fed cows had increased milk and ECM yields compared to those fed alfalfa silage. Similar milk yield increases in BFT-fed cows were also reported in 2 studies utilizing fresh BFT, without or with polyethylene glycol (Woodward et al., 1999,2009). The addition of polyethylene glycol inactivated the binding activity of forage tannins in these studies, attributing CT activity as the only dietary difference, which suggests that CT in the diets resulted in increased milk yield. However, the CT concentration in the current study was much less than that in the studies by Woodward et al. (2.6% and 1.9% in 1999 and 2009, respectively), which could imply that other differences such as enhanced NDF digestion in the BFTH used in our study and its subsequent effects on ruminal fermentation, and not CT in BFTH, likely played a role in increased milk production.

Milk fat concentration and yield were not different across dietary treatments, whereas milk true protein concentration and yield were greater for cows consuming ABT relative to those fed AHT (Table 3.2). Similar results were found in milk of cows fed BFT silage-containing diets (Hymes-Fecht et al., 2013). Cows fed BT tended to have increased feed efficiency based on milk yield (*P* = 0.09) and increased feed efficiency based on ECM yield compared to those fed ABT; however, there were no differences between AHT and BT on the feed efficiencies.

### ***Ruminal Fermentation Characteristics***

Dietary treatments in general did not influence ruminal pH profiles with minor effects on minimum and maximum ruminal pH (Table 3.3). Mean pH averaged 6.47 across treatments, which is typical in cows fed high-forage diets. However, the maximum pH pf BT-fed cows never exceeded that of AHT or ABT, which could suggest increased fermentation from that diet. Hymes-Fecht et al., (2013) reported minor effects on ruminal pH by feeding BFT silage-based diet. Dietary NDF concentration for all treatments used in the current study would be adequate to maintain optimal ruminal pH.

Total VFA concentration tended to increase (*P* = 0.09) in cows fed BT compared to those fed AHT and ABT (Table 3.3). Feeding BFTH resulted in a tendency for acetate proportion to decrease (*P* = 0.09), propionate proportion to increase (*P* = 0.07), leading to a tendency for decreased acetate-to-propionate ratio (*P* = 0.07). Effects of feeding CT-containing forages or CT extracts on VFA profiles have been variable. Carulla et al. (2005) reported that VFA concentrations remained unchanged, but molar proportion of propionate increased in sheep fed diets supplemented with CT-containing black wattle (*Acacia mearnsii*). Decreased acetate-to-propionate ratio was reported for cows fed BFT silage (Hymes-Fecht et al., 2013) and in a continuous culture study (Williams et al., 2011) for TMR diets containing BFTH. The tendency for increased total VFA concentration due to feeding the BT in the present study suggests enhanced ruminal fermentability by feeding BFTH and can support increased energy supply for lactation. In addition, increase in propionate and decrease in acetate corresponded to improvements in fiber digestion of forages (Eun and Beauchemin, 2007). It is not uncommon to observe changes in VFA proportions as a direct effect of enhanced fiber digestion in the rumen, implying that feeding BFTH may affect microbial growth, shift the metabolic pathways by which specific microbes utilize substrates, or both. Propionate is quantitatively the most important VFA precursor of glucose synthesis and, therefore, has a major impact on hormonal release and tissue distribution of nutrients (Nagaraja et al., 1997). Consequently, increased VFA concentration and propionate proportion as a result of feeding BFTH would contribute to improving nutrient supply and utilization, which may have resulted in increases in milk production as well as feed efficiency observed in the current study.

While concentration of $NH_3$-N was similar across treatments, cows fed BT exhibited greater MCP yield relative to those fed AHT and ABT (Table 3.3). The most efficient way to improve nutrient utilization in the rumen is to maximize microbial growth by capturing N-containing compounds released from RDP sources and convert that N into rumen microbial cells. Tannin-protein complexes inhibit the fermentation of forage protein to ammonia in the rumen, increasing the amount of protein that reaches the small intestine (Barry et al., 1986; Waghorn et al., 1987). Therefore, CT included in the diet as a supplement or as a component of the forage would be expected to reduce the amount of N fermented in the rumen. However, there was no effect on $NH_3$-N concentration in response to dietary treatments in the current study, indicating that CT concentration in ABT and BT may have been inadequate to form substantial tannin-protein complexes, one of the most promising effects of CT on ruminal fermentation. Besides adequate N supply, ruminal MCP synthesis also depends on supply of adequate amounts of carbohydrate as an energy and carbon source for the synthesis of peptide bonds (Bach et al., 2005). When cows are fed high-forage diets like the ones tested in the current study, contribution from ruminally fermented NDF for the synthesis of MCP would be greater than for high-concentrate diets. Enhanced NDF digestion evidenced by increased VFA concentration by feeding BT may have provided greater energy for MCP yield due to feeding BT. Berthiaume et al. (2010) examined alfalfa cultivars with high vs. low NDF digestibility in vitro and observed increased total VFA production and increased apparent digestibility of DM and OM. These factors may have contributed to increased MCP yield in this study.

### *Utilization of N*

Intake of N was not different among treatments, whereas cows on BFTH-containing diets secreted more milk N than those on AHT, resulting in 3 - 7% improvement in N utilization efficiency for milk N for ABT or BT diets, respectively (Table 3.4). That both

BFTH-containing diets resulted in improvements suggests that even small inclusions of BFTH can enhance digestion and efficiency of N utilization and increases the desirability of feeding BFTH. A similar improvement in N utilization efficiency (4%) was reported for a BFT silage diet (Hymes-Fecht et al., 2013). Concentration of MUN, which reflects inefficiency of dietary N utilization and ruminal $NH_3$-N, did not differ between treatments. Like the effect of CT on $NH_3$-N concentration discussed earlier, a relatively small concentration of CT in ABT and BT may have resulted in no effect on MUN concentration in the present study. Benchaar et al. (2008) and Aguerre et al. (2010) observed no effect of supplementation of quebracho CT extract at 0.45-0.90% DM on MUN concentration.

The observance of no change in N intake and MUN concentration led to lack of response of N excretion into urine, feces, and manure due to dietary treatments (Table 3.4). In contrast, cows fed ABT and BT tended to increase milk N-to-manure N ratio ($P = 0.07$), which is attributed to the shift of N to increased milk N secretion coupled with increased MCP yield from feeding the BFTH-containing diets. A greater milk N-to-manure N ratio is more desirable, indicating that less manure N must be managed per unit of milk N produced by the herd. Thus, feeding BFTH in dairy diets may reduce manure $NH_3$-N by increasing secretion of milk N per unit of manure N excreted. Feeding BFT silage diets shifted the route of N excretion from urine to feces in one study (Hymes-Fecht et al., 2013), which is an effective way of reducing $NH_3$ volatilization and resultant N waste. However, where reduced excretion of manure N was only numerically observed from feeding BFTH in the current study, it is difficult to make assumptions that BFTH can effectively reduce N excretion in manure.

# CONCLUSIONS

Replacing alfalfa hay with birdsfoot trefoil hay in high forage, hay-based diets decreased NDF and ADF intake, tended to increase milk yield, ECM and increased ECM efficiency. The ABT and BT diets both tended to increase milk yield by an average 3.5% more than AHT, suggesting that even a diet that partially replaced AH with BFTH shows promise to improve milk efficiency per unit of N intake, which can be a benefit to farms with limited supply of BFTH. An additional economic benefit was found if added income from increased milk protein was considered. In addition, cows fed with birdsfoot trefoil hay had improved microbial protein yield. Improved digestibility of fiber in BFT hay due to cell wall characteristics such as reduced iNDF concentration of BFTH, as suggested in this study, may have contributed to improved milk protein secretion compared to cows fed alfalfa hay diet. Result of this study suggest that dairies that feed BFT preserved as hay may see production increases when including BFTH in high forage diets, even with a 21% inclusion of BFTH. Overall industry goals of increasing digestibility of NDF from forage that can lead to improved N utilization efficiency could be met when BFTH is fed. Producers with elevated feed costs, such as organic farmers, could benefit from feeding BFTH. Future research to determine specific rumen microbe and consequential effects *in vivo* from feeding BFTH with greater CT concentration than that fed in this study and consequential dairy cow performance is well warranted. Although we reported enhanced digestion of BFTH possibly due to its favorable cell wall structure toward microbial fermentation, we have yet to explore how reduced CT-containing BFTH affects microbial community structure, particularly cellulolytic bacteria.

# REFERENCES

Aguerre, M. J., M. A. Wattiaux, M. C. Capozzolo, P. Lencioni, and C. Cabral. 2010. Effect of quebracho-chestnut tannin extracts at two dietary crude protein levels on performance and rumen fermentation of dairy cows. J. Dairy Sci. 93(Suppl. 1):445. (Abstr.)

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624.

AOAC International. 2000. Official Methods of Analysis. Vol. 1 and 2. 17th ed. AOAC International, Gaithersburg, MD.

Baah, J., M. Ivan, A. N. Hristov, K. M. Koenig, L. M. Rode, and T. A. McAllister. 2007. Effects of potential dietary antiprotozoal supplements on rumen fermentation and digestibility in heifers. Anim. Feed Sci. Tech. 137:126–137.

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

Barry, T. N., R. T. Manley, and S. J. Duncan. 1986. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. 4. Site of carbohydrate and protein digestion as influenced by dietary reactive tannin concentrations. Br. J. Nutr. 55:123–137.

Barry, T. N., and W. C. McNabb. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. Nutr. 81:263–272.

Barry, T. N., D. M. McNeill, and W. C. McNabb. 2001. Plant secondary compounds; their impact on nutritive value and upon animal production. Pages 445–452 in Proc. XIX Int. Grass. Conf., Sao Paulo, Brazil.

Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88:2117–2129.

Benchaar, C., T. A. McAllister, and P. Y. Chouinard. 2008. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extract. J. Dairy Sci. 91:4765–4777.

Berthiaume, R., C. Benchaar, A. V. Chaves, G. F. Tremblay, Y. Castonguay, A. Bertrand, and A. F. Brito. 2010. Effects of nonstructural carbohydrate concentration in alfalfa on fermentation and microbial protein synthesis in continuous culture. J. Dairy Sci. 93:693–700.

Buxton, D. R. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Anim. Feed Sci. Tech. 59:37–49.

Buxton, D. R., and J. R. Russell. 1988. Lignin constituents and cell-wall digestibility of grass and legume stems. Crop Sci. 28:553–558.

Carulla, J. E., M. Kreuzer, A. Machmuller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decrease methanogenesis and urinary nitrogen in forage-fed sheep. Aust. 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 Urinary Excretion of Purine Derivatives - an Overview of The Technical Details. Int. Feed. Res. Unit, Occas. Publ. Rowett Res. Inst., Bucksburn, Aberdeen, UK.

Chai, K., P. M. Kennedy, and L. P. Milligan. 1984. Reduction in particle size during rumination in cattle. Can. J. Anim. Sci. 64(Suppl.):339 (Abstr.).

Cooper, S. M., and N. Owen-Smith. 1985. Condensed tannins deter feeding by browsing ungulates in a South African savanna. Oecologia 67:142–146.

DHIA, 2013. Dairy Herd Improvement Association. Annual review. Provo, UT.

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

Dschaak, C. M., C. M. Williams, M. S. Holt, J. -S. Eun, A. J. Young, and B. R. Min. 2011. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. J. Dairy Sci. 94:2508–2519.

Eun, J.-S., and K. A. Beauchemin. 2005. Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, and milk production. J. Dairy Sci. 88:2140–2153.

Eun, J.-S., and K. A. Beauchemin. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using *in vitro* fermentation characteristics. Anim. Feed Sci. Tech. 132:298–315.

Grabber, J. H., W. E. Zeller and I. Mueller–Harvey. 2013. Acetone enhances the direct analysis of procyanidin– and prodelphinidin– based condensed tannins in Lotus species by the butanol–HCl iron assay. Agric. and Food Chem. 61:2669–2678.

Hoffman, P. C., S. J. Sievert, R. D. Shaver, D. A. Welch, and D. K. Combs. 1993. In situ dry matter, protein, and fiber degradation of perennial forages. J. Dairy Sci. 76:2632–2643.

Hunt, S. R., J. W. MacAdam, and T. C. Griggs. 2014. Lignification and tannin localization during the development of birdsfoot trefoil stems. Crop Sci. 54:1876–1886.

Hymes-Fecht, U. C., G. A. Broderick, R. E. Muck, and J. H. Graber. 2013. Replacing alfalfa or red cover silage with birdsfoot trefoil silage in total mixed rations increases production of lactating dairy cows. J. Dairy Sci. 96:460–469.

Ingalls, J. R., J. W. Thomas, M. B. Tesar, and D. L. Carpenter. 1966. Relations between ad libitum intake of several forage species and gut fill. J. Anim. Sci. 25:283–289.

Janicek, B. N., P. J. Kononoff, A. M. Gehman, and P. H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91:3544–3553.

Jones, S. J., D. L. Starkey, C. R. Calkins, and J. D. Crouse. 1990. Myofibrillar protein turnover in feed-restricted and realimented beef cattle. J. Anim. Sci. 68:2707–2715.

Landau, S., N. Silanikove, Z. Nitsan, D. Barkai, H. Baram, F. D. Provenza, and A. Perevolotsky. 2000. Short-term changes in eating patterns explain the effects of condensed tannins on feed intake in heifers. Appl. Anim. Behav. Sci. 69:199–213.

Leonardi, C., M. Stevenson, and L. E. Armentano. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. J. Dairy Sci. 86:4033–4042.

MacAdam, J. W., and T. C. Griggs. 2013. Irrigated birdsfoot trefoil variety trial: Forage nutritive value. Accessed November 15, 2014. http://extension.usu.edu/files/publications/publication/AG_Forages_2013-02pr.pdf.

Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85:1165–1175.

Martin, N. P., and D. R. Mertens. 2005. Reinventing alfalfa for dairy cattle and novel uses. Pages 299–311 in Proc. 35th California Alfalfa and Forage Symposium, Visalia, CA.

McCarthy, F. D., W. G. Bergen, and D. R. Hawkins. 1983. Muscle protein turnover in cattle of differing genetic backgrounds as measured by urinary N'-methylhistidine excretion. J. Nutr. 113:2455–2463.

McGraw, R. L., and G. C. Marten. 1986. Analysis of primary spring growth of four pasture legume species. Agron. J. 78:704–710.

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 temperate forages: A review. Anim. Feed Sci. Tech. 106:3–19.

Mowat, D. N., M. L. Kwain, and J. E. Winch. 1969. Lignification and in vitro cell wall digestibility of plant parts. Can. J. Plant Sci. 49:499–504.

Nagaraja, T. G., C. J. Newbold, C. J. Van Nevel, and D. I. Demeyer. 1997. Manipulation of ruminal fermentation. Pages 523–632 in The Rumen Microbial Ecosystem, 2nd ed. P. N. Hobson and C. S. Stewart, ed. Blackie Academic and Professional, New York, NY.

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

Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80:1005–1028.

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.

Priolo, A., G. C. Waghorn, M. Lanza, L. Biondi, and P. Pennisi. 2000. Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. J. Anim. Sci. 78:810–816.

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

SAS Institute. 2012. SAS/STAT User’s Guide. Release 9.3. SAS Inst. Inc., Cary, NC.

Shingfield, K. J., and N. W. Offer. 1998. Evaluation of milk allantoin excretion as an index of microbial protein supply in lactating dairy cows. Anim. Sci. 67:371–385.

Shingfield, K. J., and N. W. Offer. 1999. Simultaneous determination of purine metabolites, creatinine, and pseudouridine in ruminant urine by reversed-phase high-performance liquid chromatography. J. Chromatogr. B 723:81–94.

Tomlin, D. C., R. R. Johnson, and B. A. Dehority. 1965. Relationship of lignification to in vitro cellulose digestibility of grasses and legumes. J. Anim. Sci. 24:161–165.

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

Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44:282–287.

Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: Voluntary intake in relation to chemical composition and digestibility. J. Anim. Sci. 24:834–843.

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

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 corniculatus* L. Br. J. Nutr. 57:115–126.

Ward, R. 2012. New analysis to better describe feeds for animal agriculture. Accessed January 5, 2015. https://www.vtdairy.dasc.vt.edu/docs/cow-colleges/2012cc/pres-14-ward.pdf.

Wattiaux, M. A., and K. L. Karg. 2004. Protein level for alfalfa and corn silage-based diets: II. Nitrogen balance and manure characteristics. J. Dairy Sci. 87:3492–3502.

Whittet, K. M. 2004. Factors affecting variability in urinary creatinine and purine derivative excretion in beef cattle. MS Thesis. Univ. of Nebraska, Lincoln.

Williams, C. M., J. -S. Eun, J. W. MacAdam, A. J. Young, V. Fellner, and B. R. Min. 2011. Effects of forage legumes containing condensed tannins on methane and ammonia production in continuous cultures of mixed ruminal microorganisms. Anim. Feed Sci. Technol. 166:364–372.

Woodward, S. L., M. J. Auldist, P. J. Laboyrie, and E. B. L. Jansen. 1999. Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. Proc. NZ Soc. Anim. Prod. 59:152–155.

Woodward, S. L., P. J. Laboyrie, and E. B. L. Jansen. 2000. *Lotus corniculatus* and condensed tannins - Effects on milk production by dairy cows. Asian-Aus. J. Anim. Sci. 13(Suppl.):521–552.

Woodward, S. L., G. C. Waghorn, M. J. Ulyatt, and K. R. Lassey. 2001. Early indications that feeding *Lotus* will reduce methane emissions from ruminants. Proc. NZ Soc. Anim. Prod. 61:23–26.

Woodward, S. L., G. C. Waghorn, K. A. Watkins, and M. A. Bryant. 2009. Feeding birdsfoot trefoil (*Lotus corniculatus*) reduces the environmental impacts of dairy farming. Proc. NZ Soc. Anim. Prod. 69:1–5.

**Table 3.1.** Ingredients and chemical composition (means ± SD) of experimental diets fed to dairy cows (n = 3 sample replicates)

| | Experimental diet[1] | | |
|---|---|---|---|
| Item | AHT | ABT | BT |
| Ingredient, % of DM | | | |
| Alfalfa hay | 42.0 | 20.7 | – |
| Birdsfoot trefoil hay | – | 20.7 | 42.6 |
| Corn silage | 15.4 | 15.4 | 15.4 |
| Corn grain, flaked | 22.5 | 22.1 | 21.9 |
| Cottonseed, whole | 6.20 | 6.20 | 6.20 |
| Soybean meal | 1.30 | 3.50 | 4.80 |
| Corn DDGS[2] | 7.10 | 7.00 | 6.80 |
| Beet pulp, shreds | 1.50 | – | – |
| Fat supplement[3] | 0.40 | 1.50 | 1.20 |
| Calcium carbonate | 1.01 | 1.01 | 1.01 |
| Salt | 0.31 | 0.31 | 0.31 |
| Urea | 0.70 | 0.70 | 0.70 |
| Magnesium oxide | 0.18 | 0.18 | 0.18 |
| Vitamins and minerals[4] | 0.14 | 0.14 | 0.14 |
| Sodium bicarbonate | 0.70 | 0.70 | 0.70 |
| Nutrient composition, % of DM | | | |
| DM, % | 60.3 ± 4.12 | 60.0 ± 3.89 | 62.1 ± 1.60 |
| OM | 91.0 ± 1.64 | 90.4 ± 0.35 | 91.8 ± 0.93 |
| CP | 15.9 ± 0.73 | 15.5 ± 0.19 | 15.2 ± 0.63 |
| RDP, % of CP[5] | 53.5 | 53.4 | 53.5 |
| RUP, % of CP[5] | 46.5 | 46.6 | 46.5 |
| NDF | 41.1 ± 0.78 | 39.7 ± 3.48 | 38.5 ± 0.72 |
| ADF | 28.8 ± 0.83 | 27.6 ± 2.66 | 26.9 ± 0.17 |
| NFC[5] | 31.1 | 31.9 | 34.3 |
| NEL[6], Mcal/kg | 1.60 | 1.60 | 1.60 |
| Condensed tannins, % of DM | 0.05 ± 0.01 | 0.38 ± 0.06 | 0.51 ± 0.08 |

[1] AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

[2] DDGS = dried distillers grains with solubles.

[3] Calcium salts of palm oil (EnerGII®, Virtus Nutrition, LLC, Corcoran, CA).

[4] Formulated to contain (per kg DM): 13.4 mg of Se (from sodium selenate), 550 mg of Cu (from copper-AA complex), 2412 mg of Zn (from zinc-AA complex and zinc sulfate), 2290 mg of Mn (from manganese-AA complex), 33 mg of Co (from cobalt carbonate), 185,045 IU of vitamin A, 22,909 IU of vitamin D, 616 IU of vitamin E, and 285 mg of Rumensin® (Elanco Animal Health, Greenfield, IN).

[5] NFC = 100 – CP – NDF – ether extract – ash.

[6] Based on tabular value (NRC, 2001).

**Table 3.2.** Intake of DM and nutrients, milk yield and composition, and efficiencies of DM and N used for milk production of lactating dairy cows fed different legume hay-based diets

| | Dietary treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | AHT | ABT | BT | SEM | *P* |
| Intake, kg/d | | | | | |
| DM | 25.7 | 26.5 | 26.7 | 0.57 | 0.33 |
| CP | 4.16 | 4.13 | 4.01 | 0.10 | 0.27 |
| NDF | $10.6^{a}$ | $10.3^{ab}$ | $10.0^{b}$ | 0.30 | < 0.01 |
| ADF | $7.36^{a}$ | $7.10^{b}$ | $6.99^{b}$ | 0.218 | 0.02 |
| Digestibility, % | | | | | |
| CP | 74.2 | 72.3 | 75.5 | 1.18 | 0.18 |
| NDF | 50.5 | 52.3 | 55.0 | 1.99 | 0.10 |
| Digested NDF intake, kg/d | 5.25 | 5.36 | 5.53 | 0.28 | 0.20 |
| Undigested NDF intake, kg/d | $5.35^{a}$ | $5.12^{a}$ | $4.47^{b}$ | 0.36 | 0.01 |
| Yield, kg/d | | | | | |
| Milk | 38.3 | 39.3 | 40.0 | 1.94 | 0.09 |
| ECM | 37.4 | 38.1 | 39.3 | 1.55 | 0.09 |
| Milk composition, % | | | | | |
| Fat | 3.37 | 3.25 | 3.38 | 0.145 | 0.22 |
| True protein | $2.84^{b}$ | $2.93^{a}$ | $2.94^{a}$ | 0.080 | < 0.01 |
| Lactose | 4.83 | 4.82 | 4.82 | 0.046 | 0.83 |
| Milk component yield, kg/d | | | | | |
| Fat | 1.28 | 1.27 | 1.34 | 0.055 | 0.13 |
| True protein | $1.08^{b}$ | $1.13^{a}$ | $1.16^{a}$ | 0.047 | 0.03 |
| Lactose | 1.85 | 1.89 | 1.92 | 0.091 | 0.08 |
| Efficiency | | | | | |
| Milk yield/DMI | 1.50 | 1.47 | 1.52 | 0.086 | 0.09 |
| ECM yield/DMI | $1.45^{ab}$ | $1.44^{b}$ | $1.48^{a}$ | 0.060 | 0.05 |

$^{a\text{-}b}$Means within a row that do not have a common superscript differ at $P < 0.05$.

[1] AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

**Table 3.3.** Ruminal fermentation characteristics of lactating dairy cows fed different legume hay-based diets

| | Dietary treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | AHT | ABT | BT | SEM | *P* |
| Minimum pH | $5.70^a$ | $5.56^b$ | $5.70^a$ | 0.106 | 0.05 |
| Maximum pH | $7.21^a$ | $7.22^a$ | $6.91^b$ | 0.083 | 0.05 |
| Mean pH | 6.45 | 6.53 | 6.37 | 0.072 | 0.34 |
| pH < 5.8 | | | | | |
| Daily episodes | 6.00 | 3.33 | 3.33 | 3.707 | 0.84 |
| Duration, h/d | 2.12 | 0.53 | 1.16 | 1.408 | 0.67 |
| Area, pH × min | 16.4 | 2.75 | 8.68 | 10.72 | 0.60 |
| Total VFA, m*M* | 102 | 106 | 119 | 6.3 | 0.09 |
| Individual VFA, mol/100 mol | | | | | |
| Acetate (A) | 61.1 | 59.0 | 59.7 | 1.93 | 0.09 |
| Propionate (P) | 23.5 | 26.1 | 24.7 | 1.25 | 0.06 |
| Butyrate | 10.8 | 10.7 | 10.8 | 0.56 | 0.83 |
| A:P | 2.62 | 2.26 | 2.42 | 0.20 | 0.07 |
| Ammonia-N, mg/100 mL | 8.33 | 6.05 | 6.70 | 1.244 | 0.51 |
| MCP,[2] g/d | $1793^b$ | $1824^b$ | $2115^a$ | 93.1 | 0.01 |

[a-b] Means within a row that do not have a common superscript differ at $P < 0.05$.

[1] AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

[2] Microbial protein production, g/d = $(\{[\text{PD production} - (0.385 \times \text{BW}^{0.075})]/0.85\} \times 70 \times 6.25)/(0.13 \times 0.83 \times 1{,}000)$ (Janicek et al., 2008).

**Table 3.4**. Nitrogen utilization of lactating dairy cows fed different legume hay-based diets

| | Dietary treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | AHT | ABT | BT | SEM | *P* |
| N intake, g/d | 666 | 662 | 646 | 25.1 | 0.76 |
| Milk N, g/d | $184^{b}$ | $194^{a}$ | $197^{a}$ | 7.7 | < 0.01 |
| Milk N:N intake[2] | $0.27^{b}$ | $0.29^{a}$ | $0.30^{a}$ | 0.02 | 0.05 |
| MUN, mg/100 mL | 14.1 | 14.4 | 14.8 | 0.68 | 0.19 |
| Urinary N excretion,[3] g/d | 289 | 299 | 306 | 17.0 | 0.17 |
| Fecal N excretion,[4] g/d | 191 | 166 | 145 | 18.6 | 0.17 |
| Manure N excretion,[5] g/d | 481 | 465 | 449 | 22.2 | 0.47 |
| UN:FN[6] | 1.51 | 1.80 | 2.11 | 0.473 | 0.24 |
| MkN:MaN[7] | 0.38 | 0.43 | 0.44 | 0.021 | 0.07 |

$^{a-b}$Means within a row that do not have a common superscript differ at $P < 0.05$.

[1] AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

[2] Efficiency of use of feed N to milk N.

[3] Predicted using the following equation: 0.026 × MUN, mg/100 mL × BW, kg (Wattiaux and Karg, 2004).

[4] Predicted using the following equation: N intake, g/d – urinary N excretion, g/d – milk N, g/d.

[5] Manure N, g/d = urinary N excretion, g/d + fecal N excretion, g/d.

[6] UN:FN = ratio of urinary N to fecal N, where urinary N and fecal N are expressed in g/d.

[7] MkN:MaN = ratio of milk N to manure N, where milk N and manure N are expressed as g N/day

# CHAPTER 4

# LACTATION PERFORMANCE AND RUMEN FERMENTATION OF DAIRY COWS GRAZING MIXED GRASS OR BIRDSFOOT TREFOIL PASTURE

## INTRODUCTION

The sustainability of pasture-based dairy systems depends strongly on the quality and quantity of home-grown pasture. Nutrient availability in pasture forages is species, location and climate dependent (Van Soest et al., 1978) and is more complicated to manipulate and predict than nutrient availability in intensive systems since it includes qualitative and quantitative unknowns associated with grazing (Kilcher, 1981; Bargo, et al., 2003; Peyraud and Delagarde, 2013). Dairy cow digestion and utilization of nutrients involves interactions of the animal, its diet and the ruminal microbial population. The most important dietary aspects regarding grazing systems in this interaction are herbage characteristics and animal ingestive behavior (Chilibroste et al., 2012; Peyraud and Delagarde, 2013) and they can greatly affect productive performance and nutrient utilization of the grazing animal.

In the temperate parts of the United States, grazing dairy systems are primarily based on permanent mixtures of cool-season grasses, predominantly tall fescue, orchardgrass, perennial ryegrass (*Lolium perenne*), red and white clover (*Trifolium repens*; NRCS, 2015). The production and quality of these pastures is often limited during the summer months, due to a combination of hot weather and a shortage of rain in unirrigated systems. Botanically diverse pastures, grass/legume mixes, and legume pastures, however, have been shown to improve the yield of the pasture (Daly et al., 1996) or have superior nutritional profiles (Sanderson et al., 2003) to grass pasture alone. One major problem with common mixed species pastures is that they often contain insufficient proportion of legume to capture the added nutritional value as extra milk production (Cosgrove et al., 2006) resulting in less milk production than is possible.

Some novel plant species have been proposed to be incorporated into pasture mixtures or as stand-alone pasture forage (Ström, 2012; Woodward et al., 2013; Pembelton et al., 2014), including chicory *(Cichorium intybus*), plantain (*Plantago lanceolata*), big trefoil (*Lotus pedunculatus*) and birdsfoot trefoil (**BFT**, *Lotus corniculatus* L). How these species affect the overall utilization and management of pastures and influence the rumen environment *in vivo* needs to be investigated if they could be potentially used to improve nutrient utilization and in turn increase sustainability. In addition, few studies have been conducted on commercial farms to determine if alternative forages are feasible for use in commercial dairy systems.

Condensed tannins are phenolic plant secondary compounds present in a number of legumes, including the foliage and stems of BFT, big trefoil and in the flowers of white clover. They act by binding to plant protein, forming hydrogen-bonded complexes (Mueller-Harvey, 2006) that make protein unavailable for rumen degradation (Bae et al., 1993; Jones et al., 1994; Smith et al., 2005). As a result, the amount of ammonia released from rumen digestion is reduced (Waghorn, 2008), improving rumen N utilization efficiency as well as animal performance. In addition, these plant species offer advantages over other pasture forages for dairy cows in order to reduce the environmental impact of N waste (Ramírez-Restrepo and Barry, 2005). However, effects of CT on rumen fermentation and nutrient utilization are dependent on the concentration and chemical nature of the CT in the forage and total diet (Waghorn, 2008; Eun and Min, 2012).

Birdsfoot trefoil has been shown to be a hardy, productive and persistent legume under irrigation in the Mountain West region of the United States (MacAdam and Griggs, 2006). The unique type (based primarily on epicatechins; Waghorn, 2008) and concentration (ranging from 20 to 120 g/kg; Aerts et al., 1999) of condensed tannins in BFT increase ruminant weight gain in cattle (Wen et al., 2002; MacAdam et al., 2011) and lambs (Douglas et al., 1999) and milk production in dairy cows (Turner et al., 2005; Woodward et al., 2009; Jacobs and Woodward, 2010). Due to altitude and temperate seasons, the CT concentration of BFT grown in the Mountain West region is typically lower than that produced in other regions (18.7 vs. 24.3 g/kg for various North American locations, Grabber et al., 2013; vs. 23.6 g/kg, New Zealand, Woodward et al., 2000 and 21.6 g/kg, New Zealand, Jacobs and Woodward, 2010). In addition, little to no research has been done on commercial dairies to determine long-term, multi-year effects of low-CT, irrigated BFT pasture on nutrient utilization and milk production of lactating dairy cows. Although BFT is tolerant of dry summers and appears well adapted to rotational grazing, difficulties with establishment and low competitive ability in seedlings (Chapman et al., 2008; Woodward et al., 2000) may limit its use on dairy farms.

Our hypothesis was that nutrient utilization, ruminal fermentation, and lactational performance would be different in dairy cattle grazing monoculture BFT pasture compared to those of cows grazing typical irrigated mixed grass pasture (perennial ryegrass and white clover mixture).

## MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University. The study was conducted at a commercial organic dairy farm (Weston, ID) in May through August of 2012 (year 1) and May through July, 2013 (year 2).

### *Experimental Design, Cows, and Treatments*

For the 2-year study, an independent grazing experiment in each year was conducted using 18 Holstein and Holstein X Jersey crossbred multiparous dairy cows in mid-lactation. The experiments were conducted in a randomized complete block design, with 18 cows paired based on breed, previous milk production and BW, then they were randomly assigned to one of 2 treatments (n = 9): 1) mixed cool-season grass pasture (70% perennial ryegrass, 15% white clover, and 15% other cool-season grasses and weeds; **MGP**) and 2) BFT pasture (**BFTP**). Before the study started, cows grazed commonly on a mixed cool season grass sward. In 2012, the cows were in their second to fourth lactation (3.3 ± 0.97) and 111 ± 14.2 DIM at the start of the experiment with BW of 541 ± 58.9 and 602 ± 66.7 kg at the beginning and the end of data collection, respectively. Average milk yield was 34.8 ± 13.15 kg/d in the adaptation period. The cows used in 2013 were in their second to fifth lactation (3.5 ± 1.25) and 119 ± 16.6 DIM at the start of the experiment with BW of 618 ± 61.7 and 600 ± 48.6 kg at the beginning and the end respectively. Milk yield averaged 32.9 ± 5.43 kg/d during the adaptation period.

Sampling for both years lasted 8 weeks, with sampling in four, 2-week periods.

### ***Pasture Establishment, Management and Supplementation***

Approximately 4.5 ha of pasture for each treatment was allocated for the grazing trial in both experimental years. The field where BFT was to be planted had been cropped for alfalfa hay for 3 yr and preceded by an annual crop of barley. On August 11, 2011, BFT was planted using a broadcast seeder (Brillion Sure Stand, Brillion Farm Equipment, Brillion, WI) into a prepared seedbed and irrigated immediately. The BFT cultivar was 'Norcen' (Norfarm Seeds Inc., Bemidji, MN) coated with OMRI-certified Apex™ Green (Summit Seed Coatings LLC, Boise, ID) containing Nitragin K rhizobium inoculant (Novozymes BioAg Inc., Brookfield, WI) specific for BFT and planted at a rate of 25 kg pure live seed (PLS) per ha, including 3 kg/ha hard seed. Pasture establishment details are reported elsewhere (Hunt et al., 2014c). The MGP was of previously-established grass pasture containing mixtures of perennial ryegrass, white clover, orchardgrass (*Dactylis glomerata* L.), tall fescue [*Schedonorus arundinaceus (Schreb*.) Dumort.], and quackgrass [*Elymus repens* (L.) Gould] (Hunt et al., 2014c). Both pastures were irrigated simultaneously by a lateral roll sprinkler system. No fertilizer was used on the pastures per organic dairy requirements. Permanent fences surrounded the treatment pastures, and temporary electric fences were used to separate weekly pasture allocations and 12-h allocations (paddocks). First grazing commenced June 20, 2012 with approximately 6,000 kg/ha BFT DM or 4,800 kg/ha grass DM offered to the cows. In 2013, grazing was begun earlier to collect data for spring growth not available the first year, and consequently grazing began May 14, 2013 with 6,750 kg/ha BFT DM or 5,100 kg/ha mixed grasses offered to cows. Plastic water troughs and salt blocks were moved weekly into the grazing area to allow ad libitum access to water and salt. Cows were moved to a fresh paddock after every milking, and following grazing, pastures were irrigated within 3 d and allowed to rest for approximately 6 wk. Then, cows returned to graze those pastures. Paddock area was held constant throughout the study, with no alterations made for pasture biomass changes: area was planned so that biomass of pasture at lowest growth stage would still provide 3 × required pasture DMI for 9 cows, based on 35 kg FCM production and estimated energy content of forage from previous studies (Williams, et al., 2011).

Cows were individually fed 2.27 kg of supplement [95% ground barley grain (11.2 % CP; 18.2 % NDF, 56.4 % starch, DM basis) and 5% organic dairy mineral which provided 1 % calcium, 0.025% phosphorus, 0.007% sodium, 0.0035% magnesium, and (/kg of DM): 47 mg of Zn, 9.6 mg of Cu, 0.16 mg of Se, 181 IU of vitamin A, 300 IU of vitamin D, and 50 IU of vitamin E using organically approved ingredients) twice daily following each a.m. and p.m. milking while in headlocks. Refusal of barley grain for each cow was recorded for 2 d measured simultaneously with the milk sampling week.

### *Climate Data*

Average monthly minimum and maximum temperatures, and total monthly precipitation and evapotranspiration were calculated from data collected by the nearest weather station (Lewiston, UT). Data were provided by the Utah State University Climate Center, Climate Database Server, which gives daily evapotranspiration estimated by the ASCE-standardized Penman-Monteith method (ASCE-EWRI, 2005). Fifty-year averages were calculated from data gathered by the same weather station, provided by Western Regional Climate Center reports of National Climatic Data Center 1945-2008 monthly mean temperatures. Fig. 4.1 shows monthly temperature (a), precipitation and evapotranspiration data (b) for 2012 and 2013 as well as mean data for 1945-2008 at this weather station.

### *Chemical Analysis of Forage and Supplement*

To determine DM of the pastures, pre- and post-grazing pasture herbage samples were cut to the approximate grazing height (approx. 5 cm above soil level), and divided into two subsamples. One set of samples were weighed fresh, dried in an oven at 65°C for 48 h, and reweighed to ascertain DM. To determine the chemical composition of the pastures, a second subsample of approximately 100 to 200 g was frozen at -20°C. This subsample was later freeze-dried and ground to pass a 1 mm screen in preparation for analysis of nutrient and CT composition. Analytical DM content of samples was used to calculate intake and nutrient composition of the herbage and was determined by oven drying at 105°C overnight; OM was determined by ashing in a 550°C oven for 5 h (AOAC, 2000; method 942.05). Samples of the concentrate were collected each sample week, composited by grazing season, and analyzed as above for DM and as described below for nutrient content.

Forage and supplement CP concentration was determined by automated N dry combustion (Flash 2000 Automatic Elemental Analyzer, ThermoFischer Scientific, The Netherlands; AOAC, 2000; method 968.06). Herbage concentration of NDF and ADF were determined sequentially using an ANKOM$^{200/220}$ Fiber Analyzer (Ankom Technology Corp., Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Pretreatment with heat-stable α-amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corp., St Louis, MO) was used in the NDF analysis. Sodium sulfite was used in the analysis and NDF was expressed inclusive of residual ash. Ether extract was measured using a fat analyzer (XT20, ANKOM Technology; AOAC, 2000; method 2003.05).

The CT concentration of the forages for each sample week was determined by direct analysis of plant material extracted using the iron-butanol-HCl-acetone procedure (Grabber et al., 2013). Three samples of freeze-dried, ground material from BFT and MGP pasture samples collected each sample week were analyzed in triplicate for CT concentration. A standard curve was created from purified *L. corniculatus* tannins. The resulting tannin extracts and standard curves were analyzed colorimetrically using a BioMate3 spectrophotometer (ThermoScientific, Rochester, NY) at 550 nM absorbance.

### *Milk Production Measurements*

Milk yield was measured for all experimental cows in the a.m. and p.m. for 4 consecutive milkings every 2 wk with a calibrated portable milk meter (Waikato Milking Systems NZ Ltd, Hamilton, New Zealand). Individual milk samples (60 mL per milking) were preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA) and stored at 4°C until analysis by the Rocky Mountain DHIA Laboratory (Nibley, UT) for fat and true protein analysis using mid-infrared wave-band (2 to 15 µm) procedures by an infrared instrument (Bentley 2000; Bentley Instruments, Chaska, MN) calibrated weekly using raw milk standards provided by Eastern Laboratory Services (Fairlawn, OH). Milk urea N was analyzed using the Berthelot enzymatic procedure on a ChemSpec 150 Analyzer (Bentley Instruments, Chaska, MN). Daily milk composition was calculated from the weighted a.m. and p.m. observations. Yields of milk fat and true protein were calculated by multiplying milk yield from the respective day by fat and true protein concentrations, respectively, for each individual cow. Milk yield, components yield, and concentrations were averaged for each day for statistical analysis. In this study, we used ECM to compare solids content of milk because true milk protein was the component of the greatest interest, calculated using the equation: [(0.327 × milk yield (kg) + (12.95 × fat yield (kg)] + (7.65 × true protein yield (kg)) (DHIA, 2013). To convert milk true protein (**TP**) to milk N, a conversion factor of 6.38 was used (DePeters and Cant, 1992).

### *Sampling and Analysis of Rumen Fluid*

Ruminal fluid was sampled from individual cows 2 h after the a.m. feeding of grain supplement following milking on week 2 and 6 each year of experiment using an orally administered Geishauser probe, a solid, tube-like probe with rows of small holes on the end connected to flexible poly tubing (Geishauser, 1993). These sampling weeks were selected to reflect show sufficient change in rumen fluid over time, but minimize animal handling. The first 100 mL of ruminal fluid was discharged to avoid contamination from saliva, and then 300 mL was collected for analysis. The pH of the ruminal fluid was measured within 5 min of collecting the samples using a portable pH meter (Oakton pH 6; Oakton Instruments, Vernon Hills, IL). Fluid was then strained through a polyester screen (pore size 355 μm; B & S H Thompson, Ville Mont-Royal, QC, Canada). Five mL of the filtered ruminal fluid was added to 1 mL of 1% sulfuric acid, and samples were frozen at -20 C for later $NH_3$-N determination. Concentration of $NH_3$-N in the ruminal contents was determined as described by Rhine et al., (1998). Another 5 mL of the filtered ruminal fluid was added to 1 mL of 25% of meta-phosphoric acid, and the samples were frozen at -20 C for later VFA determination. The VFA were quantified using gas chromatography (Hewlett-Packard model 6890 series II) with a capillary column (30 m, 0.32 mm i.d., 1-µm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA), and flame-ionization detection. Crotonic acid was used as an internal standard. The oven temperature was 170°C held for 4 min, then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium.

### ***Statistical Analysis and Calculations***

Composition data from analysis of the 2 pastures were analyzed with GLMMIX in SAS (SAS Institute, 2013-2014) using a model that included sample week and treatment (pasture source). Animal production data were analyzed as a randomized block design using the PROC MIXED of SAS (SAS Institute, 2013-2014). A single mean observation was computed for each cow for each sample week (n = 9). Treatment means were determined using data from individual measurements collected from animal samples on weeks 2, 4, 6, and 8 for milk yields and components and weeks 2 and 6 for rumen fermentation parameters. Covariate analysis was included in milk yield and composition analysis for 2012 due to an outlier cow in one treatment. No effect of treatment (pasture forage source) was observed for data collected during the adaptation (week 0); hence, week 0 was excluded from the statistical analysis. Due to the different grazing start times for the 2 years, the effect of forage was found to be different for each year, so each year was reported separately. For all statistical analyses, significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

# RESULTS AND DISCUSSION

## *Climate Data*

Temperature, precipitation, and evapotranspiration in 2012 -2013 are reported as monthly means in Figure 4.1. Average monthly maximum temperature in 2012 were elevated compared with that of the 50-yr average (A), and evapotranspiration (B) for the early spring months in 2012 was increased compared to 2013. This can indicate more heat units to increase rate of growth of pasture forage. The average monthly precipitation in 2012 for the spring and early summer months was much reduced compared with that of the 50-yr average. This reduction in precipitation likely increased forage maturity and nutrient concentrations of forages through the early phase of the season prior to grazing. In 2013, precipitation was similar to the 50-yr average for the spring months, which supported growth well. Evapotranspiration was not as great in 2013 as in 2012. Reduced evapotranspiration in wet years or increased evapotranspiration in dry years can influence plant growth (Lynk et al., 1990; Litherland et al., 2002; Machado et al., 2007). Although the pastures were under irrigation during the sample weeks, irrigation only followed the cows as they grazed. Therefore, paddocks could have been stressed by weather conditions, which may have affected plant rate of maturity. Overall climate conditions indicate that forages in 2012 could have grown faster and matured at a faster rate, influencing CP and NDF concentrations.

## *Characteristics of Experimental Pasture Forages*

Table 4.1 reports the nutrient composition of the pastures grazed during the experiment in 2012. Forage DM was greater for MGP than BFTP. Concentration of CP was expected to be greater for BFTP than MGP; however, during the first year the seasonal average for the 2 pasture forages was similar, while at wk 6 and 8, CP concentration of the BFTP was less than the MGP. Concentration of NDF was much reduced for BFTP compared with MGP throughout the grazing season. Non-fiber carbohydrate concentration of pasture forages was consistently greater for BFTP compared with MGP with 10 unit increases on average for the BFTP. Concentration of CT was greater for BFTP throughout the grazing season in 2012, with two-fold greater concentration for seasonal average in the BFTP than the MGP.

In 2013, pre-grazed forage DM concentration for MGP generally exceeded that of BFTP for each sampling week and over the season (Table 4.2). Concentration of CP for the BFTP was greater than that of the MGP, exceeding it by 3 percentage units for the seasonal average. Concentration of NDF for MGP showed a 20 percentage unit increase over BFTP, with each week consistently greater for MGP. Concentration of NFC was also greater for BFTP compared with the MGP throughout the grazing season. Concentration of CT was greater for BFTP than MGP, and it increased gradually as the grazing season progressed.

When comparing the two years, CP concentration for BFTP was greater in 2013 than in 2012, but it was similar in the 2 seasons for MGP. In addition, NFC concentration was greater in 2013 compared to 2012 regardless of type of pastures. Increased evapotranspiration and increased temperature recorded for April and May of 2012 (Figure 4.1) led to increased heat units sufficient to promote an increased rate of maturity for the forages. By the time pastures were grazed for the study, increased NDF, decreased CP, and decreased NFC in both forages compared to 2013 were noted.

In a grazing study comparing spring-growth BFTP with ryegrass and clover, Jacobs and Woodward (2010) reported BFTP nutrients that are similar to our results; seasonal average DM was similar (17.9%), though CP for BFTP (22.4%) was greater in that study than our first year average for BFTP (15.6%), but similar to our second year average. Concentration of NDF in BFTP averaged 32.4% DM in that short-term study (Jacobs and Woodward, 2010), which was similar to our first year average, but was 6 percentage units greater than our second year average concentration of NDF. Woodward et al. (1999) reported BFTP with greater CP (25.6% DM), less ADF and NDF (22.9 and 30.4%, respectively), and greater NFC concentrations compared with high-quality ryegrass, with BFTP nutritive concentrations comparable to those in our study. Hymes-Fecht et al. (2013) reported 34.2% NFC as an average for the BFT silage used in their study, and the value was greater than that observed in 2012 of our study, but less than that found in 2013. Woodward et al. (2009) reported 22.3% NFC in a 45% fresh BFT diet and 18.2% NFC in a 76% ryegrass and 14% white clover diet. The concentration of NDF has the greatest effect on NFC concentration; as NDF concentration increases with plant maturity, the NFC concentration decreases (Fulkerson et al., 2007). A study of pasture grown in the same region as our study (MacAdam et al., 2011) reported low tannin variety Norcen BFTP to have similar CP (22.3% DM), but greater NDF and ADF concentrations (45 and 36% DM, respectively) compared with our findings, which suggests impacts of climate conditions on plant growth and development varies by year, climate, and region.

Most of the differences between weeks the first year was attributed to greater maturity and climatic effects on the newly seeded pasture, as it required time to mature before grazing commenced, resulting in over-matured forages grazed during the first rotation. Forages generally decline in nutritive value with increasing maturity (Van Soest et al., 1978) which can also affect intake. As NDF increases in forages, voluntary intake decreases, as longer rumen retention time restricts rumen volume to allow more forage to be consumed (Allen, 2000). As NDF increases with maturity in both legumes and grasses, CP decreases dramatically as grasses mature (Kilcher, 1981) but CP in legumes decreases only slightly (Coblentz and Grabber, 2013). Pasture species nutrient content (protein, carbohydrates including fiber, minerals and vitamins) and metabolizable energy (ME) density changes in relation to season (Fulkerson et al., 1998; Smith et al., 1998; Stockdale, 1999; Entz et al., 2002), stage of growth (Kilcher, 1981; Reeves et al., 1996; Ayres et al., 1998; Fulkerson et al., 2007), soil fertility or fertilizer application rate (particularly N; Reeves et al., 1996), and soil moisture status (Entz et al., 2002).

Since our forage was sampled over an 8 week period for each year, covering more than one grazing rotation, forage nutrient concentration was influenced by and varied according to what part of the growth phase (i.e., vegetative, flowering, mature) the pasture was in when grazed. Similarly, Cassida et al. (2000) showed that maturity and climate affected BFT; concentrations of NDF increased from 250 to 380 g $kg^{-1}$, ADF increased from 180 to 320 g $kg^{-1}$ in BFT, CP decreased from 280 to 180 g $kg^{-1}$and in situ dry matter disappearance decreased from 860 to 730 g $kg^{-1}$for spring cuttings as forage matured over 22 days. Undegradable intake protein increased from 12 to 22 g $kg^{-1}$with maturity for BFT species for spring growth in that study (Cassida et al., 2000).

Birdsfoot trefoil pasture seemed to mature more slowly than MGP in both seasons and concentration of NDF was less compared to the MGP in the same time period. This could indicate increased digestibility of BFTP compared to MGP. Initiation of digestion of fiber by rumen microbes occurs in the cell lumen, so a highly digestible layer in the cells would enhance microbial colonization of fiber and increase digestion of NDF. More recently, Hunt et al. (2014b) reported that lignification of stems and leaves of BFT compared with alfalfa was different as the stem matured and elongated, and these potential cell wall differences could increase digestion rates of NDF in the BFT.

### *Milk Production, Yield and Components*

Milk yield was greater ($P<0.05$) for cows consuming BFTP compared to MGP for week 4 for the first year (Figure 4.2). However, for the second year milk yield for BFTP cows was greater by an average of 4.2 kg/d over the study period. Increased CP, NFC and decreased NDF concentrations for the BFT forage due to spring growth and vegetative stage of production for the forage was likely a contributor to improved milk yield for year 2.

Table 4.3 shows the milk production and composition for grazed cows by sample week for 2012. Milk yield was greater for BFTP cows only for weeks 4 and 8, which contributed to a season increase of 1.5 kg/d for BFTP cows compared to MGP cows. Energy-corrected milk was greater for BFTP cows from the 4th sample week to the end of the season, due to greater milk yield or protein yield, depending on the week. There were no differences in fat concentration during the first year and milk true protein concentration was different only for week 4 ($P<0.05$) due to treatment. Milk urea N varied for each forage by sample week over the season for year 1, but the overall season averages were similar for both pasture treatments.

For the second year (Table 4.4), week of study also influenced milk yield, with yield decreasing for MGP cows as the season continued, but increasing week 4 and then declining for BFTP cows, reflecting the increase and then decrease in nutrient content of the forage. Yield of ECM also was greater ($P<0.05$) for BFTP -fed cows compared to the MGP cows for most weeks the second year. Milk yield for weeks 4, 6 and 8 for the second year was greater by an average of 5.1 kg/d for the BFTP cows compared to MGP cows. Energy- corrected milk was greater for MGP cows for week 2, but decreased for week 6 and 8 compared to BFTP cows which was influenced by greater fat concentration for the MGP cows, but greater yield for the BFTP cows. Fat concentration was greater for MGP cows for most weeks compared to BFTP cows, while protein concentration only differed by week and was not influenced by forage source. For year 2, MUN was greater for the BFTP cows compared to the MGP cows by an average of 4 mg/100 mL.

Milk yield and ECM were greater for both treatments the second year compared to the first year, but BFTP cows consistently increased in milk production over the MGP cows both years and nearly every week. However, for year 1 BFTP cows had greater milkfat, where year 2, MGP cows had greater milkfat compared to BFTP cows. Milk true protein concentration followed the same pattern as milkfat for year 1. In contrast, year 2 milk protein concentration was generally the same for both treatments. Because of these differences, yields of protein and fat were varied between treatments for both years, with protein yield higher for BFTP cows than MGP cows for both years.

Woodward et al. (1999) report greater MY of BFTP-fed cows (17.23 vs. 12.07 kg/cow/d for BFTP and ryegrass pasture, respectively), although cows in that study did not receive supplementation. Three other studies by Woodward et al., where BFTP was fed with or without polyethylene glycol (**PEG**; Woodward et al., 1999,2000, and 2009) all showed at least 5 kg/d greater milk production in BFTP cows compared to BFTP+PEG, ryegrass and ryegrass + PEG. The addition of PEG inactivated the binding activity of forage tannins in these studies, with the intent that CT activity would be the only dietary difference in the respective forage treatments, suggesting that diet CT increased milk yield. The authors also attributed greater milk yield in part to the improved pasture quality and increased DMI of cows consuming BFT (Woodward et al., 1999). The CT concentration of BFTP in the current study (average of 1.09% of DM for both seasons) was less than that in both studies by Woodward (2.7% and 1.9%, 1999 and 2000, respectively), which implies that other differences such as enhanced NDF digestion, decreased NDF and increased NFC in the legume forage and not solely CT concentration, likely played a role in increased milk production in our study.

Woodward et al. (1999) reported increased milk solids for BFTP compared to ryegrass (1.28 kg vs. 0.90 for BFTP and ryegrass, respectively) though the contribution from fat or protein was not differentiated. In another study, Woodward et al. (2000) reported increased milk protein in BFTP cows, but no difference in milkfat or lactose. Similar results were found in milk of cows fed BFT-silage-containing diets (Hymes-Fecht et al., 2013). That study also showed greater milk true protein concentration and yield in cows fed BFT silage compared to alfalfa- and red-clover silage fed cows. In contrast, Dschaak et al. (2011) supplemented TMR diets with 3% DM CT extract from quebracho and saw no increases in milk yield due to tannin supplementation, and milk composition was not different. The response in milk production in our study is likely due to increased nutrient intake of BFTP from the increased energy value of increased forage NFC (averaging 10 units increase) and decreased NDF concentration (15% reduction) of BFTP compared to the MGP, and possibly the increase of condensed tannins in BFTP (Woodward et al., 2013). Increased forage nutrient availability most likely supported greater milk and protein yield in the BFTP-grazed cows. Though forage protein was usually elevated in BFTP, it is important to note that ruminants will not improve performance in response to additional absorbed CP if total amino acid supply is not limiting performance. For example, Waghorn (2008) noted that for ruminants fed a fibrous diet (*e.g.* over 500 g/kg dietary DM), productive performance is first limited by diet energy concentration. The yield of VFA will be low when stemmy, mature, fibrous material requires extensive chewing before rumen microbes can attach for fermentation, and addition of CT to the diet may inhibit some fiber-degrading bacteria (Waghorn, 2008). In these cases, increases in AA absorption will only contribute to the energy balance, rather than protein synthesis.

During the first year, sample weeks with the least MUN also coincided with the greatest concentration of CT in BFTP over the MGP (average of 1.21 CT compared to 0.45 % of DM for BFTP and MGP, respectively), which may have contributed to an increase in milk protein. Dschaak et al. (2011) detected decreased concentration of MUN by 16.2% in cows supplemented with condensed tannin extract (CTE). Milk urea N decreased by 7.2% in cows supplemented with CTE at 1.8% DM, but not at lower concentrations of 0.45 to 0.9% DM (Aguerre et al., 2010). Likewise, Benchaar et al. (2008) observed no effects of CTE supplemented at 0.64% of DM on MUN. It seems at least 1% of CT in diet DM must be present to see effects on MUN; however, in year 2 when the CT concentration of BFTP was consistently greater for BFTP than MGP, MUN excretion was also greater for BFTP cows.

Though our study showed no difference in MUN concentration between treatments the first year, milk protein secretion was greater that year for cows grazing BFTP, possibly indicating an increase in N efficiency. In the second year, cows grazing early spring growth of the forages led to increased intake protein, and less CT present in the grass forage, though BFTP had slightly greater CT concentration compared to both the year 1 BFTP and MGP both years. Jonker et al. (1998) indicated that MUN is indirectly affected by efficiency of ruminal N fermentation and carbohydrate digestibility, through an increase in milk N secretion, a decrease in N intake, or an increase in fecal N. Diet energy availability may have been greater in the BFTP in the first year compared to the MGP and rumen ammonia was probably utilized for rumen microbial crude protein production rather than diffused to the rumen wall and contributing to MUN. Though the CT content for BFTP was greater for most weeks in year 2, excessive protein degradation could have exceeded CT-binding capacity for protein, which could explain the increased MUN concentrations for BFTP cows. The relationship of total available protein in the rumen to CT concentration and complexing availability is still not completely understood, and requires more research. Broderick (1995) reported that MUN more clearly reflected dietary CP intake than did ruminal ammonia concentration. In our study it is evident that forming CT-protein complexes decreased protein degradation and NH3-N production in the rumen only for weeks with forage CT concentration exceeding 1% and CP below 18% DM.

### *Rumen Fermentation Characteristics*

Rumen pH was affected by pasture species (Table 4.5) for both sample weeks of year 2012; BFTP pH was 0.8 units below the treatment average for the first year. Total VFA was 20 mM less for the BFTP compared to the MGP for both sample weeks of year 1. None of the dietary treatments nor sample weeks influenced proportions of acetate or propionate, though butyrate was increased ($P<0.05$) for the MGP treatments for week 2, contributing to a seasonal increase in butyrate for grass-fed cows. Ratio of acetate to propionate (A: P) was not different between treatments or weeks the first year.

For year 2013, pH was not different between weeks, though BFTP was 0.20 units greater compared to MGP ($P<0.05$) for the season average. Total VFA was 20 mM greater for MGP cows for both weeks of year 2. No differences in acetate or propionate proportions were noted for year 2, though butyrate was increased for MGP cows for week 2. Ratio of acetate to propionate was not different, though rumen ammonia was greater for both sample weeks for BFTP cows.

When comparing both years, rumen pH was greater for both treatments the second year compared to the first year. The largest difference in rumen pH was 0.21 units between MGP and BFTP in week 6 of the second year. The milkfat the first season was less for both treatments compared to the second season, which may have been related to the reduced rumen pH effect of the forages. Peyraud (1993) reported that ruminal pH rapidly decreases as intake increases for fresh grass, though increased intake of white clover (**WC**) showed no effect on pH. Average rumen pH for grasses in that study were 6.6 and 5.8 for 15 kg and 20 kg DM intake, respectively. Average rumen pH for WC was 6.5 for both levels of intake, and the authors attributed lower sugar content and greater CP content of WC to increase buffering efficiency in the rumen. The increased CP concentration of BFTP could have been attributed to increased buffer capacity in our study the second year, when BFTP was consistently greater in CP. Total VFA was greater both years for MGP compared to BFTP cows. Dschaak et al. (2011) reported that diets with supplementation of 2.2 % DM CTE decreased total VFA concentration, which corresponded to the decreased DMI in that study. In contrast, Waghorn and Shelton (1997) reported that, in sheep fed a 37% fresh BFT: 63 % ryegrass/clover pasture diet containing 1 % CT, there were no differences in VFA concentration (100 mmol/L) compared to the 100% ryegrass/clover pasture and the PEG-drenched, BFT-fed groups, suggesting that CT did not affect VFA production. Beauchemin et al., (2007) reported that increasing supplementation of quebracho CTE (up to 2% of DMI) tended ($P = 0.08$) to decrease total VFA concentration by 8.4 %, decreased acetate molar proportion by 0.8 mol/ 100mol and A: P ratio by 0.19 units. Waghorn et al. (1987, 1994) noted that CT in temperate forages can slow rates of digestion in the rumen, so the benefits to animal performance could represent a higher efficiency of feed utilization, associated with decreased dry matter intake. The reduction in total VFA in our study for BFTP is difficult to explain, since rudimentary DMI measurements suggested increased DMI for BFTP-grazed cows (data not published), and milk production was increased. It is possible the CT could have reduced fiber digestion through interactions with microbial digestion enzymes, or toxic effects on specific cellulolytic bacteria, which could have reduced total VFA in BFTP, but these factors are difficult to measure in vivo, and were not addressed in this study.

In this study, increased rumen concentration of acetate was generally influenced by dietary fiber concentration that increased as the pastures matured, and was greater in MGP cows due to elevated forage NDF. Increased NDF concentration of MGP could have also contributed to the increase in butyrate, and likely influenced A:P ratio as the season progressed. Elevated rumen pH and A:P ratio during year 2 compared to year 1 for both treatments could also have been influenced by the forage to grain ratio of the cows' diet, as we recorded decreased intake of grain supplement in year 2 (data not shown) compared to year 1.

In our study, we did not see a difference in propionate due to treatment (Table 4.5), though BFTP consistently had elevated NFC. In contrast, Noviandi et al. (2014) reported in a continuous culture experiment that propionate increased ($P$=0.03) by 10.2 % due to 75% legume proportion in the diet and BFTP content ($P$< 0.01), which they attributed to increased NFC concentration from the BFTP.

Rumen $NH_3$–N ranged between 4.8 and 13.92 mg/100 mL for both years. We expected rumen ammonia concentration to be increased for both treatments, based on *in vitro* results reported previously in our lab (averaging 14.5 mg/100mL for grass treatments and 20.2 mg/100mL for BFT-dominant treatments; Noviandi et al., 2014) and especially for weeks when pasture was increased in protein content in the early part of the grazing season. When grazing non-tannin containing legumes, increased legume proportion in the diet of grazing cows increases protein content in the rumen which leads to the inefficient utilization of $NH_3$–N for microbial protein synthesis. It is believed that energy is the most limiting factor in microbial growth in these cases (Bach et al., 2005), and thus, increasing NFC as a proportion of carbohydrates typically has positive effects by providing carbohydrate substrates for microbial protein synthesis. However, Lykos et al. (1997) found that inclusion of NFC in the range of 35 to 42% DM was needed to increase energy density in the diets. In our experiment, the NFC concentrations of MGP and BFTP were between 14 and 39% DM, and therefore, for most weeks during the study the NFC concentrations were less than that thought necessary to improve $NH_3$–N utilization. Hristov et al. (2005) suggested that increased concentrations of NFC improve the ability of ruminal microbes to capture and utilize $NH_3$–N for protein synthesis. In the present study, $NH_3$–N concentrations in MGP-grazed cows remained around the minimum indicated for optimizing microbial synthesis (5 mg/10 mL; Satter and Slyter, 1974), but this did not alter milk protein concentration. Cows grazing BFTP had greater ruminal $NH_3$-N year 2 compared to the grass-grazed cows that year; however, increased MUN secretion by these same cows (13.4 mg/100 mL on average; $P < 0.01$; Table 4.4) could indicate reduced N utilization efficiency, despite increased forage CT concentration at this same time.

Most studies of BFT pasture source show that the net effect of dietary CT is a reduction in ammonia released from rumen protein digestion and increased flow of plant protein to the intestine for absorption with an increased concentration of N in feces and reduced urinary N output (Waghorn, 2008; Woodward et al., 2009; Patra and Saxena, 2011). Though CT concentrations of BFTP in this study were less than other BFTP reported in New Zealand studies of BFTP (2.7 %; Woodward et al., 2009, 2011) and those reported for our region (3.8% DM; MacAdam et al., 2011), some effects on rumen $NH_3$–N were present but appear to be influenced by forage CP concentration. Since we did not see reduction of ruminal $NH_3$–N or MUN, our low CT concentration did not have as great an impact on N as some studies have reported. While inclusion of forages with CT in animal diets may not be profitable for temperate agriculture at the present time (Waghorn and Clark, 2006), changes in ruminant diets may be brought about by consumer demands and legislation. Were this to be the case, the value of CT for reducing environmental N waste brought about by excess use of urea for fertilizer, or excessive N concentration in high-quality legume and grass pastures and improved animal health when forages containing CT are fed, will be apparent if chemical nitrogen fertilizer is taxed and drug use is further limited (Waghorn, 2008). Alternative, natural sources of rumen modifiers that improve efficiency of nutrient use in dairy cows to reduce waste may be more desirable than other inputs for animal production, and offer a strong incentive to maintain CT research.

## CONCLUSIONS

This study quantified pasture nutrients and effects of forage maturity for 2 types of pasture over 8 sample weeks for 2 grazing seasons. Lactating cows grazing BFT pasture as a sole forage source in grazing commercial dairy farms improved milk yield an average of 18% over traditional grass pasture. Response in milk component yield varied, depending on the stage of forage maturity at grazing and growing conditions that affected pasture quality. Lactating cows grazing birdsfoot trefoil had greater season average for protein yield, reduced ruminal $NH_3$–N for some weeks, as well as reduction in MUN in some but not all sample weeks. We found that differences in MUN and ruminal $NH_3$–N concentrations between the cows grazing different forage sources did not correlate with CT concentrations. We conclude that CT contribute to increased milk production N efficiency only when CT concentrations in the forage were greater than 1% DM. Advantages of reduced NDF and increased NFC content of the BFTP likely contributed to improvements in milk production detected in this study more so than the tannin concentration contribution to improved milk production. If BFT pasture were to be adopted on a system-wide level, management of the pasture forage maturity would be critical in order to provide the best quality and highest concentration of tannins in order to increase sustainability. More research is needed to determine long-term, year-to-year variations in the forage nutrients and their effects on milk production and overall efficiency of nutrient utilization, with the use of increased tannin-concentration varieties of BFT to be considered.

## REFERENCES

Aerts, R. J., T. N. Barry, and W. C. McNabb. 1999. Polyphenols and agriculture: Beneficial effects of proanthocyanidins in forages. Agric. Ecosyst. Environ. 75:1–12.

Aguerre, M. J., M. A. Wattiaux, M. C. Capozzolo, P. Lencioni, and C. Cabral. 2010. Effect of quebracho-chestnut tannin extracts at two dietary crude protein levels on performance and rumen fermentation of dairy cows. J. Dairy Sci. 93(Suppl. 1):445 (Abstr.).

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624.

ASCE–EWRI. 2005. The ASCE standardized reference evapotranspiration equation. Page 173 in Technical Committee Report to the Environmental and Water Resources Institute of the American Society of Civil Engineers from the Task Committee on Standardization of Reference Evapotranspiration. ASCE–EWRI, Reston, VA.

AOAC, 2000. Association of Official Analytical Chemists, International. Official Methods of Analysis. 17th ed. AOAC, Arlington, VA.

Ayres, J. F., K. S. Nandra, and A. D. Turner. 1998. A study of the nutritive value of white clover (Trifolium repens L.) in relation to different stages of phenological maturity in the primary growth phase in spring. Grass and Forage Sci. 53: 250–259.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88, E9–E21.

Bae, H. D., T. A. McAllister, L. J. Yanke, K. J. Cheng, and A. D. Muir. 1993. Effect of condensed tannins on endoglucanase activity and filter paper digestion by Fibrobacter succinogenes S85. Appl. Environ. Microbio. 59:2132–2138.

Bargo F., L. D. Muller, E. S. Kolver and J. E. Delahoy. 2003. Invited Review: Production and digestion of supplemented dairy cows on pasture. J Dairy Sci. 86:1–42

Benchaar, C., T. A. McAllister, and P. Y. Chouinard. 2008. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or Yucca schidigera saponin extracts. J. Dairy Sci. 92:4765–4777.

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.

Broderick, G. A. 1995. Desirable characteristics of forage legumes for improving protein utilization in ruminants. J. Anim. Sci. 73:2760–2773.

Cassida, K. A., T. S. Griffin, J. Rodriguez, S. C. Patching, O. B. Hunterman, and S. R. Rust. 2000. Protein degradability and forage quality in maturing alfalfa, red clover, and birdsfoot trefoil. Crop Sci. 40:209–215.

Chapman D. F., J. Tharmaraj, and Z. N. Nie. 2008. Milk production potential of different sward types in a temperate southern Australian environment. Grass and For. Sci. 63:221–228

Chilibroste, P., D. A. Mattiauda, O. Bentancur, P. Soca, and A. Meikle. 2012. Effect of herbage allowance on grazing behavior and productive performance of early lactation primiparous Holstein cows. Anim. Feed Sci. Technol. 173:201–209.

Coblentz, W. K., and J. H. Grabber. 2013. In situ protein degradation of alfalfa and birdsfoot trefoil hays and silages as influenced by condensed tannin concentration. J. Dairy Sci. 96:3120–3137.

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

Cosgrove, G. P., J. L. Burke, A. F. Death, G. A. Lane, K. Fraser and D. Pacheco. 2006. The effect of clover-rich diets on cows in mid lactation: production, behaviour and nutrient use. Proc. NZ Grass. Assoc. 68:267–275.

DHIA, 2013. Dairy Herd Improvement Association. Annual Review. Provo, UT.

Daly, M. J., R. M. Hunter, G. N. Green, and L. Hunt. 1996. A comparison of multi–species pasture with ryegrass–white clover pasture under dryland conditions. Proc. NZ Grass. Assoc 58:53–58.

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

Douglas, G. B., M. Stienezen, G. C.Waghorn, A. G Foote, and R. W. Purchas. 1999. Effect of condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) and sulla

(*Hedysarum coronarium*) on body weight, carcass fat depth, and wool growth of lambs in New Zealand. NZ J. Agric. Res. 42:55–64.

Dschaak, C. M., C. M. Williams, M. S. Holt, J. S. Eun, A. J. Young, and B. R. Min. 2011. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. J. Dairy Sci. 94:2508–2519.

Entz, M. H.,V. S. Baron, P.M. Carr, D. W. Meyer, S. Smith, and W.P. McCaughey. 2002. Potential of forages to diversify cropping systems in the Northern Great Plains. Agron. Journal, 94:240–250.

Eun, J.–S., and B. R. Min. 2012. Emerging opportunities and challenges on exploitation of bioactive plant secondary compounds to mitigate environmental impacts by ruminants. J. Anim. Sci. 90 (Suppl. 3):491. (Abstr.)

Fulkerson, W. J., K. Slack, D. W. Hennessy, and G. M. Hough. 1998. Nutrients in ryegrass (Lolium spp), white clover (Trifolium repens) and kikuyu (Pennisetum clandestinum) pastures in relation to season and stage of regrowth in subtropical environment. Aust. J. Exp. Agric. 38:227–240.

Fulkerson, W. J., J. S. Neal, C. F. Clark, A. Horadagoda, K. S. Nandra, and I. Barchia. 2007. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. Livestock Sci. 107:253–264.

Geishauser, T. 1993. An instrument for the collection and transfer of ruminal fluid and for the administration of water soluble drugs in adult cattle. Bovine Pract. 27:38–42.

Grabber, J. H., W. E. Zeller, and I. Mueller–Harvey. 2013. Acetone enhances the direct anlysis of procyanidin- and prodelphinidin-based condensed tannins in Lotus species by the butanol-HCl iron assay. Agric. and Food Chem. 61:2669–2678.

Hristov, A. N., J. K. Ropp, K. L. Grandeen, S. Abedi, R. P. Etter, A. Melgar, and A. E. Foley. 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. J. Anim. Sci. 83:408–421.

Hunt, S. R., T. C. Griggs, and J. W. MacAdam. 2014a. Change in birdsfoot trefoil (Lotus corniculatus) nutritive value with stem elongation, flowering and pod formation. In A. Hopkins et al., ed. Proceedings of the European Grassland Federation, Aberystwyth, Wales, 7–11 September 2014. Grass. Sci. in Europe 19:884–886.

Hunt, S. R., J. W. MacAdam, and T. C. Griggs. 2014b. Lignification and tannin localization during the development of birdsfoot trefoil Stems. Crop Sci. 54:1876–1886.

Hunt, S. R., J. W. MacAdam, and J. R. Reeve. 2014c. Establishment of birdsfoot trefoil (*Lotus corniculatus*) pastures on organic dairy farms in the Mountain West USA. Organic Agricult. 1–15.

Hymes–Fecht, U. C., G. A. Broderick, R. E. Muck, and J. H. Grabber. 2013. Replacing alfalfa or red clover silage with birdsfoot trefoil silage in total mixed rations increases production of lactating dairy cows. J. Dairy Sci. 96:460–469.

Jacobs, J., and S. Woodward. 2010. Capturing the benefits of alternative forages for increased dairy farm profitability. Pages 292-304 in Australasian Dairy Sci. Symp. Proc, Hamilton, NZ.

Jones, G. A., T. A. McAllister, A. D. Muir, and K. J. Cheng. 1994. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacterium. Appl. Environ. Microbiol. 60:1374–1378.

Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81: 2681–2692.

Kilcher, M. R. 1981. Plant development, stage of maturity and nutrient composition. J. Range Manag. Archives 34:363–364.

Litherland, A. J., S. J. Woodward, D. R. Stevens, D. B. Mcdougal, C. J. Boom, T. L. Knight, and M. G. Lambert. 2002. Seasonal variations in pasture quality on New Zealand sheep and beef farms. Proc. NZ Soc. Anim. Prod. 62:138–142.

Lykos, T., G. A.Varga, and D. Casper. 1997. Varying degradation rates of total nonstructural carbohydrates: effects on ruminal fermentation, blood metabolites, and milk production and composition in high producing Holstein cows. J. Dairy Sci. 80:3341–3355.

Lynk, S. O., G. W. Gee, and J. L. Downs. 1990. The effects of water stress on phenological and ecophysiological characteristics of cheatgrass and Sandberg's bluegrass. J. Range Manag. 43:507–513.

MacAdam, J. W., and T. C. Griggs. 2006. Performance of birdsfoot trefoil, white clover, and other legume–grass mixtures under irrigation in the Intermountain West USA. Proc. NZ Grass. Assoc. 68:355.

MacAdam, J. W., R. E. Ward, T. C. Griggs, B. R. Min, and G. E. Aiken. 2011. Case Study: Average daily gain and blood fatty acid composition of cattle grazing the nonbloating legumes birdsfoot trefoil and cicer milkvetch in the Mountain West. Prof. Anim. Sci. 27:574–583.

Machado, C. F., S. T. Morris, J. Hodgson, C. Matthew, and N. Auza. 2007. Seasonal variation in the quality of a lucerne-based pasture and its relationship with morphological and maturity estimates. Anim. Prod. Sci. 47:575-582.

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

NRCS. 2015. Pasture resources. Accessed 6 Feb. 2015. http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/landuse/rangepasture/pasture/

Noviandi, C. T., J. S. Eun, M. D. Peel, B. L. Waldron, B. R. Min, D. R. ZoBell, and R. L. Miller. 2014. Effects of energy supplementation in pasture forages on in vitro ruminal fermentation characteristics in continuous cultures. Prof. Anim. Sci. 30:13–22.

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.

Pembleton, K. G., K. N. Tozer, G. R. Edwards, J. L. Jacobs, and L. R. Turner. 2014. Simple versus diverse pastures–opportunities and challenges in dairy systems. Page 207 in Proc. 5th Australasian Dairy Sci. Symp.

Peyraud, J. L. 1993. Comparison of the digestion of white clover to perrenial ryegrass in lactating cows. Fourrages 135:465–473.

Peyraud, J. L., and R. Delagarde. 2013. Managing variations in dairy cow nutrient supply under grazing. Animal (Suppl.) 7:57–67.

Ramírez-Restrepo, C. A., and T. N. Barry. 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. Animal Feed and Technol. 120:179–201.

Reeves, M., W. J. Fulkerson, and R. C. Kellaway. 1996. Forage quality of kikuyu (Pennisetum clandestinum): The effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass (Lolium perenne). Crop and Pasture Sci., 47:1349–1359.

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.

Sanderson, M. A., M. Labreveux, M. B. Hall, and G. F. Elwinger. 2003. Nutritive value of chicory and English plantain forage. Crop Sci. 43:1797–1804.

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

SAS Institute, 2013-2014. ver. 9.3 SAS Institute Inc., Cary, NC.

Smith, A. H., E. G. Zoetendal, and D. I. Mackie. 2005. Bacterial mechanism to overcome inhibitory effects of dietary tannins. Microb. Ecol. 50:197–205.

Smith, K. F., R. J. Simpson, R. N. Oram, K. F. Lowe, K. B. Kelly, P. M. Evans, and M. O. Humphreys. 1998. Seasonal variation in herbage yield and nutritive value of perennial ryegrass (*Lolium perenne L.*) cultivars with high or normal herbage water–soluble carbohydrate concentrations grown in three contrasting Australian dairy environments. Aust. J. Exp. Agric. 38:821–830.

Stockdale, C. R. 1999. Effect of season and the time since defoliation on the nutritive characteristics of three irrigated perennial pasture species in northern Victoria. 1. Energy, protein and fibre. Aust. J. Exp. Agric. 39:555–565.

Ström, G. 2012. Effect of botanically diverse pastures on the milk fatty acid profiles in New Zealand dairy cows. Dissertation. Swedish University of Agricultural Sciences, Ultuna.

Turner, S. A., G. C. Waghorn, S. L. Woodward and N. A. Thomson. 2005. Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) affect the detailed composition of milk from dairy cows. Proc NZ Soc. Anim. Prod. 65:283–289.

Van Soest, P. J., Mertens, D. R., and B. Deinum. 1978. Preharvest factors influencing quality of conserved forage. J. Anim. Sci. 47:712–720.

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

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. Pages 107–110 in Greenhouse Gases and Animal Agriculture; An Update. C. R. Soliva, J. Takahashi, M. Kreuzer, ed. Int. Congress Series 1293. Elsevier, Amsterdam.

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. 128:365–372.

Waghorn, G.C., I. D.Shelton, and W. C. McNabb. 1994. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 1. Non-nitrogenous aspects. J. Agric. Sci. Camb. 123:99–107.

Waghorn, G.C., Ulyatt, M.J., John, A., Fisher, M.T., 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.

Wen, L., R. L. Kallenbach, J. E. Williams, C. A. Roberts, P. R. Beuselinck, R. L. McGraw, and H. R. Benedict. 2002. Performance of steers grazing rhizomatous and nonrhizomatous birdsfoot trefoil in pure stands and in tall fescue mixtures. J. Anim. Sci. 80:1970.

Williams, C.W., J-S. Eun, J. W. MacAdam, A. J. Young, V. Fellner, and B. R. Min. 2011. Effects of forage legumes containing condensed tannins on methane and ammonia production in continuous cultures of mixed ruminal microorganism. Anim. Feed Sci. and Technol. 166:364–372.

Woodward, S. L., M. J. Auldist, P. J. Laboyrie and E. B. L. Jansen. 1999. Effect of Lotus corniculatus and condensed tannins on milk yield and milk composition of dairy cows. Proc. NZ Soc. Anim. Prod. 59:152–155

Woodward, S. L., P. J. Laboyrie, and E. B. L. Jansen. 2000. Lotus corniculatus and condensed tannins–effects on milk production by dairy cows. Asian– Aus. J. Anim. Sci (Suppl.) 13:521–525.

Woodward, S. L., G. C. Waghorn, K. A. Watkins, and M. A. Bryant. 2009. Feeding birdsfoot trefoil (Lotus corniculatus) reduces the environmental impacts of farming. Proc. NZ Soc. Anim. Prod. 69:1–5.

Woodward, S. L., C. D. Waugh, C. G. Roach, D. Fynn, and J. Phillips. 2013. Are diverse species mixtures better pastures for dairy farming? Proc. NZ Grass. Assoc. 75:79–84.

**Table 4.1.** Nutrient concentration of mixed grass pasture or birdsfoot trefoil pasture in 2012

| Week and treatment[1] | Pasture forage nutrient, % DM | | | | | | |
|---|---|---|---|---|---|---|---|
| | DM, % | CP | NDF | ADF | EE[2] | NFC[3] | CT[4] |
| Wk 2 | | | | | | | |
| MGP | $24.6^{a}$ | $11.2^{b}$ | $45.2^{a}$ | $26.3^{b}$ | 2.1 | $15.2^{b}$ | $0.45^{b}$ |
| BFTP | $16.8^{b}$ | $16.6^{a}$ | $31.7^{b}$ | $24.1^{a}$ | 3.3 | $24.3^{a}$ | $1.58^{a}$ |
| Average | 20.7 | $13.4^{d}$ | $38.6^{e}$ | $25.2^{d}$ | 2.7 | $19.8^{c}$ | $1.02^{d}$ |
| Wk 4 | | | | | | | |
| MGP | 24.2 | 12.7 | $48.6^{a}$ | $28.0^{a}$ | 2.8 | $7.9^{b}$ | $1.11^{b}$ |
| BFTP | 20.9 | 12.8 | $32.5^{b}$ | $25.4^{b}$ | 2.4 | $26.9^{a}$ | $1.23^{a}$ |
| Average | 22.6 | $12.7^{d}$ | $40.6^{d}$ | $26.7^{c}$ | 2.6 | $17.4^{d}$ | $1.17^{c}$ |
| Wk 6 | | | | | | | |
| MGP | 21.0 | $19.5^{a}$ | $50.8^{a}$ | 26.8 | 3.0 | 13.5 | $0.57^{b}$ |
| BFTP | 19.7 | $17.9^{b}$ | $36.6^{b}$ | 27.4 | 2.7 | 15.4 | $0.91^{a}$ |
| Average | 20.4 | $18.7^{c}$ | $43.7^{c}$ | $27.1^{c}$ | 2.7 | $14.6^{d}$ | $0.74^{e}$ |
| Wk 8 | | | | | | | |
| MGP | $30.8^{a}$ | $20.5^{a}$ | $45.3^{a}$ | 24.1 | $3.5^{a}$ | $16.8^{b}$ | $0.60^{b}$ |
| BFTP | $17.1^{b}$ | $17.8^{b}$ | $31.4^{b}$ | 23.3 | $1.1^{b}$ | $26.4^{a}$ | $0.98^{a}$ |
| Average | 24.0 | $19.2^{c}$ | $38.3^{d}$ | $23.7^{e}$ | 2.3 | $21.6^{c}$ | $0.79^{e}$ |
| Wk 2 to Wk 8 | | | | | | | |
| MGP | $24.3^{f}$ | 15.8 | $45.7^{f}$ | 24.1 | 2.7 | $13.3^{g}$ | $0.56^{g}$ |
| BFTP | $19.5^{g}$ | 15.5 | $32.9^{g}$ | 24.8 | 2.5 | $23.3^{f}$ | $1.15^{f}$ |
| Pooled SEM | 1.75 | 0.35 | 0.51 | 0.14 | 0.52 | 1.80 | 0.004 |

[a-b] Within a column and week, means with different superscripts are different ($P < 0.05$).
[c-e] Within a column, averages with different superscripts are different ($P < 0.05$).
[f-g] Within a column, means with different superscripts are different ($P < 0.05$).
[1] MGP = mixed grass pasture containing perennial ryegrass, orchardgrass, and white clover; BFTP = birdsfoot trefoil pasture.
[2] EE = ether extract.
[3] NFC = nonfiber carbohydrates = 100 – CP – NDF – EE – ash.
[4] CT = condensed tannins.

**Table 4.2.** Nutrient concentration of mixed grass pasture or birdsfoot trefoil pasture in 2013

| Week and | Pasture forage nutrient, % DM | | | | | | |
|---|---|---|---|---|---|---|---|
| treatment[1] | DM, % | CP | NDF | ADF | EE[2] | NFC[3] | CT[4] |
| Wk 2 | | | | | | | |
| MGP | $22.6^{b}$ | 20.9 | $41.9^{a}$ | $25.7^{a}$ | $3.8^{a}$ | $22.8^{b}$ | 0.24 |
| BFTP | $17.0^{a}$ | 21.5 | $26.2^{b}$ | $18.8^{b}$ | $3.2^{b}$ | $38.3^{a}$ | 0.58 |
| Average | $19.8^{de}$ | $21.1^{c}$ | $34.0^{d}$ | $22.2^{d}$ | $3.5^{c}$ | $30.6^{d}$ | $0.41^{d}$ |
| Wk 4 | | | | | | | |
| MGP | $21.1^{b}$ | $16.5^{b}$ | $40.1^{a}$ | $21.0^{a}$ | 3.7 | $29.5^{b}$ | $0.21^{b}$ |
| BFTP | $15.5^{a}$ | $21.7^{a}$ | $20.9^{b}$ | $15.5^{b}$ | 3.6 | $43.4^{a}$ | $0.85^{a}$ |
| Average | $18.3^{d}$ | $19.1^{c}$ | $30.6^{e}$ | $18.2^{e}$ | $3.6^{c}$ | $36.4^{c}$ | $0.52^{cd}$ |
| Wk 6 | | | | | | | |
| MGP | $23.1^{b}$ | $10.4^{b}$ | $44.1^{a}$ | $29.5^{a}$ | $3.9^{a}$ | $30.2^{b}$ | $0.15^{b}$ |
| BFTP | $19.8^{a}$ | $16.9^{a}$ | $25.1^{b}$ | $25.0^{b}$ | $3.6^{b}$ | $44.4^{a}$ | $1.14^{a}$ |
| Average | $21.5^{de}$ | $13.6^{d}$ | $34.6^{d}$ | $27.2^{c}$ | $3.7^{c}$ | $37.3^{c}$ | $0.64^{c}$ |
| Wk 8 | | | | | | | |
| MGP | $28.8^{b}$ | $12.1^{b}$ | $56.5^{a}$ | $37.0^{a}$ | $2.5^{b}$ | $19.4^{b}$ | $0.20^{b}$ |
| BFTP | $23.5^{a}$ | $14.4^{a}$ | $31.7^{b}$ | $21.3^{b}$ | $2.9^{a}$ | $29.7^{a}$ | $1.30^{a}$ |
| Average | $26.2^{c}$ | $13.3^{d}$ | $44.1^{c}$ | $29.2^{c}$ | $2.7^{d}$ | $24.6^{e}$ | $0.75^{c}$ |
| Wk 2 to Wk 8 | | | | | | | |
| MGP | $23.9^{f}$ | $15.0^{g}$ | $45.6^{f}$ | $28.3^{f}$ | $3.5^{f}$ | $25.5^{g}$ | $0.20^{f}$ |
| BFTP | $18.9^{g}$ | $18.6^{f}$ | $26.0^{g}$ | $20.1^{g}$ | $3.3^{g}$ | $39.7^{f}$ | $0.97^{g}$ |
| Pooled SEM | 0.65 | 0.66 | 1.6 | 1.32 | 0.04 | 2.3 | 0.29 |

[a-b] Within a column and week, means with different superscripts are different ($P < 0.05$).

[c-e] Within a column, averages with different superscripts are different ($P < 0.05$).

[f-g] Within a column, means with different superscripts are different ($P < 0.05$).

[1] MGP = mixed grass pasture containing perennial ryegrass, orchardgrass, and white clover; BFTP = birdsfoot trefoil pasture.

[2] EE = ether extract.

[3] NFC = nonfiber carbohydrates = 100 – CP – NDF – EE – ash.

[4] CT = condensed tannins.

**Table 4.3.** Milk production and composition of dairy cows grazing mixed grass pasture or birdsfoot trefoil pasture in 2012

| | Yield, kg/d | | Milk composition, % | | Milk component yield, kg/d | | MUN, mg/100 mL |
|---|---|---|---|---|---|---|---|
| Treatment$^{1}$ | Milk | ECM | Fat | Protein | Fat | Protein | |
| Wk 2 | | | | | | | |
| MGP | 31.3 | 31.2 | 3.50 | 2.89 | 1.10$^{b}$ | 0.90 | 12.2 |
| BFTP | 31.9 | 33.3 | 3.63 | 3.01 | 1.21$^{a}$ | 0.96 | 10.9 |
| Average | 31.6$^{c}$ | 32.3$^{c}$ | 3.56 | 2.95$^{d}$ | 1.15$^{c}$ | 0.93$^{c}$ | 11.6$^{c}$ |
| Wk 4 | | | | | | | |
| MGP | 24.9$^{b}$ | 23.6$^{b}$ | 3.68 | 2.89$^{b}$ | 0.92$^{b}$ | 0.72$^{b}$ | 11.1$^{a}$ |
| BFTP | 28.7$^{a}$ | 27.3$^{a}$ | 3.71 | 3.17$^{a}$ | 1.06$^{a}$ | 0.91$^{a}$ | 9.2$^{b}$ |
| Average | 26.7$^{c}$ | 25.5$^{d}$ | 3.69 | 3.03$^{c}$ | 0.99$^{d}$ | 0.82$^{d}$ | 10.2$^{d}$ |
| Wk 6 | | | | | | | |
| MGP | 26.1 | 25.9$^{b}$ | 3.48 | 2.67 | 0.91$^{b}$ | 0.69$^{b}$ | 6.3$^{b}$ |
| BFTP | 26.8 | 27.5$^{a}$ | 3.81 | 2.91 | 1.02$^{a}$ | 1.02$^{a}$ | 10.1$^{a}$ |
| Average | 26.5$^{d}$ | 26.7$^{d}$ | 3.56 | 2.79$^{e}$ | 0.97$^{d}$ | 0.86$^{d}$ | 8.2$^{e}$ |
| Wk 8 | | | | | | | |
| MGP | 24.3$^{b}$ | 23.9$^{b}$ | 3.41 | 2.75 | 0.83$^{b}$ | 0.67$^{b}$ | 11.8 |
| BFTP | 25.4$^{a}$ | 26.2$^{a}$ | 3.73 | 2.98 | 1.05$^{a}$ | 0.94$^{a}$ | 13.4 |
| Average | 24.8$^{e}$ | 26.6$^{d}$ | 3.57 | 2.87$^{f}$ | 0.94$^{d}$ | 0.81$^{d}$ | 12.6$^{f}$ |
| Wk 2 to Wk 8 | | | | | | | |
| MGP | 26.7$^{h}$ | 26.2$^{h}$ | 3.52 | 2.80$^{h}$ | 0.94$^{h}$ | 0.75$^{h}$ | 10.3 |
| BFTP | 28.2$^{g}$ | 28.5$^{g}$ | 3.71 | 3.02$^{g}$ | 1.09$^{g}$ | 0.96$^{g}$ | 10.9 |
| Pooled SEM | 1.47 | 1.19 | 0.01 | 0.07 | 0.004 | 0.031 | 0.58 |

$^{a\text{-}b}$Within a column and week, means with different superscripts are different ($P < 0.05$).

$^{c\text{-}f}$Within a column, averages with different superscripts are different ($P < 0.05$).

$^{g\text{-}h}$Within a column, means with different superscripts are different ($P < 0.05$).

[1] MGP = mixed grass pasture containing perennial ryegrass, orchardgrass, and white clover; BFTP = birdsfoot trefoil pasture.

**Table 4.4.** Milk production and composition of dairy cows grazing mixed grass pasture or birdsfoot trefoil pasture in 2013

| | Yield, kg/d | | Milk composition, % | | Milk component yield, kg/d | | MUN, mg/100 mL |
|---|---|---|---|---|---|---|---|
| Treatment[1] | Milk | ECM | Fat | Protein | Fat | Protein | |
| Wk 2 | | | | | | | |
| MGP | 34.7 | 37.3$^{b}$ | 3.98 | 3.08 | 1.37 | 1.06 | 14.8$^{b}$ |
| BFTP | 35.2 | 36.7$^{a}$ | 3.77 | 2.99 | 1.32 | 1.05 | 16.4$^{a}$ |
| Average | 35.0$^{c}$ | 37.0$^{c}$ | 3.87$^{c}$ | 3.04$^{d}$ | 1.35$^{c}$ | 1.06$^{c}$ | 15.6$^{c}$ |
| Wk 4 | | | | | | | |
| MGP | 31.4$^{b}$ | 32.0 | 3.51$^{a}$ | 3.11 | 1.10 | 0.98$^{b}$ | 13.6 |
| BFTP | 36.5$^{a}$ | 34.8 | 2.97$^{b}$ | 3.11 | 1.09 | 1.14$^{a}$ | 13.8 |
| Average | 33.9$^{c}$ | 33.4$^{d}$ | 3.25$^{d}$ | 3.11$^{c}$ | 1.10$^{d}$ | 1.06$^{c}$ | 13.7$^{d}$ |
| Wk 6 | | | | | | | |
| MGP | 28.2$^{b}$ | 29.0$^{b}$ | 3.72$^{a}$ | 2.93 | 1.05 | 0.82$^{b}$ | 6.3$^{b}$ |
| BFTP | 34.8$^{a}$ | 33.8$^{a}$ | 3.25$^{b}$ | 2.93 | 1.13 | 1.02$^{a}$ | 12.8$^{a}$ |
| Average | 31.5$^{d}$ | 31.4$^{de}$ | 3.49$^{de}$ | 2.93$^{e}$ | 1.09$^{d}$ | 0.92$^{d}$ | 9.5$^{e}$ |
| Wk 8 | | | | | | | |
| MGP | 25.0$^{b}$ | 25.9$^{b}$ | 3.81 | 2.87 | 0.94$^{b}$ | 0.72$^{b}$ | 7.9$^{b}$ |
| BFTP | 32.9$^{a}$ | 33.6$^{a}$ | 3.60 | 2.95 | 1.19$^{a}$ | 0.97$^{a}$ | 21.0$^{a}$ |
| Average | 28.9$^{e}$ | 29.7$^{e}$ | 3.71$^{ce}$ | 2.91$^{e}$ | 1.07$^{d}$ | 0.84$^{e}$ | 14.5$^{f}$ |
| Wk 2 to Wk 8 | | | | | | | |
| MGP | 29.8$^{h}$ | 31.1$^{h}$ | 3.76$^{a}$ | 2.99 | 1.12 | 0.89$^{h}$ | 10.7$^{h}$ |
| BFTP | 34.9$^{g}$ | 34.7$^{g}$ | 3.40$^{b}$ | 2.99 | 1.18 | 1.04$^{g}$ | 15.9$^{g}$ |
| Pooled SEM | 1.15 | 1.14 | 0.024 | 0.04 | 0.004 | 0.033 | 0.33 |

$^{a\text{-}b}$Within a column and week, means with different superscripts are different ($P < 0.05$).

$^{c\text{-}f}$Within a column, averages with different superscripts are different ($P < 0.05$).

$^{g\text{-}h}$Within a column, means with different superscripts are different ($P < 0.05$).

[1] MGP = mixed grass pasture containing perennial ryegrass, orchardgrass, and white clover; BFTP = birdsfoot trefoil pasture.

**Table 4.5.** Ruminal fermentation profiles of dairy cows grazing mixed grass pasture or birdsfoot trefoil pasture in 2012 and 2013

| Week and Treatment[1] | pH | Total VFA[2] | Individual VFA[3] | | | A:P | $NH_3$-N[4] |
|---|---|---|---|---|---|---|---|
| | | | A | P | B | | |
| Year 2012 | | | | | | | |
| Wk 2 | | | | | | | |
| MGP | $6.49^{a}$ | $97.5^{a}$ | 64.8 | 20.2 | $13.1^{a}$ | 3.2 | $4.80^{b}$ |
| BFTP | $6.30^{b}$ | $77.1^{b}$ | 61.8 | 20.3 | $10.4^{b}$ | 3.2 | $7.10^{a}$ |
| Average | $6.41^{c}$ | 87.2 | 63.3 | 20.3 | 11.8 | 3.13 | $5.95^{d}$ |
| Wk 6 | | | | | | | |
| MGP | $6.15^{a}$ | $106.2^{a}$ | 64.3 | 19.2 | 11.9 | 3.3 | 7.09 |
| BFTP | $5.99^{b}$ | $79.4^{b}$ | 63.5 | 19.5 | 12.0 | 3.4 | 8.22 |
| Average | $6.07^{d}$ | 92.8 | 63.9 | 19.3 | 11.9 | 3.31 | $7.66^{c}$ |
| Wk 2 to Wk 6 | | | | | | | |
| MGP | $6.32^{a}$ | $101.9^{a}$ | 64.6 | 19.9 | $12.6^{a}$ | 3.2 | $5.6^{b}$ |
| BFTP | $6.15^{b}$ | $78.3^{b}$ | 62.6 | 19.8 | $11.2^{b}$ | 3.3 | $7.7^{a}$ |
| Average | 6.23 | 90.1 | 63.6 | 19.8 | 11.9 | 3.3 | 6.8 |
| Pooled SE | 0.09 | 1.16 | 0.93 | 0.38 | 0.589 | 0.48 | 0.689 |
| Year 2013 | | | | | | | |
| Wk 2 | | | | | | | |
| MGP | $6.87^{b}$ | $82.5^{a}$ | 66.8 | 17.7 | $12.1^{a}$ | 3.8 | $6.10^{b}$ |
| BFTP | $7.04^{a}$ | $67.5^{b}$ | 70.1 | 17.4 | $8.3^{b}$ | 4.1 | $11.04^{a}$ |
| Average | 6.96 | 80.6 | 68.40 | 17.54 | 10.20 | 3.89 | 8.57 |
| Wk 6 | | | | | | | |
| MGP | $6.80^{b}$ | $75.0^{a}$ | 69.7 | 16.4 | 11.1 | 4.3 | $4.90^{b}$ |
| BFTP | $7.05^{a}$ | $56.7^{b}$ | 69.4 | 16.7 | 9.5 | 4.2 | $13.92^{a}$ |
| Average | 6.93 | 75.6 | 69.50 | 16.53 | 10.30 | 4.21 | 9.41 |
| Wk 2 to Wk 6 | | | | | | | |
| MGP | $6.84^{b}$ | $78.8^{a}$ | 68.23 | 17.0 | $11.6^{a}$ | 4.1 | $5.5^{b}$ |
| BFTP | $7.05^{a}$ | $62.1^{b}$ | 69.70 | 17.0 | $8.9^{b}$ | 4.2 | $12.5^{a}$ |
| Average | 6.93 | 70.4 | 69.50 | 17.0 | 10.30 | 4.2 | 8.9 |
| Pooled SE | 0.05 | 1.21 | 0.62 | 0.44 | 0.647 | 0.16 | 0.485 |

$^{a\text{-}b}$Within a column and week, means with different superscript letter are different ($P < 0.05$).

$^{c\text{-}d}$Within a column, averages with different superscript letter are different ($P < 0.05$).
clover; BFTP = birdsfoot trefoil pasture.

[1] MGP = mixed grass pasture containing perennial ryegrass, orchardgrass, and white

[2] Total volatile fatty acid concentration is expressed as m*M*.

[3] Molar proportion is expressed as mol/100 mol. A = acetate; P = propionate; and B = butyrate.
[4] Ammonia-N expressed as mg/100 mL.

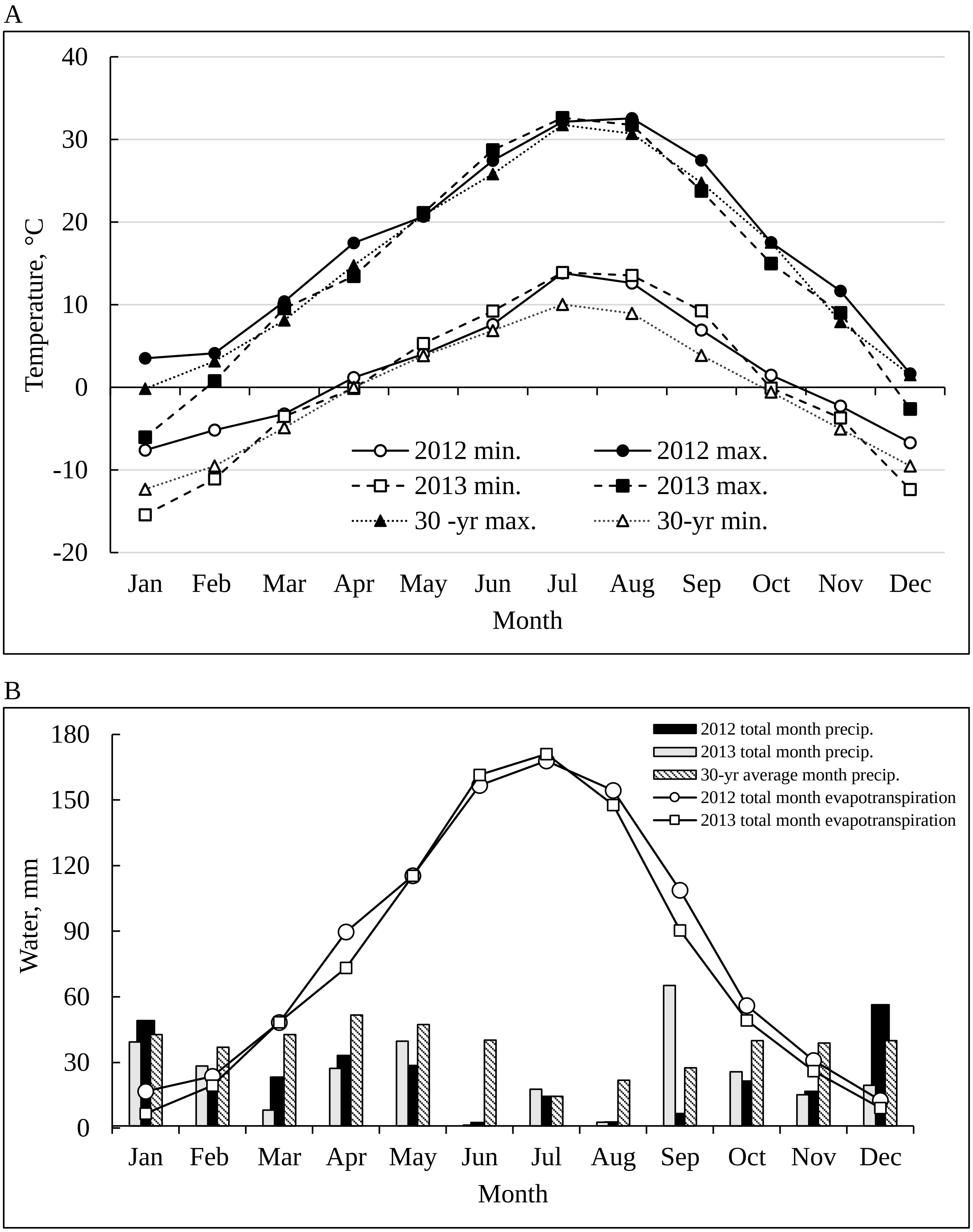

**Fig. 4.1** Average monthly minimum (min.) and maximum (max.) temperatures for nearest weather station (Lewiston, UT) in 2012 and 2013 with 30-year average for comparison (A). Total monthly precipitation (precip.) and evapotranspiration in 2012 and 2013, and 30-yr average monthly precipitation for comparison (B).

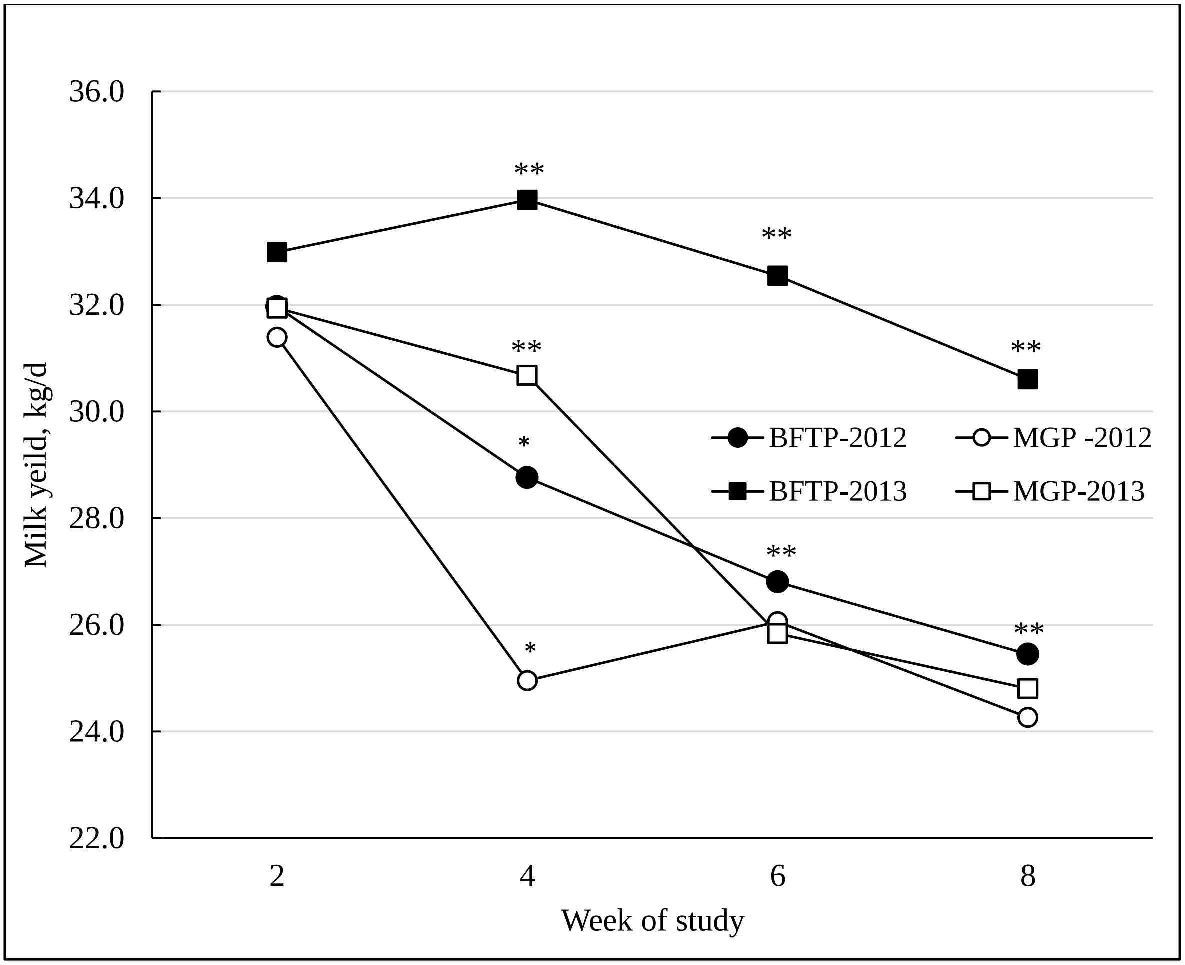

**Figure 4.2.** Pasture forage effect on milk yield by week of study and treatment in 2012 and 2013. Grazing start date was June 20 and May 14 in 2012 and 2013, respectively. Overall season effect of treatment was *P* = 0.42 and *P* = 0.02 in 2012 and 2013, respectively. $^{*}$Treatment means by week in 2012 differ ($P < 0.05$). $^{**}$Treatment means by week in 2013 differ ($P < 0.05$).

# CHAPTER 5

# BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS*) AFFECTS *IN VITRO* RUMEN MICROBIAL ECOSYSTEM AND METHANOGENESIS IN CONTINUOUS CULTURE DUE TO CONDENSED TANNINS

## INTRODUCTION

Pasture-based dairies are most often small family-owned operations with the goal of improving farm profitability through increased reliance on pasture as a natural, local forage resource. Dairy pastures in the western USA are typically composed of cool-season grass varieties. If managed under intensive rotational stocking they may contain more crude protein (**CP**) than can be efficiently used by dairy cows (Muller and Fales, 1998). The protein in pasture forage is highly degradable, and when consumed by ruminants, excess protein is quickly converted to ammonia, resulting in elevated excretion of nitrogenous waste by the animal. If digestible carbohydrate sources are insufficient, inefficient utilization of pasture nitrogen (**N**) in the rumen occurs, limiting optimal microbial protein synthesis. This increases metabolic energy cost to convert ruminal ammonia-N (**$NH_3$-N**) to urea. Therefore, N utilization would be improved by matching protein and carbohydrate supply, reducing the amount of N consumed by dairy cows, or incorporating more dietary N into milk protein (Higgs et al., 2013).

Birdsfoot trefoil (*Lotus corniculatus* L.; **BFT**) is persistent in the cool, dry, alkaline soils of the Intermountain West in USA and is a condensed-tannin (**CT**) containing legume that does not cause bloat in cattle when grazed. Two effects of CT are the reduction of ruminal protein degradation by soluble protein precipitation in the rumen at typical ruminal pH and reduced ruminal methane (**$CH_4$**) production (Min et al., 2003; Williams et al., 2011). Methane is produced by methanogens in the rumen during anaerobic fermentation of soluble and structural carbohydrates contained in forage-based diets (Waghorn et al., 2002; Tavendale et al., 2005). Ruminal $CH_4$ production represents 3 to 9% feed energy loss and contributes to greenhouse gas emissions in the environment (Moss et al., 2000). Reducing enteric $CH_4$ emissions has been of great interest for increasing sustainability of ruminant production. Diets containing 2.6% CT from BFT resulted in decreased $CH_4$ emission in dairy cattle compared to grass-based diets (Woodward et al., 2001), while similar reduction on $CH_4$ production was reported in a continuous culture study due to the presence of CT of BFT (Williams et al., 2011). The inhibitory effects of CT on ruminal methanogenesis have been attributed to direct effects on methanogenic archaea, protozoa-associated $CH_4$ production, or an indirect result of a depression of fiber digestion in the rumen (Patra et al., 2012).

Orchardgrass has become one of the mostly widely distributed grasses in the Northeast and Midwest (van Santen and Sleper, 1996). However, research regarding the supplementation and relationships between forage quality, $CH_4$ emissions, and rumen fermentation is scarce. In contrast, these factors have been studied in perennial ryegrass (Lolium perenne L.) more than in any other grass due to its productivity, nutritive value, and prevalence in major grassland regions. The popularity of orchardgrass as a forage option to replace perennial ryegrass for grazing dairy farms has not been supported by detailed studies regarding supplementation or efficiency of nutrient utilization.

Microbial population changes in the gut of sheep fed CT-containing diets were reported using a 16S PCR technique (Min et al., 2002). To date, however, little research has focused on changes in microbial population diversity, particularly methanogenic archaeal population, in response to feeding BFT of OG pasture-based diets and additional effects of supplementation. Therefore, the objective of this study was to investigate effects of feeding BFT pasture-based diets without or with concentrate supplementation on ruminal fermentation with a focus on $CH_4$ production in continuous cultures. We were particularly interested in methanogenic archaea in response to feeding BFT-based diets, effects of supplementation, and resultant $CH_4$ production.

## MATERIALS AND METHODS

### *Pasture Forages, Dietary Treatments and Experimental Design*

Orchardgrass (*Dactylis glomerata L.*; **OG**) assessed in this experiment was planted in a randomized complete block design with 4 replications on August 4, 2010 at the Utah State University Experiment Station Intermountain Irrigated Pasture Project Farm in Lewiston, UT, USA. Irrigation was used for establishment of the pastures and during production. Nitrogen fertilizer was applied to OG monocultures in 3 applications of 20 kg N/ha during the growing season. Plots were harvested twice (August 5 and September 17, 2013) by cutting to a height of 8 cm with a sickle-bar harvester (Swift Machine & Welding LTD, Swift Current, SK, Canada) while OG was in a vegetative stage.

Birdsfoot trefoil was planted in 2012 at the Greenville Farm (Logan, UT, USA) in a randomized block with 3 replications. Irrigation was applied for establishment and during production. No fertilizer was applied to the BFT plots. The second and third regrowth were harvested using a hand sickle on July 16 and September 9, 2013.

Herbage samples were frozen at −4°C and freeze-dried (FreeZone 12 L Freeze Dry Systems, Labconco Corp., Kansas City, MO, USA). Herbage and TMR components samples were ground to pass a 4.0-mm screen (Wiley Mill, Model 4; Thomas Scientific Co., Swedesboro, NJ, USA), while subsamples of all dietary ingredients for proximate analyses were ground to pass a 1.0-mm screen.

Six dietary treatments were randomly applied to an 8-unit dual-flow continuous culture fermentor system according to a 2 (pasture forages) × 3 (supplementations) factorial design. Treatments were replicated three times, and the experimental unit was the independent run of continuous cultures (n = 3). The six diets used in this study were control pasture forages (OGP and BFTP) only or forage with two supplements [ground barley (GB), and total mixed ration (TMR)]: OG pasture and no supplement (OGP–NS); OGP supplemented with GB (OGP–GB); OGP supplemented with TMR (OG–TMR); BFT pasture and no supplement (BFTP–NS); BFT pasture supplemented with GB (BFTP–GB); and BFT pasture supplemented with TMR (BFTP–TMR). The GB and the TMR were supplemented at 30% DM in the corresponding diets. These levels and type of supplementation were chosen based on data collected from organic dairy farms currently using these feeding strategies for mixed grass species pastures (R. Christensen, unpublished data). The barley grain was supplied from a local organic dairy, dried at 60°C for 24 h, and ground to 4 mm (Wiley Mill Model 4). The TMR diets were balanced for each forage so that the nutrients were consistent between the forage treatments. It was composed of 70% DM forage and 30% TMR consisting of 15% canola meal (for OG) or 15% barley straw (for BFT), 20% corn silage, 25% alfalfa hay, and 40% barley grain. They contained 16.3, 42.9, and 25.3% CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF), respectively for the OGP-TMR and 16.9, 37.3, and 25.8% CP, NDF, and ADF, respectively for the BFTP-TMR (Table 5.1). The GB or TMR supplementation is typical of late grazing season diets used on commercial organic dairy farms in the Intermountain West, USA (i.e., Utah, Idaho, Wyoming, Montana and parts of Arizona and Nevada). Diets were balanced to support a Holstein cow weighing 690 kg, producing 27 kg milk with 3.5% milk fat and 3.1% true protein using the Cornell-Penn-Miner System (CPM Dairy, Version 3.0).

### *Continuous Culture Operation*

Whole ruminal contents were collected from 2 dry, ruminally cannulated Holstein cows prior to a.m. feeding (average pH of 6.9). Cows were fed a 100% oat hay diet. All animal care protocols were approved by the Utah State University Institutional Animal Care and Use Committee. Rumen fluid was collected from various locations within the rumen, placed into preheated insulated containers, and transported to the laboratory. In the lab, rumen contents were strained through a polyester screen (PeCAP, pore size 355 μm; B & SH Thompson, Ville Mont-Royal, QC, Canada), mixed from both cows and then with constant stirring, the filtered ruminal inoculum (750 ml) was added to a dual-flow continuous culture fermentor (Prism Research Glass, Inc., Research Triangle Park, NC, USA), which was modified in construction and operation from the design described by Teather and Sauer (1988). The main modifications were a reconfigured overflow sidearm that angled downward at approximately 45° to facilitate emptying, and a faster stirring rate (45 rpm) that still allowed stratification of particles into an upper mat, a middle liquid layer of small feed particles, and a lower layer of dense particles.

An anaerobic condition was maintained in the fermentors by infusion of $CO_2$ at a rate of 20 ml/min. Artificial saliva prepared according to Slyter et al. (1966) was continuously infused into fermentors at a rate of 0.85 ml/min using a pump (Model 323, Watson-Marlow Inc., Wilmington, MA, USA) to maintain a fractional dilution rate of 7.2%/h. To mimic rumen motility, cultures were continuously stirred by a central paddle attached to an electric motor. Each fermentor received a total of 15 g of DM/day that was fed in 4 equal portions at 0600, 1200, 1800 and 2400 h.

### *Sample Collection*

Cultures were adapted to experimental diets by increasing the proportion of the treatment diet as follows: cultures received 100% of the respective forage on day 1, then 2:1 of the experimental diet forage to experimental diet ratio on day 2, and then 1:2 on day 3, and on day 4 100% of the treatment diet was fed. Three d adaptation to treatment diet was allowed (days 4 to 7) prior to data collection, sampling, and analysis of culture content, which were independently performed in each run. On d 8 and 9 of each run, ruminal culture pH data and 2 sets of 5 ml culture fluid samples for volatile fatty acids (**VFA**) and $NH_3$-N analysis were collected. Culture pH was measured hourly, and $CH_4$ samples were collected from the headspace gas of each fermentor at 0600, 0900, 1200, 1500 and 1800 h using a 10 μl gastight syringe (Hamilton Co., Reno, NV) and analyzed for $CH_4$ with a GLC (Model CP-3900, Varian, Walnut Creek, CA, USA). Daily $CH_4$ production (mM/day) was calculated as reported by Jenkins et al. (2003) using the equation: $CH_4$ proportion in fermentor headspace (mM/ml) × $CO_2$ gas flow through the fermentor headspace (20 ml/min) × 60 min × 24 h.

Immediately after $CH_4$ sampling at 0800 and 1400 h, 5 ml of culture contents were taken, added to 1 ml of 25% meta-phosphoric acid and stored at -40°C for VFA determination. At the same times as VFA sample collection, another 5 ml of culture content was collected from each fermentor, mixed with 1% sulfuric acid, and stored frozen (-40°C) for $NH_3$-N analysis.

On the final day of each run (day 9), the total volume of fermentor contents was collected and blended using a blender (Master Prep, EURO-PRO Operating LLC, Boston, MA, USA) for 1 min. The homogenate was filtered through polyester material (PeCAP, pore size 355 μm) and stored frozen (-40°C) for microbial population assays.

### ***DNA Extraction from Ruminal Culture Content***

Genomic bacterial DNA was isolated from 1 ml of each unknown rumen fluid sample according to the method described in the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA). Extracted DNA (2 μl) was quantified using a Nanodrop ND-1000 spectrophotometer (Nyxor Biotech, Paris, France) and run on 0.8% agarose gel with 0.5 M tris-borate-EDTA (TBE) buffer. The samples were then stored -80°C for the real-time PCR analysis.

### ***Primers, PCR amplification, and Gel Analysis***

A set of PCR primers was designed and validated (Koike and Kobayashi, 2001; Tajima et al., 2001; Tymensen and McAllister, 2011) for specific detection of species listed in Table 2. Primers [50 pmol of each per reaction mixture; primers 1 to 6 (Integrated DNA Technologies, Inc., Coralville, IA, USA; Sheffield et al., 1989; Muyzer et al., 1993)] were mixed with Jump Start Red-*T*aq Ready Mix (Sigma-Aldrich Corporation, St. Louis, MO, USA) according to Reysenbach et al. (1992).

Amplifications of PCR were performed on the PTC-200 Peltier Thermal Cycler (MJ Research Inc., Waltham, MA, USA) with the following program: 1) denaturation at 95°C for 3 min; 2) subsequent 35 denaturing cycles at 95°C for 30 sec; 3) various annealing temperatures described in Table 2 for 30 sec and extension at 72°C for 1 min (Tajima et al., 2001). Fifteen µL aliquots were resolved in a 7.5% polyacrylamide gel (37.5:1). The DGGE gel was run at 60°C and 82 V for 15 h using a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, USA). The DGGE gel was then stained with GelStar (Cambrex, USA), and the gel images were captured using a FluorChem Imager (Alpha Innotech).

Gels were analyzed using the Quantity One software package, version 4.62 (Bio-Rad Laboratories, USA). After normalization, bands were defined for each sample by using band detection based on parameters and comparison to the standard lanes to determine the values of the experimental bands using those standards. Peak heights in the densitometric curves were used to determine the diversity indices based on the Shannon-Weiner diversity index, calculated as $H = -\Sigma[P_i \ln(P_j)]$, where $H$ is the diversity index and $P_i$ is the importance probability of the bands in a lane ($P_j = n_j/n$ where $n_i$ is the height of an individual peak and n is the sum of all peak heights in the densitometric curves).

### ***Chemical Analysis***

Analytical DM concentration of forages and diet samples was determined by oven drying at 105°C for 3 h (AOAC, 2000; method 930.15), and organic matter (OM) was determined by ashing at 550ºC for 5 h (AOAC, 2000; method 942.05). Concentration of

N was determined using an organic elemental analyzer (Flash 2000; CE Elantech Inc., Lakewood, NJ, USA; AOAC, 2000; method 990.03). Concentrations of NDF and ADF were sequentially determined using an ANKOM200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treated with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation). Ether extract was measured (AOAC, 2000; method 2003.05) using a fat analyzer (XT20, ANKOM Technology). Total extractable CT concentration in forage samples and experimental diets was determined using a butanol-HCl colorimetric procedure (Terrill et al., 1992).

Culture VFA were separated and quantified using a GLC (Model 6890 series II, Hewlett Packard Co., Avondale, PA, USA) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA, USA) and flame ionization detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of 5°C/min, then increased by 3°C/min to 220°C and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C, respectively, and the carrier gas was helium (Eun and Beauchemin, 2007). Concentration of $NH_3$-N was determined using colorimetric analysis as described by Rhine et al. (1998) using a plate reader (MRX$^e$, Dynex Technologies, Chantilly, VA, USA).

### S*tatistical Analysis*

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA) using the model described below:

$Y_{ijkl} = \mu + R_i(F_j) + P_k + S_l + (PS)_{kl} + e_{ijkl}$, where $Y_{ij}$ = individual response variable measured, $\mu$ = overall mean, $R_i(F_j)$ = random effect of fermentor j within independent run i, $P_k$ = fixed effect of forage k (OG vs. BFT; k = 1 to 2), $S_l$ = fixed effect of supplement l (NS vs. GB vs. TMR; l = 1 to 3), $(PS)_{kl}$ = interaction between forage k and supplement l and $e_{ijkl}$ = residual error. Denominator degrees of freedom were estimated using the Kenward-Roger option. The same mixed model was used for variables that were repeated in time (culture pH and $CH_4$), but sampling time and a repeated statement were added to the model.

Significant effects were accepted when $P \leq 0.05$, and trends were discussed when $0.5 < P \leq 0.10$. When the interaction between type of forages and supplements was $P \leq 0.10$, Bonferroni-adjusted *P*-values were used to assess the supplements within the type of forage. Results are reported as least square means.

## RESULTS

### *Nutrient Composition of Diets*

Nutrient composition and CT concentration of diets are presented in Table 5.1. In comparison with grasses, legumes contain greater CP and less fiber concentrations, so all diets containing BFTP were greater in CP and less in NDF and ADF concentrations except for the TMR-supplemented diet, which were quite similar to OGP-TMR, as was intended. There was a considerable difference of non-fiber carbohydrates (NFC) concentration between OGP and BFTP (14.6 vs. 38.2%). With regard to the NS and GB diets, OGP–NS and OGP–GB had less CP concentrations compared with the BFTP–NS and BFTP–GB (11.5 and 11.9% vs. 20.6 and 18.3%, respectively), whereas fiber concentrations of the BFTP–GB were the least (25.6% NDF and 18.6% ADF). Ether extract concentration declined with supplementation in the OGP diets but were greater than the BFTP diets (average of 3.48 vs 2.37%, respectively). A noticeable CT concentration was detected only in BFTP forage (4.96% DM; Table 5.1). Consequently, dietary treatments containing BFTP had considerable concentrations of CT (2.70 and 2.83% for BFTP–GB and BFTP–TMR, respectively).

### ***Culture pH, $NH_3$-N and VFA Profiles***

Culture pH was maintained at least 6.01 across dietary treatments, and there was no difference among treatments in culture pH (Table 5.3). Concentration of $NH_3$-N decreased when cultures were offered BFTP-based diets compared with OGP-based diets, while supplementing with GB or TMR did not affect $NH_3$-N concentration regardless of source of pasture.

Neither forage type nor supplementation had an effect on the concentration of total VFA (Table 5.3). Feeding BFTP-based diets increased molar proportion of acetate compared with OGP-based diets, whereas molar proportion of propionate was similar between OGP- and BFTP-based diets, leading to an increase in acetate-to-propionate ratio (A:P) due to feeding BFTP-based diets. Molar proportions of butyrate and all branched-chain VFA were reduced by offering BFTP-based diets compared with OGP-based diets. Adding supplements decreased acetate proportion, but increased propionate proportion, regardless of forage type, resulting in a decrease in A:P. In addition, supplementing GB or TMR increased proportions of butyrate, valerate and isovalerate both in OGP- and BFTP-based diets.

### ***Microbial Community Structure and $CH_4$ Production***

Microbial population densities of *F. succinogenes* and *R. flavefaciens* decreased when cultures were fed BFTP-based diets compared with OGP-based diets, while *R. albus* was not affected by type of forage (Table 5.4). Adding supplements in general decreased the cellulolytic bacteria, but the decreases were greater in the BFTP-based diets compared with OGP-based diets, resulting in interactions between type of forage and supplementations.

Density of *Methanobrevibacter* spp. decreased in cultures fed BFTP-based diets, but density of *Methanomicrobium* spp. was not affected (Table 5.4). Adding supplements did not influence methanogens. Likewise, $CH_4$ production was reduced by feeding BFTP-based diets; however, supplementation of GB or TMR led to no effect on $CH_4$ production, regardless of type of forage (Fig. 1).

## DISCUSSION

### ***Nutrient Composition of Diets***

Birdsfoot trefoil deposits less NDF in the stem and leaf as it matures compared with OG (Mowat et al., 1969). Thus, BFTP consistently had less fiber concentration, despite the fact that forages were harvested at a comparable maturity. Although the intent of the 2 TMR supplements was to formulate the diets with similar nutrient concentrations, NDF concentrations were more than 5 percentage units greater in the OGP-TMR compared with the BFTP-TMR. Birdsfoot trefoil contains 0.5 to 4.7% CT for various cultivars and growth conditions (Barry and McNabb, 1999; Grabber, 2009), and consequently in this study it was greater than most values reported in the literature. The concept of using CT-containing forages in ruminant production is sound only when CT improve overall ruminal fermentation without interfering with other ruminal functions. Condensed tannins in BFTP may have negative effects on fiber fermentation in the rumen because of a toxic property of CT against cellulolytic bacteria (Patra and Saxena, 2011), particularly with elevated concentration of CT is contained in the diet (Patra et al., 2012).

### *Culture pH, $NH_3$-N and VFA Profiles*

Because cultures were fed 100% or 70% pasture-based diets, it was expected that dietary treatment would not result in a negative effect on culture pH. Noviandi et al., (2014) reported that culture pH decreased when forages were supplemented with 30% corn, while it was similar between forage mixtures of grass and legume without or with distiller's grain supplementation. Starch is often supplemented in pasture rations primarily as an energy source, because most pasture forages lack starch at an adequate amount to support extensive microbial biomass production (Kolver et al., 2007). However, increased starch fermentation can have adverse effects on rumen microbes, and ruminal pH is often reduced when supplemented with starch-containing concentrates. Calsamiglia et al. (2008) reported that the effect of ruminal pH on NDF digestibility was relatively small when ruminal pH exceeded 6.0, but digestibility of NDF decreased sharply when pH was below this threshold. Therefore, in our study, supplementing GB or TMR in pasture-based diets did not interfere with digestibility of fiber or apparent other ruminal physiological conditions.

Ruminal $NH_3$-N concentration decreased due to feeding BFTP-based diets and was not affected by the increased CP concentration in BFTP-based diets compared with OGP-based diets (18.6 and 13.2% on average, respectively). Activity of CT was not inhibited despite reduced concentration of CT in BFTP diets with GB or TMR supplementation.

Protein-binding activity of CT in the BFT decreases ruminal $NH_3$-N concentration in 2 ways: 1) reducing dietary protein degradation via formation of insoluble tannin-protein complexes or decreasing the solubility of protein (Tanner et al., 1994; Min et al., 2000); and 2) inhibiting proteolytic bacteria and/or proteolytic enzymatic activity (Patra et al., 2012). *In vivo*, these functions would shift an increased proportion of protein from ruminal degradation to digestion and absorption in the small intestine and result in increased N utilization efficiency. Under typical cattle feeding conditions, manipulation of ruminal protein degradation or increase of the efficiency of N utilization in the rumen is the most effective strategy to reduce N losses (Tamminga, 1996). Using data obtained from continuous culture studies, Bach et al., (2005) reported that as efficiency of N utilization increases, $NH_3$-N accumulation in the fermentors decreases ($R^2 = 0.78$). Thus, the reduction in the $NH_3$-N concentration through CT in BFTP suggests an improvement in utilization of dietary N in ruminal fermentation.

Ruminal $NH_3$-N concentration is typically much less in defaunated animals compared to faunated ones (Jouany and Ushida, 1998; Santra and Karim, 2000). Some of these results could, on the one hand, be attributed to greater microbial synthesis, and on the other hand, to less bacterial recycling (Firkins et al., 1998; Koenig et al., 2000) and bacterial proteolysis when protozoa are missing (Onodera et al., 1977; Demeyer and Van Nevel, 1979). In order to assess effect of protozoa on ruminal $NH_3$-N concentration, we contrasted estimated $NH_3$-N concentration under faunated ($NH_3\text{-N} = 30.9 + 9.7$ CP % DM) and defaunated condition ($NH_3\text{-N} = 8.4$ CP % DM) using 2 separate equations obtained from a meta-analysis study using 75 concerned trials (Eugène et al., 2004). For faunated conditions, expected concentrations were 14.2, 14.6, 18.9, 23.1, 20.8, and 19.5

mg/100 ml, whereas under defaunated condition, 9.66, 10.0, 13.7, 17.3, 15.4, and 14.2 for OGP–NS, OGP–GB, OGP–TMR, BFTP–NS, BFTP–GB, and BFTP–TMR, respectively. Based on these estimates, there were sizable differences between culture $NH_3$-N concentrations and estimated $NH_3$-N concentrations under faunated conditions when BFTP-based diets were offered, but less differences when OGP-based diets were fed. This contrasting result infers that CT in BFTP may have reduced protozoal population, which likely contributed to decreased culture $NH_3$-N concentration when BFTP-based diets were fed in the current study. Khiaosa-Ard et al. (2009) reported that a diet containing 7.9% CT extract from black wattle (*Acasia mearnsii*) decreased the total protozoal population relative to a control diet (1.91 vs. $7.26 \times 10^3$ cells/ml, respectively) in continuous cultures. Makkar et al. (1995) also reported that quebracho CT (0, 0.1, 0.2, and 0.4 mg/ml) linearly decreased the total protozoal population (2.75, 1.53, 1.46, and $0.98 \times 10^3$ cells/ml, respectively).

Due to potentially toxic effects of CT in BFTP, we expected some negative responses on ruminal fermentation profiles when cultures were fed BFTP-based diets in this study. Tannins affect ruminal fermentation by forming complexes with numerous types of molecules including carbohydrates, proteins, polysaccharides and enzymes involved in protein and carbohydrate digestion and bacterial cell membranes (Scalbert, 1991; Reed, 1995). For example, cellulose can have direct surface interactions with tannins (Chiquette et al., 1988), and the tannins can alter microbial colonization on fiber particles, leading to reduced fiber digestibility. In the current study, it was expected that shifts in VFA profiles toward a decrease in acetate and an increase in propionate would occur when fiber digestion was altered due to interactions of tannins with rumen microbes. On the contrary, feeding BFTP-based diets resulted in an increase in A:P with no effect on total VFA concentration. Feeding toxic compounds such as CT can have a profound impact on the growth rate of bacteria and alter the metabolic pathways of fermentation. However, given the complex interactions between microbial growth and fermentation environment, the rumen ecosystem may have developed a strategy to maintain a normal rate of substrate fermentation by changing the concentration and/or shifting the metabolic pathways by which specific microbes utilize substrates.

In the present study, feeding BFTP-based diets decreased branched-chain VFA proportions which arise almost exclusively from the oxidative deamination of amino acids. The inability of the cellulolytic bacteria to transport preformed branched-chain amino acids across their cell wall makes the branched-chain VFA essential for normal growth of fiber-digesting bacteria (Bryant, 1973). The decreases in branched-chain VFA proportions due to feeding BFTP-based diets suggests reduced deaminative activity coupled with decreased ruminal N degradation through direct effects of CT in BFTP.

### ***Microbial Community Structure and $CH_4$ Production***

Tannin toxicity has been hypothesized to result from selective inhibition of microbial cell wall synthesis (Jones et al., 1994; Smith and Mackie, 2004). The antibacterial activity of tannins is mediated by formation of complexes with the cell wall membrane of bacteria to cause morphological changes and secretion of extracellular enzymes (Smith et al., 2005). The major cellulolytic bacteria in the rumen such as *R. albus, F. succinogenes* and *R. flavefaciens* have been found to be inhibited by CT, although degree of inhibition and sensitivity of bacteria varied among the studies, depending upon the dose and type of CT. In a pure culture study, CT of BFT inhibited the growth of *F. succinogenes* at a concentration of 400 µg/ml, but had no appreciable inhibitory effect on the growth at concentrations below 400 µg/ml (Bae et al., 1993). Min et al. (2005) observed that addition of 200, 400 and 600 µg CT/ml reduced the growth rate of *F. succinogenes*; however, the growth of *R. albus* transiently increased at low (50-100 µg/ml), but not at high (> 200 µg/ml) concentrations of CT. The CT reduced the extracellular endoglucanase activity at concentrations as low as 25 µg/ml, whereas cell-associated endoglucanase activity increased at CT concentrations of up to 300 µg/ml, and then decreased at 400 µg/ml (Min et al., 2005). The exposure of *F. succinogenes* to CT appears to cause the formation of tannin-protein complexes on the cell surface, which can induce interference with the adhesion process of bacterial cells to the cellulose. The selective antibacterial action of CT-containing BFT diets against *F. succinogenes* (gram-negative) and *R. flavefaciens* (gram-positive) but not *R. albus* (gram-positive) is not clear in the current study. Generally, gram-positive bacteria have been known to be more susceptible to tannins than gram-negative bacteria (Jones et al., 1994). In the present study, *R. flavefaciens* may have been inhibited by *R. albus* in the reduced-cellulose condition of BFTP-based diets compared to OGP-based diets. Meanwhile, greater decreases in all cellulolytic bacteria through feeding BFTP-based diets evidenced by interactions between type of forage and supplementations indicate an additive effect between CT and readily fermentable carbohydrates from GB and TMR on the cellulolytic microbial ecosystems of the rumen.

In the current study, concentration of $CH_4$ decreased when cultures were fed BFTP-based diets compared with OGP-based diets with a selective depression against *Methanobrevibacter* spp., but not *Methanomicrobium* spp. One possible mechanism for this effect could be that the protozoa-associated methanogens are inhibited in the rumen by an anti-protozoal effect of the CT in BFTP. While *Methanobrevibacte*r spp. are known as major protozoa-associated methanogens, *Methanomicrobium* spp. are classified as protozoa-free methanogens (Sharp et al., 1998). It is evident that the CT in BFTP at a relatively great concentration exerted their toxic effects against protozoa and protozoa-associated methanogens, leading to the decrease in culture $CH_4$ production. There is a body of evidence to indicate that CT decrease protozoal population (Makkar et al., 1995; Khiaosa-Ard et al., 2009; Tan et al., 2011), and the decrease in $CH_4$ production can be mediated through decreased protozoal number (Bodas et al., 2012). Min et al. (2014) reported that feeding CT-containing pine bark (10.3% CT) selectively altered methanogenic archaeal populations in goats; among methanogens, *Methanobrevibacte*r linearly decreased with increasing pine bark supplementation in goats (75, 72, and 49% for 0, 15 and 30% pine bark, respectively). The authors indicated that the decrease in *Methanobrevibacter spp*. prevalence was assumed to be linked to the substantial relationship between these archaea and protozoa and subsequent reduction in reducing equivalent ($H_2$) cross-feeding between protozoa and archaea.

## CONCLUSIONS

We assessed *in vitro* rumen microbial ecosystem response to feeding CT-containing BFTP diets in comparison with non-CT-containing OGP diets as pasture only controls or with 2 different types of supplementation. The CT in BFTP exerted anti-microbial properties against cellulolytic bacteria and methanogens, but those effects were species-dependent. In addition, the CT of BFTP sizably reduced $NH_3$-N concentration with its toxic effect on protozoal population. Type of supplementation did not affect CT activity on rumen ammonia concentration, and suggested that various types of BFTP diets could be fed and still exert reductions in $CH_4$ output. Care must be taken in extrapolating these results to *in vivo* conditions, as overall rumen microbial ecosystem function is likely feed intake dependent. Intake will eventually influence the amount of CT and supplement actually consumed by animals in their diets. Function of chemical activity of CT in BFTP on microbial physiology remains to be identified. A future direction for use of BFT in ruminants needs to consider the dynamic effects of CT on rumen microbial ecosystem with target diets and farming systems.

## REFERENCES

AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

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

Bae, H. D., T. A. McAllister, J. Yanke, K. J. Cheng, and A. D. Muir. 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl. Environ. Microbiol. 59:2132–2138.

Barry, T. N., and W. C. McNabb. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. Nutr. 81:263–272.

Bodas, R., N. Prieto, R. García-González, S. Andrés, F.J. Giráldez, and S. López. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed Sci. Technol. 176:78–93.

Bryant, M. P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. FASEB J. 32:1889–1913.

Calsamiglia, S., P. W. Cardozo, A. Ferret, and A. Bach. 2008. Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. J. Anim. Sci. 86:702–711.

Chiquette, J., K. J. Cheng, J. W. Costerton, and L.P. Milligan. 1988. Effect of tannins on the digestibility of two isosynthetic strains of birdsfoot trefoil (*Lotus corniculatus* L.) using *in vitro* and *in sacco* techniques. Can. J. Anim. Sci. 68:751–760.

Demeyer, D. I., and C. J. Van Nevel. 1979. Effect of defaunation on the metabolism of rumen microorganisms. Br. J. Nutr. 42:515–524.

Eugène, M., H. Archimede, and D. Sauvant. 2004. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. Livest. Prod. Sci. 85:81–97.

Eun, J.-S., and K. A. Beauchemin. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using *in vitro* fermentation characteristics. Anim. Feed Sci. Technol.**132**, 298–315.

Firkins, J. L., M. S. Allen, B. S. Oldick, and N. R. St-Pierre. 1998. Modeling ruminal digestibility of carbohydrates and microbial protein flow to the duodenum. J. Dairy Sci. 81:3350–3369.

Grabber, J. H. 2009. Protein fractions in forage legumes containing protein-binding polyphenols: Freeze-drying vs. conservation as hay or silage. Anim. Feed Sci. Technol. 151:324–329.

Higgs, R. J., A. J. Sheahan, K. Mandok, M. E. Van Amburgh, and J. R. Roche. 2013. The effect of starch-, fiber-, or sugar-based supplements on nitrogen utilization in grazing dairy cows. J. Dairy Sci. 96:3857–3866.

Jenkins, T. C., V. Fellner, and R. K. McGuffey. 2003. Monensin by fat interactions on trans- fatty acids in cultures of mixed ruminal microorganisms grown in continuous fermentors fed corn or barley. J. Dairy Sci. 86:324–330.

Jones, G. A., T. A. McAllister, A. D. Muir, and K. G. Cheng. 1994. Effects of sainfoin (*Onobrychis viciifolia* scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol. 60:1374–1378.

Jouany, J. P., and K. Ushida. 1998. The role of protozoa in feed digestion. Asian-australas. J. Anim. Sci. 12:113–128.

Khiaosa-Ard, R., S. F. Bryner, M. R. L. Scheeder, H. R. Wettstein, F. Leiber, M. Kreuzer, and C. R. Soliva. 2009. Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. J. Dairy Sci. 92: 177–188.

Koenig, K. M, C. J. Newbold, F. L. McIntosh, and L.M. Rode. 2000. Effects of protozoa on bacterial nitrogen recycling in the rumen. J. Anim. Sci. 78:2431–2445.

Koike, S., and Y. Kobayashi. 2001. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens*. FEMS Microbiol. Lett. 204:361–366.

Kolver, E. S., J. R. Roche, C. R. Burke, J. K. Kay, and P. W. Aspin. 2007. Extending lactation in pasture-based dairy cows: I. Genotype and diet effect on milk and reproduction. J. Dairy Sci. 90:5518–5530.

Makkar, H. P. S., K. Becker, H. Abel, and C. Szegletti. 1995. Degradation of condensed tannins by rumen microbes exposed to quebracho tannins (QT) in rumen simulation technique (RUSITEC) and effects of QT on fermentative process in the RUSITEC. J. Sci. Food Agric. 69:495–500.

Min, B. R., W. C. McNabb, T. N. Barry, and J. S. Peters. 2000. Solubilization and degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39; Rubisco) protein from white clover (*Trifolium repens*) and *Lotus corniculatus* by rumen microorganisms and the effect of condensed tannins on these processes. J. Agri. Sci. (Camb.) 134:305–317.

Min, B. R., G. T. Attwood, K. Reilly, W. Sun, J. S. Peters, T. N. Barry, and W. C. McNabb. 2002. *Lotus corniculatus* condensed tannins decrease *in vivo* populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. Can. J. Microbiol. 48:911–921.

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 temperate forages: A review. Anim. Feed Sci. Technol. 106:3–19.

Min, B. R., W. E. Pinchak, J. D. Fulford, and R. Puchala. 2005. Wheat pasture bloat dynamics, *in vitro* ruminal gas production, and potential bloat mitigation with condensed tannins. J. Anim. Sci. 83:1322–1331.

Min, B. R., S. Solaiman, R. Shange, and J.–S. Eun. 2014. Gastrointestinal bacterial and methanogenic archaea diversity dynamics associated with condensed tannin-containing pine bark diet in goats using 16S rDNA amplicon pyrosequencing. Int. J. Microbiol. Article ID 141909. Accessed 5 Dec. 2014, http://dx.doi.org/

10.1155/2014/141909.

Moss, A. R., J. P. Jouany, and J. Newbold. 2000. Methane production by ruminants: its contribution to global warming. In Annales de Zootechnie, EDP Sciences 49:231-253.

Mowat, D. N., M. L. Kwain, and J. E. Winch. 1969. Lignification and in vitro cell wall digestibility of plant parts. Can. J. Plant Sci. 49:499–504.

Muller, L. D., and S. L. Fales. 1998. Supplementation of cool-season grass pastures for dairy cattle. Pages 335–350 in Grass for Dairy Cattle. J. H. Cherney, D. J. R. Cherney, D. J. R. ed. CABI Publishing, New York, NY.

Muyzer, G., E. C. de Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695–700.

Noviandi, C. T., J.-S Eun, M. D. Peel, B. L. Waldron, B. R. Min, D. R. Zobell, and R. L. Miller. 2014. Effects of energy supplementation in pasture forages on in vitro ruminal fermentation characteristics in continuous cultures. Prof. Anim. Sci. 30:13–22.

Onodera, R., H. Yamaguchi, C. Eguchi, and M. Kandatsu. 1977. Limits of survival of the mingled rumen bacteria in the washed cell suspension of rumen ciliate protozoa. Agric. Biol. Chem. 41:2465–2466.

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.

Patra, A. K., J. Stiverson, and Z. Yu. 2012. Effects of quillaja and yucca saponins on communities and select populations of rumen bacteria and archaea, and fermentation in vitro. J. Appl. Microbiol. 113:1329–1340.

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

Reysenbach, A. L., L. J. Giver, G. S. Wickham, and N. R. Pace. 1992. Differential amplification of rRNA genes by polymerase chain reaction. Appl. Environ. Microbiol. 58:3417–3418.

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.

Santra, A., and S. A. Karim. 2000. Growth performance of faunated and defaunated Malpura weaner lambs. Anim. Feed Sci. Technol. 86:251–260.

Scalbert, A. 1991. Antimicrobial properties of tannins. Phytochemistry 30:3875–3883.

Sharp, R., C. J. Ziemer, M. D. Stern, and D. A. Stahl. 1998. Taxon-specific associations between protozoal and methanogens populations in the rumen and a model rumen system. FEMS Microbiol. Ecol. 26:71–78.

Sheffield, V. C., D. R. Cox, L. S. Lerman, and R. M. Myers. 1989. Attachment of a 40-base-pair G + C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc. Natl. Acad. Sci. USA 86:232–236.

Slyter, L. L., M. P. Bryant, and M. J. Wolin. 1966. Effect of pH on population and fermentation in a continuously cultured rumen ecosystem. Appl. Microbiol. 14:573–578.

Smith, A., and R. I. Mackie. 2004. Effect of condensed tannins on bacterial diversity and metabolic activity in the rat gastrointestinal tract. Appl. Environ. Microbiol. 70:1104–1115.

Smith, A., E. Zoetendal, and R. I. Mackie. 2005. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. Microb. Ecol. 50:197–205.

Tajima, K., T. Nagamine, H. Matsui, M. Nakamura, and R. I. Aminov. 2001. Phylogenetic analysis of archaeal 16S rRNA libraries from the rumen suggests the existence of a novel group of archaea not associated with known methanogens. FEMS Microbiol. Lett. 200:67–72.

Tan, H. Y., C. C Sieo, N. Abdullah, J. B. Liang, X. D. Huang, and Y. W. Ho. 2011. Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. Anim. Feed Sci. Technol. 169:185–193.

Tanner, G. L., A. E. Moore, and P. J. Larkin. 1994. Proanthocyanidins inhibit hydrolysis of leaf proteins by rumen microflora in vitro. Br. J. Nutr. 71:947–958.

Tamminga, S. 1996. A review on environmental impacts of nutritional strategies in ruminants. J. Anim. Sci. 74:3112–3124.

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. 123:403–409.

Teather, R. M. and F. D. Sauer. 1988. A naturally compartmented rumen simulation system for the continuous culture of rumen bacteria and protozoa. J. Dairy Sci. 71:666–673.

Terrill, T. H., A. M. Rowan, G. B. Douglas, and T. N. Barry. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric. 58:321–329.

Tymensen, L. D., and T. A. McAllister. 2012. Community structure analysis of methanogens associated with rumen protozoa reveals bias in universal archaeal primers. Appl. Environ. Microbiol. 78:4051–4056.

van Santen, E., and D. A. Sleper. 1996. Orchardgrass. Pages 503–534 in Cool-Season Forage Grasses, L.E. Moser, ed. Agron. Monogr. 34. ASA, CSSA, and SSSA, Madison, WI.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:473–481.

Waghorn, G. C., M. H. Tavendale, and D. R. Woodfield. 2002. Methanogenesis from forages fed to sheep. Proc. NZ Grassl. Assoc. 64:159–165.

Williams, C. M., J.-S. Eun, J. W. MacAdam, A. J. Young, V. Fellner, and B. R. Min. 2011. Effects of forage legumes containing condensed tannins on methane and ammonia production in continuous cultures of mixed ruminal microorganisms. Anim. Feed Sci. Technol.166:364–372.

Woodward, S. L., G. C. Waghorn, M. J. Ulyatt, and K. R. L. Lassey. 2001. Early indications that feeding Lotus will reduce methane emissions from ruminants. Proc. NZ Soc. Anim. Prod. 61:23–26.

**Table 5.1.** Nutrient composition of dietary treatments provided to continuous cultures

| | Diet | | | | | |
|---|---|---|---|---|---|---|
| | OGP | | | BFTP | | |
| Item, % DM | NS | GB | TMR | NS | GB | TMR |
| OM | 85.0 | 88.7 | 89.5 | 91.0 | 89.5 | 90.6 |
| CP | 11.5 | 11.9 | 16.3 | 20.6 | 18.3 | 16.9 |
| NDF | 56.5 | 44.8 | 42.9 | 29.8 | 25.6 | 37.3 |
| ADF | 31.6 | 24.2 | 25.3 | 23.3 | 18.6 | 25.8 |
| Ether extract | 3.91 | 3.40 | 3.15 | 2.42 | 2.31 | 2.35 |
| NFC[*] | 14.6 | 28.6 | 27.2 | 38.2 | 43.3 | 34.0 |
| Condensed tannins | 0.28 | 0.16 | 0.12 | 4.96 | 2.70 | 2.83 |

OGP–NS = orchardgrass (*Dactylis glomerata L.* as freeze-dried pasture and no supplement, OGP–GB = orchardgrass supplemented with ground barley grain (30%), OGP–TMR = orchardgrass supplemented with TMR (30%), BFTP–NS = birdsfoot trefoil (*Lotus corniculatus* L) as freeze-dried pasture and no supplement, BFTP–GB = birdsfoot trefoil supplemented with ground barley grain (30%) and BFTP–TMR = birdsfoot trefoil supplemented with TMR (30%).

[*] Non-fiber carbohydrates = 100 – CP – NDF – ether extract – ash.

**Table 5.2.** Species-specific primers sequences for 16S RNA used in this experiment

| Bacterium | Primer | Sequence (5´-3´) | Annealing temperature, °C | Reference* |
|---|---|---|---|---|
| Cellulolytic bacteria | | | | |
| *Ruminococcus albus* | Ra1281f | CCCTAAAAGCAGTCTTAGTTCG | 60.0 | 1, 2 |
| | Ra1439r | CCTCCTTGCGGTTAGAACA | | |
| *Ruminococcus flavefaciens* | Rf154f | TCTGGAAACGGATGGTA | 60.0 | 1, 2 |
| | Rf425r | CCTTTAAGACAGGAGTTTACAA | | |
| *Fibrobacter succinogenes* | Fs219f | GGTATGGGATGAGCTTGC | 62.0 | 1, 2 |
| | Fs654r | GCCTGCCCCTGAACTATC | | |
| Methanogens | | | | |
| *Methanobrevibacter* spp. | NestMbbF | TGGGAATTGCTGGWGATACTRTT | 65.3 | 3 |
| | NestMbbR | GGAGCRGCTCAAAGCCA | | |
| *Methanomicrobium* spp. | NestMmF | GTTTAAAACACATGGGAAGA | 59.5 | 3 |
| | NestMmR | ATTCCCAGTATCTCTTAGACGC | | |

*1 = Koike and Kobayachi (2001); 2 = Tajima et al., (2001); 3 = Tymensen and McAllister (2012).

**Table 5.3.** Ruminal fermentation characteristics in continuous cultures receiving grazing lactating cow diets of grass or birdsfoot trefoil forages unsupplemented or supplemented with ground barley or TMR

| | Diet | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OGP | | | BFTP | | | | Significance of effect[*] | | |
| Item | NS | GB | TMR | NS | GB | TMR | SEM | PF | SUP | INT |
| Culture pH | 6.23 | 6.11 | 6.01 | 6.08 | 6.19 | 6.22 | 0.085 | 0.53 | 0.77 | 0.07 |
| $NH_3$-N, mg/100 ml | 14.4 | 16.7 | 14.5 | 9.17 | 10.4 | 8.17 | 2.60 | <0.01 | 0.14 | 0.87 |
| Total VFA, mM | 41.2 | 44.1 | 45.7 | 35.9 | 41.6 | 43.1 | 3.54 | 0.17 | 0.22 | 0.85 |
| Individual VFA, mol/100 mol | | | | | | | | | | |
| Acetate (A) | $70.6^{a}$ | $62.3^{b}$ | $60.0^{c}$ | $73.1^{a}$ | $67.8^{b}$ | $66.8^{b}$ | 1.79 | <0.01 | <0.01 | 0.01 |
| Propionate (P) | 19.2 | 20.5 | 22.1 | 19.2 | 20.4 | 21.9 | 1.06 | 0.80 | <0.01 | 0.99 |
| Butyrate | $7.13^{b}$ | $12.1^{a}$ | $13.0^{a}$ | $5.50^{b}$ | $8.70^{a}$ | $8.03^{a}$ | 1.075 | <0.01 | <0.01 | 0.04 |
| Valerate | 1.13 | 1.55 | 2.03 | 0.78 | 1.24 | 1.68 | 0.269 | 0.02 | <0.01 | 0.99 |
| Isobutyrate | 0.57 | 0.53 | 0.59 | 0.41 | 0.29 | 0.21 | 0.194 | 0.02 | 0.76 | 0.76 |
| Isovalerate | 1.08 | 2.59 | 2.19 | 0.94 | 1.84 | 1.44 | 0.498 | 0.02 | <0.01 | 0.41 |
| A:P | 3.78 | 3.00 | 2.69 | 3.94 | 3.27 | 3.13 | 0.228 | <0.01 | <0.01 | 0.30 |

OGP–NS = freeze-dried orchardgrass (*Dactylis glomerata L*) and no supplement, OGP–GB = orchardgrass supplemented with ground barley grain (30%), OGP–TMR = orchardgrass supplemented with TMR (30%), BFTP–NS = freeze-dried birdsfoot trefoil pasture and no supplement, BFTP–GB = birdsfoot trefoil (*Lotus corniculatus* L) supplemented with ground barley grain (30%) and BFTP–TMR = birdsfoot trefoil supplemented with TMR (30%).

Means in the same row within OG and BFT subgroups with different superscripts differ ($p < 0.05$).

[*] $CH_4$ = methane and $NH_3$-N = ammonia-N.

[†] PF = effect of forage (OGP vs. BFTP), SUP = effect of supplementation and INT = interaction between PF and SUP.

**Table 5.4.** Densitometric quantification (% intensity) of rumen bacteria and archaeal methanogen diversity in continuous cultures receiving pasture forages without or with barley or dairy TMR supplementation

| | Diet | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OGP | | | BFTP | | | | Significance of effect* | | |
| Item | NS | GB | TMR | NS | GB | TMR | SEM | PF | SUP | INT |
| Cellulolytic bacteria | | | | | | | | | | |
| *Ruminococcus albus* | 6.18 | 5.91 | 5.82 | 8.89$^a$ | 5.48$^b$ | 3.01$^c$ | 0.739 | 0.90 | 0.06 | 0.06 |
| *Fibrobacter succinogenes* | 6.21$^b$ | 8.58$^a$ | 6.49$^b$ | 6.10$^a$ | 4.51$^b$ | 1.87$^c$ | 0.481 | <0.01 | <0.01 | <0.01 |
| *Ruminococcus flavefaciens* | 9.78$^a$ | 9.62$^a$ | 8.11$^b$ | 2.92$^a$ | 2.03$^b$ | 1.02$^b$ | 0.432 | <0.01 | <0.01 | <0.01 |
| Methanogens | | | | | | | | | | |
| *Methanobrevibacter* spp. | 8.07 | 7.81 | 5.53 | 4.92 | 4.26 | 3.87 | 0.729 | 0.02 | 0.20 | 0.20 |
| *Methanomicrobium* spp. | 4.51 | 5.37 | 3.63 | 3.89 | 3.50 | 3.32 | 0.450 | 0.18 | 0.52 | 0.52 |

OGP–NS = orchardgrass (*Dactylis glomerata L*) forage and no supplement, OGP–GB = orchardgrass supplemented with ground barley grain (30%), OGP–TMR = orchardgrass supplemented with TMR (30%), BFT–NS = birdsfoot trefoil foragae (*Lotus corniculatus* L) and no supplement; BFTP–GB = birdsfoot trefoil supplemented with ground barley grain (30%) and BFTP–TMR = birdsfoot trefoil supplemented with TMR (30%).

Means in the same row within OGP and BFTP subgroups with different superscripts differ ($P < 0.05$).

*F = effect of forage (OG vs. BFT), SUP = effect of supplementation and INT = interaction between F and SUP

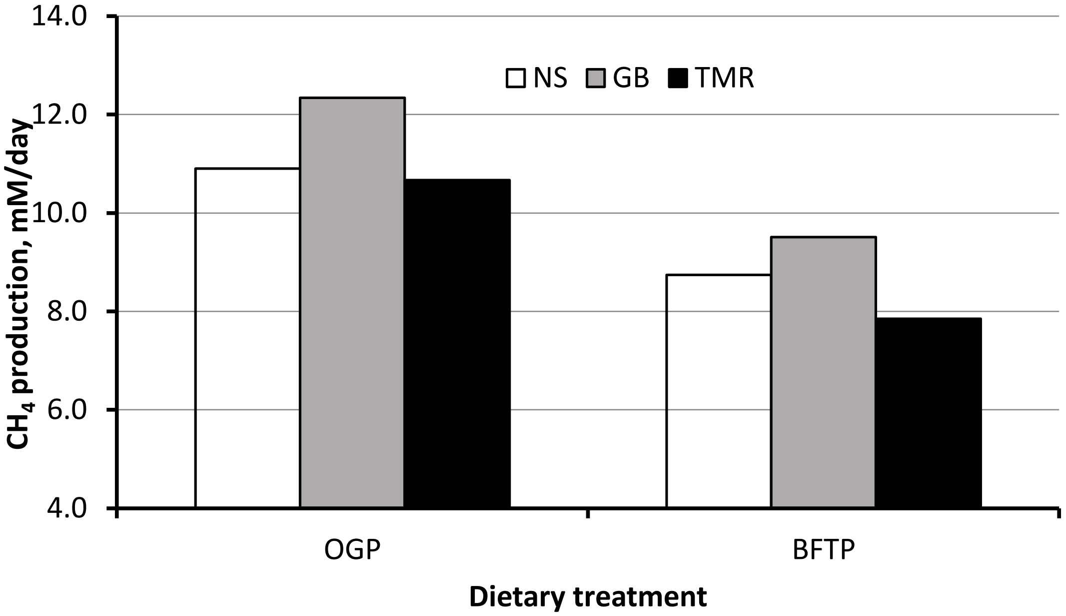

**Fig.5.1.** Methane ($CH_4$) production as affected by pasture forage type and energy supplementation in continuous cultures. OGP–NS = orchardgrass (*Dactylis glomerata L*) forage and no supplement; OGP–GB = orchardgrass forage supplemented with ground barley grain (30%); OGP–TMR = orchardgrass forage supplemented with TMR (30%); BFTP–NS = birdsfoot trefoil (*Lotus corniculatus* L) forage and no supplement; BFTP–GB = birdsfoot trefoil supplemented with ground barley grain (30%); BFTP–TMR = birdsfoot trefoil supplemented with TMR (30%).

Effects of pasture forage, supplementation, and interaction between forage and supplementation were $P < 0.01$, $P = 0.34$ and $P = 0.95$, respectively, with the SEM of 1.50.

# CHAPTER 6

# CONCLUSION

In the United States, legume forages such as alfalfa and white clover are an important digestible fiber- and N- providing component of the diet for lactating cows. The economic sustainability of dairy systems depends strongly on the quality and quantity of home-grown hay and pasture, but their environmental sustainability depends on reduction of wastes from dairy cows consuming high-forage diets. Forage quality affects feed intake and feed energy density, lactational performance, cow health, and feed cost. The protein in high quality hay or pasture forage degrades quickly in the rumen, and when consumed by ruminants, most in excess of available energy sources is converted to ammonia, resulting in excessive excretion of nitrogenous waste. This process results in inefficient utilization of dietary nitrogen (N) in the rumen and limits optimal microbial protein synthesis. It also increases metabolic energy cost to convert ruminal ammonia-N ($NH_3$-N) to urea and increases N waste excretion into the environment. Therefore, opportunities to improve N utilization would be found in either reducing the amount of N fermented to $NH_3$-N in the rumen, reducing excess dietary N consumed by dairy cows, incorporating more dietary N into milk protein, or shifting N excretion from urine to less-volatile fecal forms of excretion.

Due to the extensive use of high- quality legume and grass forages in dairy diets, both as a freshly grazed forage and as preserved hay and silage, research to improve utilization of the protein in these forages is warranted. Novel approaches for reducing the amount of N waste generated by dairy cows or incorporating more dietary N into milk protein are opportunities to improve N utilization. In addition to N waste, methane ($CH_4$)

is also of concern. From 3 to 9 % of energy from feed is lost through ruminal $CH_4$ production, so reducing enteric $CH_4$ emissions can also improve sustainability of ruminant production.

Three research projects described in this dissertation address the improvement of the sustainability of dairy systems through the use of birdsfoot trefoil (*Lotus corniculatus* L.; **BFT**) in the diets of lactating cows. Birdsfoot trefoil is a non-bloating forage legume that is similar to alfalfa in feeding value, and has the potential to be fed as the forage component of dairy cow diets. It has cell wall digestion characteristics unique from alfalfa and contains condensed tannins (**CT**). Condensed tannins have been shown to reduce the degradation of plant protein by complexing with the protein in the rumen to prevent fermentation. In addition, research has shown an inhibitory effect of CT on ruminal methanogenesis, which is due in part to direct effects on methanogenic archaea, protozoa-associated $CH_4$ production, or indirect effects through a depression of fiber digestion in the rumen.

The hypothesis of the first study was that replacing alfalfa hay partially or completely with birdsfoot trefoil hay (**BFTH**) in high forage diets would improve lactational performance of cows. It was found that BFT hay diets had decreased NDF and ADF intake, tended to increase milk yield, and increased ECM efficiency compared to diets based on AH. In addition, cows fed with BFTH had improved milk N efficiency and microbial protein yield over cows fed AH diets. Improved digestibility of fiber in BFTH due to potentially more digestible cell wall structure of BFTH, supported increased milk N efficiency and ECM yield through greater milk protein concentration compared to cows fed the alfalfa hay diet. Results of this study suggest that dairies feeding BFT preserved as hay may see production advantages when including BFTH in high forage diets. Improving sustainability by utilizing high forage diets that include forages with increased digestibility of NDF and improved N utilization efficiency could be met when BFTH is fed.

In the second study of this dissertation, the use of BFT pasture (**BFTP**) for a sole forage source in grazing commercial dairy farms was explored. It was found that the BFT pasture can have advantages over traditional grass pasture, depending on the management and growing conditions that affect pasture quality. Lactating cows grazing BFT had greater milk yield and ECM yield for most sample weeks, but had reduced ruminal $NH_3$–N only some sample weeks, and reduction in MUN for BFTP cows in some but not all sample weeks during this two year study. It was found that decreases in MUN and ruminal $NH_3$–N in BFTP cows could not be attributed to reduction of rumen degradable protein due to CT. We conclude that CT at low concentrations in BFTP are ineffective at consistently reducing environmental losses of N through MUN or ruminal $NH_3$–N, as improvements occurred only when CT concentrations in the BFT forage were greater than 1% DM and forage CP was less than 18% DM. Advantages of reduced NDF and increased NFC concentration of the BFTP likely contributed to increased intake which could have led to the improvements in milk production detected in this study more than CT contribution to improved milk production. If BFTP were to be adopted on a system-wide level, management of the pasture forage maturity at time of grazing is critical in order to provide the best quality forage base through a moderate NDF concentration and highest concentration of tannins in order to increase sustainability.

In the third study, response of *in vitro* rumen microbial ecosystems to feeding CT-containing BFT diets was assessed, in comparison with non-CT-containing orchardgrass (**OG**) diets, as well as 2 different types of supplementation that is common to grazing dairies. Concentration of $NH_3$-N decreased when cultures were offered BFT-based diets compared with OG-based diets, while supplementing ground barley (**GB**) or TMR did not affect $NH_3$-N concentration regardless of source of forage. Feeding BFT-based diets increased the molar proportion of acetate compared with OG-based diets, whereas molar proportion of propionate was similar between OG- and BFT-based diets, which affected the acetate-to-propionate ratio (A:P). The CT in BFT exerted anti-microbial properties against cellulolytic bacteria and methanogens, but those effects depended on the species of interest. Microbial populations of *F. succinogenes* and *R. flavefaciens* decreased when cultures were offered BFT-based diets compared with OG-based diets, while *R. albus* was not affected by type of pasture forage. Adding supplements in general decreased the cellulolytic bacteria, but the decreases were greater in the BFT-based diets compared with OG-based diets, resulting in interactions between type of forage and supplements. *Methanobrevibacter* spp. decreased due to feeding BFT-based diets, but *Methanomicrobium* spp. was not affected, likely due to the former being associated with protozoa, which may have been affected by CT toxicity. In addition, the CT of BFT sizably reduced $NH_3$-N concentration with its toxic effect on protozoal population. Extrapolating these results to *in vivo* conditions is challenging, as the overall rumen microbial ecosystem is likely feed intake-dependent, which determines the actual amount of CT consumed by animals. Consideration of the dynamic effects of CT on the rumen microbial ecosystem with target diets (i.e., high- concentrate or high-forage) and farming systems (i.e., farms where cows are pasture- grazed vs cows conventionally fed preserved forages) is a direction that future research could address.

It can be surmised from the results of these three studies utilizing BFT in dairy diets that effects of CT from BFT grown in the western U.S. are highly dependent on diet concentration, which is supported in the literature, and that the CP concentration of the diet or forage being consumed has an overarching effect on N utilization efficiency. Improvements in milk yield and ECM yield were evident in the first two studies, but cannot be said to be due only to CT action, as MUN and ruminal $NH_3$-N were largely unaffected by the presence of CT in the rumen. This suggests that a critical concentration of CT in the rumen must be reached before a reduction in protein degradation can occur, and likely some type of interaction with diet protein type, CP concentration, and possibly energy substrates in the diet needs to be considered. Though not directly addressed in these studies, the unique type of cell wall structure and nutrient constituents such as reduced NDF and increased NFC in BFT compared to other forages appeared to affect rate and extent of digestion, which in turn affected intake and nutrient extraction from the diet. These factors were likely greater contributors to the improvement of lactational performance noted in these studies than an effect due to CT. The third study utilized varieties of BFT that were greater in CT concentration compared to the first two studies. The third study showed expected ruminal $NH_3$-N reduction due to CT and revealed that specific methanogenic, cellulolytic and proteolytic bacteria are inhibited by CT while others are not. This effect was measured for only a few species of rumen bacteria, and could have had similar effects on other types of rumen microbes, which could exert overall effects on rumen energy and N use efficiency. It can be suggested that rumen CT activity, which depends on DMI, CT concentration, and CT chemical structure of the CT-containing forage being fed will all affect overall N utilization. Further studies *in vivo* are needed, particularly with varieties of BFT that consistently contain more than 1.5% CT. It was evident that the decreased $NH_3$-N concentration due to feeding BFT-based diets in the third study was a result of CT in BFT, and its effect was not diluted despite reduced concentration of CT in BFTP diets with GB or TMR supplementation.

In terms of improving sustainability, BFT increased milk yield and components in the first and second study, which could have improved the economic sustainability of those cows consuming BFT. Since N waste (in terms of rumen $NH_3$-N and MUN reduction) was numerically reduced in study 1 for the BFTH- containing diets and affected by forage CT concentration due to sample week in study 2, (reduced ruminal ammonia was evident when CT was increased above about 1% DM), it is likely that CT diet concentration needs to be at least 1% to see N waste reduction in order to improve environmental sustainability. Reduction in rumen $NH_3$-N was found in study 3 for diets with CT concentrations above 1% (2.7 and 2.83% DM for BFT- GB and BFT-TMR, respectively) even with supplementation compared to the non-CT forage. Thus, the reduction in the $NH_3$-N concentration through CT in higher CT-containing BFT can contribute to improving utilization of dietary N in ruminal fermentation and reducing N excretion.

These studies collectively show that BFT included as a major forage in dairy diets of either conventional hay-based TMR or as a pasture forage can improve lactational performance of dairy cows. The improvement of N utilization efficiency, however, was only evident in some of the study results. Milk N:manure N was only slightly improved in study 1 due to feeding BFTH. Measures of N utilization in study 2 could only be based on milk production and MUN data, as intake data were not possible on an organic dairy due to organic constraints. Milk Urea N seemed to correlate with CP concentration of the pasture forages, and when pasture was lush and in a vegetative state (most of year 2) the MUN was increased for BFTP cows compared to MGP cows. Milk protein concentration was for the most part similar between treatments, and protein yields only fluctuated with the overall milk yield according to sample week, and seemed unconnected with the CT concentration of the BFTP. I cannot conclude that CT in BFT forage always improved N utilization- in these presented cases, it appears to depend on forage quality and CT concentration of the forage, and it was probably influenced by overall diet CP concentration, as well as chemical activity of the CT in the forages, which was not measured.

Sustainability goals can be supported utilizing BFT, however, more research for *in vivo*, commercial dairy, and different BFT varieties is necessary to see how CT in BFT as well as the unique cell wall characteristics affect the interaction of diet components, and microbes in the rumen. Forages containing CT are acceptable for the dairy industry and offer much to enhance sustainability in ruminants despite many unknowns relative to the use of BFT in lactation dairy diets.

# APPENDIX

**Table A.1**. Least squares mean fatty acid composition of the milk from lactating cows grazing grass or birdsfoot trefoil pasture during 2 yr grazing study.

| Year 2012 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid | Week 2 | | Week 6 | | | Significance of effect | | |
| | Grass | BFT | Grass | BFT | SEM | week | treatment | W × T |
| C16:0 | 24.75 | 30.47 | 25.18 | 28.76 | 0.714 | <0.0001 | 0.0002 | 0.9802 |
| C18:0 | 8.21 | 6.90 | 8.29 | 6.62 | 0.358 | <0.0001 | <0.0001 | <0.0001 |
| trans C18:1 | 0.07 | 0.791 | 0 | 0 | 0.198 | 0.008 | 0.0032 | 0.0004 |
| cis C18:1 | 17.49 | 13.99 | 20.89 | 16.55 | 0.868 | <0.0001 | <0.0001 | 0.0749 |
| cis-trans | 3.45 | 2.49 | 2.76 | 2.56 | 0.267 | <0.0001 | 0.0240 | 0.3332 |
| C18:2, n-6 | 2.76 | 2.76 | 3.01 | 2.72 | 0.137 | 0.438 | 0.0001 | 0.045 |
| C18:3, n-3 | 1.25 | 1.95 | 1.33 | 1.69 | 0.081 | 0.267 | 0.0001 | 0.0653 |
| cis 9, t 11 CLA | 0.08 | 0.06 | 0.47 | 0 | 0.089 | 0.023 | 0.1638 | 0.112 |
| trans 10, cis 12 CLA | 0.02 | 0.10 | 0.08 | 0 | 0.019 | 0.0773 | 0.7831 | 0.0047 |
| Others | 9.90 | 10.7 | 10.51 | 9.04 | 0.163 | 0.0051 | <0.0001 | 0.3681 |
| MUFA | 24.68 | 21.84 | 27.49 | 23.30 | 0.958 | 0.0029 | 0.0002 | 0.856 |
| PUFA | 17.12 | 12.84 | 14.24 | 16.51 | 0.649 | <0.0001 | 0.177 | 0.0394 |
| SFA | 56.84 | 64.91 | 56.54 | 59.96 | 0.927 | 0.298 | <0.0001 | 0.256 |
| PUFA:SFA | 0.302 | 0.198 | 0.253 | 0.277 | 0.013 | <0.0001 | 0.9248 | 0.0523 |

| Year 2013 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Week 2 | | Week 6 | | | Significance of effect | | |
| Fatty acid | Grass | BFT | Grass | BFT | SEM | week | treatment | W × T |
| C16:0 | 24.41 | 23.87 | 28.57 | 30.14 | 0.714 | <0.0001 | 0.0002 | 0.9802 |
| C18:0 | 7.30 | 3.43 | 8.06 | 7.81 | 0.358 | <0.0001 | <0.0001 | <0.0001 |
| trans C18:1 | 0.07 | 1.09 | 0 | 0 | 0.198 | 0.008 | 0.0032 | 0.0004 |
| cis C18:1 | 18.07 | 11.44 | 19.6 | 18.11 | 0.868 | <0.0001 | <0.0001 | 0.0749 |
| cis-trans | 3.38 | 5.22 | 2.03 | 2.56 | 0.267 | <0.0001 | 0.0240 | 0.3332 |
| C18:2, n-6 | $1.79^a$ | $3.07^b$ | $2.10^a$ | $2.86^b$ | 0.137 | 0.438 | 0.0001 | 0.045 |
| C18:3,n-3 | $1.18^a$ | $1.95^b$ | $0.78^c$ | $1.69^d$ | 0.081 | 0.267 | 0.0001 | 0.0653 |
| c 9, t 11 CLA | 1.27 | 1.33 | 0.79 | 0.87 | 0.089 | 0.023 | 0.1638 | 0.112 |
| t 10, c 12 CLA | 0.00 | 0.00 | 0.05 | 0.07 | 0.019 | 0.0773 | 0.783 | 0.0047 |
| MUFA | 24.63 | 24.33 | 25.86 | 24.41 | 0.958 | 0.0029 | 0.0002 | 0.856 |
| PUFA | 16.26 | 20.30 | 11.72 | 12.56 | 0.649 | <0.0001 | 0.177 | 0.0394 |
| SFA | 57.55 | 58.25 | 60.04 | 62.17 | 0.927 | 0.298 | <0.0001 | 0.256 |
| PUFA:SFA | 0.283 | 0.351 | 0.203 | 0.196 | 0.013 | <0.0001 | 0.9248 | 0.0523 |

# VITA

## Rachael G. Christensen

---

90 North Main Street crcfarm@gmail.com
Lewiston, UT 84320 (435) 881-0017

**Positions Held:**

- Christensen Research and Consulting Services (Lewiston, UT): May, 2009 – current
  - Dairy nutrition consultation, nutrient management, pasture and forage management, and records evaluation
  - Owner and sole proprietor of business
  - Distribute Renaissance Nutrition products and services
- Intermountain Farmers Association (Lewiston, UT): 2004 – 2009
  Dairy feed sales and nutrition consulting
- Cargill Animal Nutrition (Rupert, ID and Ogden, UT): 2001 – 2004
  Dairy Solutions Consultant
- Brigham Young University Dairy (Spanish Fork, UT: 1998 – 1999)
  Assistant Dairy Herdsman

**Skills:**

- Excellent oral and written skills
- Effectively present technical information to wide variety of audiences- academic, industry, community
- On-farm communication with dairy owners/operators
- Dairy nutrition software proficiency, CPM, AMTS, Feed Mill Manager, Excel
- Statistical analysis, SAS 9.3 proficiency, Excel
- Sustainability surveys of dairy farms
- Word, Excel, PowerPoint proficiency
- Proximate analysis of feeds and forages, lab analysis and interpretation of feed samples

**Education:**

- PhD, Utah State University, January 2012 – May 2015;
  Major emphasis: Animal Nutrition; Major Professor: Dr. Jong-Su Eun
  - Projects: Evaluate effects of feeding birdsfoot trefoil fed to lactating dairy cows
  - Increase nutrient utilization efficacy, reduce waste on target farms
  - Apply research to improve sustainability of organic dairy farms
  - Evaluate management and feeding practices to increase sustainability of farms

- Evaluate use of alternative forages (birdsfoot trefoil) to replace traditional forages in lactation dairy rations and subsequent sustainability improvements.

- MS: Utah State University, 2002;
  Major emphasis: Dairy Management; Major Professor: Dr. Allen Young
  - ➢ Project: Using DHIA records and MUN values to evaluate reproductive measures of dairy cows in Intermountain Region

- BS: Brigham Young University, 1999
  Major: Animal Science (Cellular Physiology and Genetics);
  Honors research advisor: Dr. Roy Silcox

**Teaching Experience:**

- ADVS 3500 Animal Nutrition and Metabolism, Laboratory Section, Utah State University, Logan UT Campus, 2012- 2014
  - ➢ Plan curriculum, prepare lectures, proctor tests and quizzes, and teach principles of livestock ration balancing. Plan laboratory activities, sample collection and processing, and feed sample identification
- ADVS 2050 Dairy Herdsman Nutrition and Ration Balancing Utah State University, Logan UT Campus, 2005- 2010
  - ➢ Plan laboratory activities, sample collection and processing, and feed sample identification, forage analysis, rumen physiology

**Membership and Leadership:**

- Member, American Registry of Professional Animal Scientists, Dairy Specialist, 2010 – current
- Women's Committee Chairperson, Board of Directors, Cache County Farm Bureau Federation, 2005 – 2012
- President, Cache County Dairy Women, 2007 – 2008

**Publications:**

**Johnson, R. G.**, and A. J. Young. 2003. The association between milk urea nitrogen and DHI production variables in western commercial dairy herds. J. Dairy Sci. 86:3008–3015.

**Johnson, R. G.** 2001. The relationship of milk urea nitrogen and DHIA production variables in western commercial dairy herds. M.S. Thesis. Utah State Univ., Logan