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Mohammad Ghelich Khan

Utah State University

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NITROGEN EXCRETION OF LACTATING DAIRY COWS FED AN ALFALFA HAY- OR BIRDSFOOT TREFOIL HAY-BASED HIGH-FORAGE DIET

by

Mohammad Ghelich Khan

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

of

MASTER OF SCIENCE

in

Animal, Dairy, and Veterinary Sciences

Approved:

Jong-Su Eun, PhD Major Professor	Allen J. Young, PhD Committee Member
Jennifer W. MacAdam, PhD Committee Member	Mark R. McLellan, PhD Vice President for Research and Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2017

ABSTRACT

Nitrogen Excretion of Lactating Dairy Cows Fed an Alfalfa Hay- or Birdsfoot Trefoil Hay-Based High-Forage Diet

by

Mohammad Ghelich Khan

Utah State University, 2017

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

Extensive research has been conducted to decrease the environmental impacts of dairy farming by using forages containing condensed tannins (CT). In this study, it was hypothesized that feeding CT-containing birdsfoot trefoil (Lotus corniculatus, BFT) to lactating dairy cows would result in a decrease in N degradation in the rumen, causing a shift in N partitioning into milk and manure outputs, compared with alfalfa hay. Urine N is more volatile and harmful to the environment compared with fecal N. By reducing urine N, overall N utilization efficiency can improve. It was our goal to verify how the changes in N partitioning would affect the overall N utilization efficiency by dairy cows fed BFT hay-based high-forage diet. The results in this report showed that feeding BFT-hay diet reduced protein degradation in the rumen, decreased N excreted to

milk and urine, and increased N excretion into feces, resulting in decreased urinary-N:fecal-N ratio due to feeding BFT-hay diet. However, the change in the N excretion routes was not associated with N utilization efficiency, which may have resulted from poor nutritive quality of BFT hay. Although the BFT hay fed in the current study was in a very mature condition and was of poor quality, DM intake and milk yield were similar in both treatments. Therefore, it is implied that BFT hay can replace alfalfa hay in dairy rations, because even poor quality BFT hay compared with alfalfa hay led to similar lactational performance and a beneficial shift in N excretion into environment.

(108 pages)

PUBLIC ABSTRACT

Nitrogen Excretion of Lactating Dairy Cows Fed an Alfalfa Hay- or Birdsfoot Trefoil Hay-Based High-Forage Diet

Mohammad Ghelich Khan

Legumes that contain condensed tannins (CT) may have lower protein degradability than alfalfa. The present study investigated the effects of feeding birdsfoot trefoil (Lotus corniculatus L.) hay on lactation performance and N utilization. Eight multiparous Holstein cows in mid lactation (150 ± 22.3 d-in-milk) were randomly assigned to 1 of 2 rations [alfalfa hay-based TMR (AHT) or birdsfoot trefoil hay-based TMR (BFTT)] in a crossover design with 2 experimental periods. Each experimental period lasted 16 d (14 d of adaptation and 2 d of total collection), and the 2 experimental periods were separated by a 7-d washout period. In the experimental diets, alfalfa hay or birdsfoot trefoil hay was included at 50% DM to AHT or BFTT, respectively. There were no treatment effects on DMI (21.4 vs. 20.7 kg/d; $P = 0.46$), milk yield (29.4 vs. 28.1 kg/d; $P = 0.47$), milk fat concentration (3.20 vs. 3.21%; $P = 0.67$), and milk protein concentration (3.20 vs. 3.16%; $P = 0.35$) for AHT and BFTT, respectively. In addition, dietary treatments did not affect milk yield/DMI ($P = 0.59$) and energy-corrected milk yield/DMI ($P = 0.49$). In contrast, CP digestibility decreased in BFTT compared with AHT (69.1 vs. 60.7%; $P < 0.01$). Concentration of milk urea-N decreased by feeding BFTT compared with feeding AHT

(11.9 vs. 13.3 mg/100 mL; $P < 0.01$), whereas total N excretion was similar ($P = 0.82$) between the diets. However, cows fed BFTT excreted more N in feces (194 vs. 168 g/d; $P < 0.01$), in contrast urinary N excretion decreased in BFTT cows compared to AH treatment ($P < 0.01$), leading to a decrease in urinary-N:fecal-N ratio ($P = 0.03$) in cows fed BFTT relative to those fed AHT. Overall results in the current study suggest that feeding BFT hay in dairy diet with a relatively great concentration (50% DM) did not affect overall lactational performance, whereas it shifted routes of N excretion evidenced by the decreased urinary-N:fecal-N ratio compared with feeding alfalfa hay. The positive impact on environment may be attributed to a functional effect of CT as well as a unique cell wall structure of birdsfoot trefoil.

Key Words: alfalfa hay, birdsfoot trefoil hay, lactating dairy cow, nitrogen excretion, total collection

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LIST OF ABBREVIATIONS

A = acetate

A:P = acetate to propionate ratio

AA = amino acid

ADF = acid detergent fiber

AH = alfalfa hay

AHT = alfalfa hay TMR

AIA = acid-insoluble ash

BFT = birdsfoot trefoil

BFTT = birdsfoot trefoil TMR

BUN = blood urea nitrogen

BW = body weight

CH4 = methane

CP = crude protein

DM = dry matter

DMI = dry matter intake

EAA = essential amino acids

ECM = energy corrected milk

FCM = fat corrected milk

ha = hectare

MCP = microbial crude protein

ME = metabolizable energy

MJ = megajoules

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

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

MP = metabolizable protein

MUN = milk urea nitrogen

CT = condensed tannin

MY = milk yield

N = nitrogen

NDF = neutral detergent fiber

NEL = net energy lactation

NFC = non fiber carbohydrates

NH3-N = ammonia nitrogen

NRC = national research council

OM = organic matter

P = propionate

RDP = ruminal degradable protein

RUP = ruminal undegradable protein

SF = sainfoin

TMR = total mixed ration

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

UUN = urinary urea nitrogen

VFA = volatile fatty acids

INTRODUCTION

By definition, sustainability is “meeting society’s present needs without compromising the ability of future generations to meet their own needs”. The three contributing pillars of sustainability are environmental responsibility, economic viability and social acceptability (USDA-EPA, 2010). This has increased public awareness and investigation of sustainability of animal production agriculture.

Many studies have shown that production agriculture is a major contributing source of environmental pollution and releases excess nutrients to the air and water. In the United States, Asia, and Europe animal agricultural production systems are receiving increased public attention as a major contributor to pollution affecting the quality of air, streams, and groundwater resources (Wang et al. 2010; von Keyserlingk et al. 2013)

Among all, N and P are the two nutrients which have caused greater concerns due to their effects on water and air quality and eutrophication. Consequently, nutrient management research has been conducted to discover strategies that might decrease N and P pollution (USDA-EPA 2012).

Alfalfa (Medicago sativa: AF) and birdsfoot trefoil (Lotus corniculatus L.: BFT) are legume forages that are valuable sources of crude and true protein for animals. However, the protein available in alfalfa is quickly degradable in rumen and silo which causes inefficient use of N by ruminants. Crude protein concentration affects profitability, productivity and N utilization efficiency. In maintenance conditions, which means no weight gain or any growth, all N fed to the cows should be excreted from the body unless it completely converts to milk N. The efficiency of feed N conversion to milk N rarely exceeds 30% which means 70% of feed N generally leaves the body, 30% of which is through feces and the remaining through urine often in the form of urea (VandeHaar and St-Pierre 2006). Obtaining efficient use of N for protein production goals such as milk and meat is difficult. Improved sustainability has been achieved through feeding lower CP levels. Nonetheless, very low protein feed decreases production in early lactation and high producing dairy cows. Protein feeding advances and detailed investigation of diet amino acids (AA) may improve efficiency via decreasing diet CP while providing essential amino acids to the animals (NRC 2001).

Feeding low amounts of protein can substantially reduce urinary N excretion and increase N utilization efficiency. Over-fed N is deaminated and excreted as urea in milk and urine, whereas metabolic N (which includes hind gut fermentation products, sloughed intestinal cells, and ruminal undegradable protein which is indigestible) can be excreted via feces (VandeHaar and St-Pierre 2006). Excreted N amount and pathway is one of the most critical environmental concerns; urinary N is more volatile than fecal N and can quickly convert into ammonia. Simply, a decrease in diet protein can have negative effects on productivity if the diet is not well-balanced. Nutrient management is the accounting of excretion and intake of nutrients.

In consideration of the relationship among crop production, manure management, animal nutrition, soil conservation, and economic results, nutrient management is an intricate issue. Sustainability will not be achieved unless economic viability is obtained. Many researchers believe that local farm- grown forage in the form of harvested or fresh pasture is the best way of utilizing resources to decrease environmental risks resulting from purchasing feed for the farm (Kohn et al. 1997; Jonker et al. 2002; Rotz 2004).

The most common forages in US dairy farms are corn silage and alfalfa (as hay and silage), grass hay and grass silage, respectively. Many efforts such as forage agronomic management, utilizing better facilities for harvesting forages and plant breeding have been made to improve forage quality. On the other hand, some other aspects of plants like lignin concentration, and cell-wall composition which influence degradability and digestibility in ruminants require further studies. There are other physiological parameters of forages as well which need to be considered. These include high degradable protein amount of alfalfa and high levels of methane production resulting from rations with high forage portion which can each lead to a reduced efficiency.

BFT is a forage which contains polyphenolic compounds with a high bioactivity that increases productivity (Patra and Saxena 2011). Condensed tannins (CT) available in BFT can attach to soluble proteins in the rumen and form CT-protein complexes, move to the small intestine and release the protein there to be absorbed (Waghorn 2008) which causes a reduction in protein degradation to NH3-N (Weiss et al. 2009). Grabber et al. (2001) showed that rations containing moderate levels of CT, around 2-4%, decrease

proteolysis in rumen and silage up to 50%. The characteristic of CT-protein complex provides a natural alternative bioactive compound which assists in the reduction of N waste in dairy.

Moreover, BFT is characterized as non-bloating which enables farmers to use it as a pure pasture. It is most useful to them in increasing fresh forage feeding in a fast growing season.

Total collection is process that measures all feed and excreted nutrients in animal nutrition studies. In the current total collection study, we tried to collect all excreted nutrients from dairy cows to have a reliable data set to compare BFT and alfalfa hay. The overall hypothesis in this dissertation was that feeding BFT hay to lactating dairy cows would change N excretion pathway from urine toward feces and that CT in BFT-containing diets would reduce milk urea N and urinary N excretion compared to alfalfa hay.

REVIEW OF LITERATURE

Decreasing nitrogen (N) excretion to environment is one of the most important public concerns in this era, as N is very harmful for the environment. Indeed, as N, which derives from protein is a very expensive nutrient of dairy cow's rations, its amounts and complications need to be reconsidered precisely. However, for farmers, production and profitability are more important than N environmental impacts. Therefore, the purpose of this review is to update recent findings on N nutrition with its focus on forage nutrition and its influence on lactational performance and N excretion to environment.

Sustainable Dairy Production

Understanding Sustainability

Improvements in agriculture have been massive, in consideration that before agriculture, hunting (wild animals) and fishing supplied food for four million people on the earth, but modern agriculture nourishes six billion people. Many of these increases in increments have resulted from water management and use of pesticides, fertilizers, new crop cultivars, and technologies in agriculture (FAO 2010). Food supply per capita has increased the human lifespan, reduced hunger and improved human nutrition. By the year 2050, earth's population will be 50% more than today, and grain requirements are projected to be doubled (Cassman 1999). Simultaneously, per capita income will be 2.4 times what it is now, which means demand for animal products will increase. Therefore, more agricultural output will be necessary to meet the demands for upcoming years, and thus more pressure on agricultural lands and farms are predicted to occur. Among all these changes, it will be important to consider effects on environmental integrity and sustainability as the increased population will also put strains on water, natural resource and utility use (Tilman et al. 2002).

In the animal production area, the dairy industry has experienced a vast amount of improvements from 1940s (Martin and Mitra 2001). The number of dairy farms and cows has decreased, but size of herds have increased, and now 63% of the milk production in US comes from farms which have more than 500 milking cows (USDA-NASS 2012). This evolution happened despite the increase in urban sprawl and reduction in farmland. Consequently, more concerns have arisen regarding dairy industry sustainability by society and environmental advocacy groups. Considering these concerns, many scientists in different areas (nutrient management, animal welfare, emission of greenhouse gases, animal science, agronomy, microbiology, agricultural engineering, and economics) have focused on sustainability. During recent years, agricultural production has been investigated in greater detail and sustainability is frequently brought up. Many studies show that increased agricultural output is strongly correlated with increased environmental factors that include byproducts, wastes, and pollution release to the environment. These concerns have placed a global focus on animal production, emphasizing management of excreted nutrients since it has been shown to be one of the most significant sources of water pollution (Wang et al. 2010; von Keyserlingk et al. 2013). The concept of sustainability, which consists of viability, environmental responsibility, and social acceptability factors (Figure 1) receives continued attention as

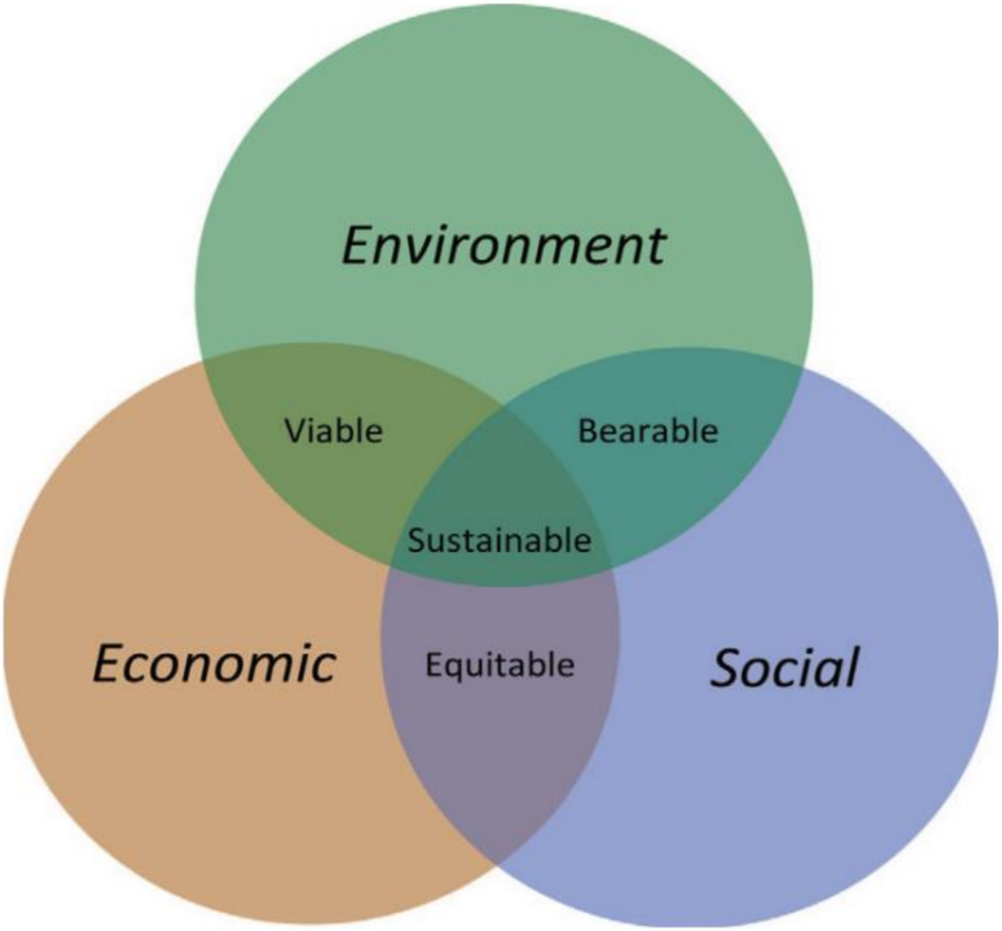

Figure 1. Sustainability pillars (von Keyserlingk et al. 2013)

time progresses (Thompson 2007). Agricultural professionals are the main managers of useable lands which are defined as all land which is not desert, rock, tundra, or boreal. Some researchers have concluded that about 50% of all usable lands in the world are already under intensive agriculture or pastoral farming (Vitousek 1997; Carpenter et al. 1998). Increasing hectares in production agriculture not only leads to possibly losing natural ecosystems, but also leads to the potential release of large amounts of N and phosphorus (P) which are extremely harmful for the environment (Tilman et al. 2002).

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 remain over the long-term;
- The ability of a system to satisfy human food and fiber needs, enhance environmental quality and the natural resource upon which the agricultural economy depends;
- Promotion of the most efficient use of nonrenewable and on-farm resources;
- Integrate, and where appropriate, enhance the natural biological cycles and controls that benefit the natural resources available;
- Sustain the economic viability of farm operations, and enhance the quality of life for farmers and society as a whole.

Some researchers feel that while on one hand sustainability favors only the increased productivity of the agricultural land as it is intensively managed by the owner or manager, it must also on the other hand provide or enhance products and environments which are valued by society (Thompson 2007). Education of all members of the system and increasing awareness of sustainability principles are the only means -whereby the balance in these systems will be achieved.

Current Sustainable Farming Practices

Farmers work and raise crops and animals in order to feed their families or to sell them in markets that are intensively globalized and competitive. Sustainable agriculture services and guidance are mostly beneficial for the society and of less direct benefit to the farmers. Thus, farmers should overlook their higher production and benefits if they want to follow guidelines for sustainable agriculture. Therefore, how is it possible to keep both the society and the people satisfied? The answer may simply lie in providing the farmers with some incentives and hence persuade them to consider environmental risks and public concerns. One such incentive can be a subsidy which is paid to farmers in different countries, others being reduced tax, higher taxes on fertilizers, new feedstuffs, and higher fines on environment (soil or water) polluters (Tilman et al. 2002).

The practices in agriculture are divided into sustainable and unsustainable. The most important factor in classifying sustainability is the person who defines it. Sustainable agriculture sometimes may mean low inputs such as fossil fuels and energy to optimize farming or maximize yield (referred to as common agriculture in practice, Thompson (2007) or reduce harmful waste or agricultural byproducts to environment. However, Thompson (2007) stated that neither sustainability nor unsustainability can be fully applied to each agricultural practice. Agricultural sustainability in true meaning can be defined as a comprehensive view where wide varieties of practices in farms are considered by small and large operations. Animal agriculture sustainability is broadly defined as a scalable food producing system which provides enough food where society and agriculture show intricate interactions.

Governments and law-makers agree upon the fact that environmental and human food safety should be among critical subjects to pay attention, since the global population is increasing daily and according to the predictions will reach more than 10 billion in the next 30 years (FAO 2009). However, even now there exists the food safety and hunger problem in many countries (FAO 2009). Such an increase in the population will cause tight competition on land (urban and rural) as well as water and food resources. Also, agricultural lands for supplying human food will decrease, while the byproducts of human nutrition simultaneously increase. As a result animal production, specifically ruminant production, plays a critical role in providing nutrition resources to human (Gill et al. 2010); ruminants are capable of converting low value matters such as agricultural byproducts that are not suitable for human consumption and pasture into very high-quality food appropriate for supplying humans and thus maintain their food safety (Knapp et al. 2014).

Nitrogen and P are two nutrients which are of the most concern in animal production because of their environmental impacts. It is clear N and P utilization in livestock is inefficient, so 60-80% of these two nutrients are excreted in the environment (ASAE 2005). Hence, the tremendous amount of nutrients which entered dairy farms remain on the farm instead of converting to meat or milk (von Keyserlingk et al. 2013).

Nitrates and salt are the most important polluting parameters of underwater sources in the US, and salt pollution is a big challenge in many parts of it. Recently, more attention has been drawn to animal production as one the outstanding polluting factors toward underwater sources and streams which strongly degrades their quality. In past decades, many researchers and investors in the agriculture sector have tried to decrease polluting excretions of industrial farms. Along the same way, much research has been directed on decreasing N and P excretion to environment as much as possible. Specifically, many researchers have tried to decrease protein intake in animals while supplying required amino acids to compensate for the loss of proteins and also optimize productivity. Some studies tried to replace concentrate with forages which are less pollutant for environment as shown in Table. 1 which shows forage production is less pollutant than concentrate (Hoekstra 2012). Also, some works put their effort to include some forages with condensed tannins (CT) in ruminant rations to decrease N excretion and methane to the environment (Christensen et al. 2015). Methane (CH_4), a greenhouse gas, has become critical as an air pollutant, and it is capable of polluting the air 21 times more than carbon dioxide (IPCC 2007) which is more related to forage feeding.

Productive Efficiency

Globally, agriculture requires to yield “more with less”. Producing a kilogram of meat requires 3-10 kg grain (3-4 kg for chicken and pigs, 4-6 kg for lamb, and 7-10 kg for beef GAO 1995). During the last half-century, annual meat production per capita has increased more than 60%. Simultaneously, salary per capita has increased, whereas grain production per capita has been reduced (GAO 1995). Animal production is becoming to an industry where millions of cattle, swine and poultry are being fed grains. The number of animals in average per livestock operation has increased 1.6 times for cattle, 2.8 times for egg production, 2.3 times for swine and 2.5 times for boilers in the US for 14 years (GAO 1995). For example, pigs average number per operation increased 2.6 times from 1990 to 2000 in Canada (Statistics Canada 2002).

Nutrients provide necessary precursors for microbial productions in the rumen, while differences in nutrient digestibility, dry matter intake (DMI), passage rate, and chemical content affect the extracted energy by microbes. Hence, concentration of metabolites produced after microbial digestion affects profiles of volatile fatty acids (VFA), CH_4 production, and amino acid compositions (Mbanzamihigo et al. 1996).

Leng (1993) stated that 75% of total ruminant CH_4 emissions originate from ruminants grazing feeds with low-quality. Increasing the feed quality is a complicated concept related to efficient feed and animal productivity, which can reduce CH_4 emissions per product unit. Enhancing quality of forage is desirable for livestock farmers as feeding high-quality forages is core to good farming practice and almost always increases profitability (Waghorn and Clark 2006). Quality of forage can be increased by grazing or harvesting less mature forages, genetic selection strains, or species which have the best digestibility (e.g., sorghum and brown midrib corn), proper storage management, especially ensiling, to preserve digestible nutrient content, enhancing nutrient utilization efficiency of feed (Boadi et al. 2004).

Environmental Issues

There is a general agreement that agriculture has a potential to produce food for eight to ten billion people while markedly decreasing population proportion of those who experience hunger, but there is less agreement how it can accompany meaningful sustainability. Sustainability refers to high production (although with major impacts) and practices in agriculture which are acceptable by society and government. The most important environmental concerns are the conversion of natural ecosystems to agricultural lands, contamination of ground waters and terrestrial and aquatic habitat by nutrients, and accumulation of pesticides from crops and resistant organic pollutants. Nutrients in agriculture pollute other ecosystems via volatilization, leaching, and waste of livestock streams.

Agriculture is one of the most important water consumers globally and water insufficiency can have serious impacts on production of food (Strzepek and Boehlert 2010). An increase in population increases the contest for resources, hence escalating the risk of pollution. In US, nitrates and salt contaminations are the widest spread contaminates for groundwater, and salt pollution (with proven harmful impacts on cattle) is a growing concern in many areas in the US (Grout et al. 2006).

Another pollutant which is a big challenge in recent years is pollution of nutrients. Recent researches in Washington (USDA-EPA 2012) and California (Harter et al. 2012) showed that approximately 20 and 10% of sampled public wells exceeded the nitrate peak contamination concentration (which is 10 mg of nitrate-N/L), respectively. Harter et al (2012) showed in some areas of California, with high dairy farm concentrations, about one-third of wells have higher nitrate amounts than maximum concentrations allowed. The nitrate pollution becomes worse if some mitigating efforts such as inserting high quality water to polluted wells are not deployed. A complex of dairy farms in Yakima Valley in Washington which had 17,240 dairy cows and 7,000 young heifers and calves in 2009, was the main source of monensin and tetracycline pollution in this area (USDA-EPA 2012). Sources for this estimation included four dairy applications, eight residential drinking wells, ten dairy lagoons, four field samples, three dairy supply wells, and four manure piles.

Globally, animal-agriculture is considered to contribute to one-third of water pollution which originates from animal-human related activities (Hoekstra and Mekonnen 2012). Mekonnen and Hoekstra (2010) demonstrated that the water footprint of animal food products, which accounts for total water usage to produce, process, deliver and distribute a food stuff from grower to consumer, the largest portion of which, about 98%, is attributed to the production of animal feedstuffs. In another study, Gerbens-Leenes et al. (2013) found that there are two major resources that contribute to footprints for animal products. The efficiency of feed conversion is the first factor, which is considered to be the required feed amount to produce a given quantity of eggs, meat, or milk. Since animals in a grazing system have a longer lifespan before reaching slaughter weight, these animals consume a greater amount of feed to produce 1-unit of meat. Based on this definition, feed conversion efficiency in grazing systems is outperformed by a conventional feeding system as well as a mixed system (grazing and conventional feeding system), the latter leaving a smaller water footprint. The second parameter has an opposite direction which refers to the feed composition eaten by animals in any system. As the amount of concentrate increases in the ration, the water footprint increases as well, since concentrates have a larger water footprint than roughages (crop residues or grass). Because of higher concentrate proportion in the diet in conventional feeding systems, the water footprint is smaller for grazing and mixed compared to conventional feeding systems. Overall, concentrates have a five times higher water footprint compared to forages (Table. 1). The total water footprint is approximately 200 and 1,000 m^3/ton to forage mixtures and concentrate mixtures, respectively, because water to forages are basically supplied by rain, but grain crops are mostly irrigated and fertilized, so the water footprints for concentrates are even 50 times those of forages (Hoekstra 2012; Table 1). Thus, how can these costs be minimized but production be simultaneously increased? The answer is very simple; livestock and crop production must grow without increase in use of harmful materials released into the environment which are often associated with agriculture. This means significant increases in water, pest, N, and P utilization efficiency

Table 1. The global-average water footprint of crop and animal products (Mekonnen and Hoekstra 2010)

Food item	Total water footprint	Nutritional content			Water footprint of nutritional value		
	L/kg	Energy density, kcal/kg	Protein, g/kg	Fat, g/kg	L/kcal	L/g Protein	L/g fat
Sugar crops	197	285	0.0	0.0	0.69	0.0	0.0
Vegetables	322	240	12	2.1	1.34	26	154
Starchy roots	387	827	13	1.7	0.47	31	226
Fruits	962	460	5.3	2.8	2.09	180	348
Cereals	1644	3208	80	15	0.51	21	112
Oil crops	2364	2908	146	209	0.81	16	11
Pulses	4055	3412	215	23	1.19	19	180
Nuts	9063	2500	65	193	3.63	139	47
Milk	1020	560	33	31	1.82	31	33
Eggs	3265	1425	111	100	2.29	29	33
Chicken meat	4325	1440	127	100	3.00	34	43
Butter	5553	7692	0.0	872	0.72	0.0	6.4
Pig meat	5988	2786	105	259	2.15	57	23
Sheepor goat meat	8763	2059	139	163	4.25	63	54
Bovine meat	15415	1513	138	101	10.19	112	153

management (Smith et al. 1999).

Waste removal and management is one of the most important issues in farms today. Manure collections can release high amounts of hydrogen sulfide and other toxic gases into the environment and also strongly increase volatile ammonia which causes N pollution, leading to the contamination of ground and surface waters and damages the environment. Such animal excretions, similar to human waste, can cause environmental and health threats and need effective management. For instance, animal excretions should be managed for use as fertilizers so as to avoid their pathogenic functioning. This handling should include efficient time, speed, amount, and methods to minimize nutrient drainage which if done correctly, decreases chemical fertilizer requirements. The use of chemical fertilizer in agriculture has increased over the last decades (Tilman et al. 2002) but if proper nutrient management is conducted, the use of chemical fertilizers to improve crop production can be reduced and simultaneously, important nutrients that would normally have been released to the environment can now be used to contribute to food production.

Nutrient Management

Discussing nutrient management requires understanding many factors such as crop production, animal nutrition, water conservation, manure management, environmental issues, and economic effects. All the aforementioned factors influence farm economy and profitability, but any negative impacts these may have on the environment will lead to unsustainability (VandeHaar and St-Pierre 2006). Among many studies done in this area, Kohn et al. (1997) tested a model to reduce N excretion on dairy farms with consideration of the relative importance of the above parameters associated with nutrient management and concluded that managing feed and feeding is the most critical parameter in reducing N excretion to the environment. (Rotz 2004; VandeHaar and St-Pierre 2006; Figure 2).

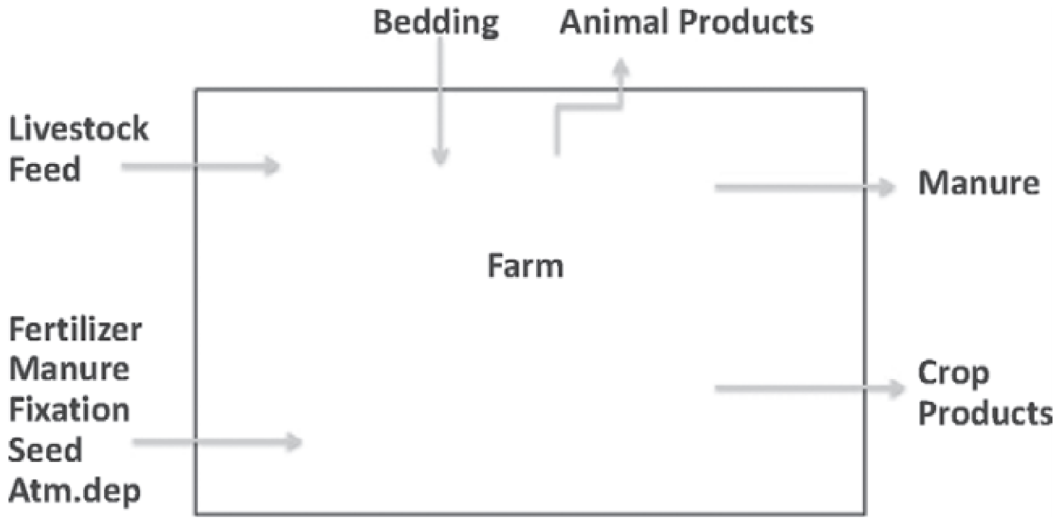

Figure 2. Farm N balance constituents (Toma et al. 2013).

Nitrogen Metabolism

There are various forms of N that are consumed by ruminants. Overall N metabolism in ruminants is a complicated pathway consisting of several mechanisms (Van Soest 1994; Figure 3). Non-protein N (NPN) consists of recycled urea infused into saliva and rumen, supplemental urea in the diet, ammonia formed by protein degradation in wet feedstuff and silage, and highly hydrolysable protein fraction in the diet. Feedstuff protein or true protein of the diet can be classified as rumen degradable (RDP) or rumen undegradable protein (RUP) (NRC 2001). The most important protein source for animals is microbial protein (MCP) which is formed by rumen microbes using true protein derived from RDP and NPN to help their growth and reproduction. Between 50 – 80% of the protein in dairy cows is supplied by the MCP which is a high-quality source of amino acids and contains higher concentrations of methionine and lysine, the two most important limiting amino acids (AA) in milk production (NRC 2001). The CP derived from endogenous resources cells and some components derived from intestinal wall and RUP in addition to MCP are digested with various proteolytic enzymes and then

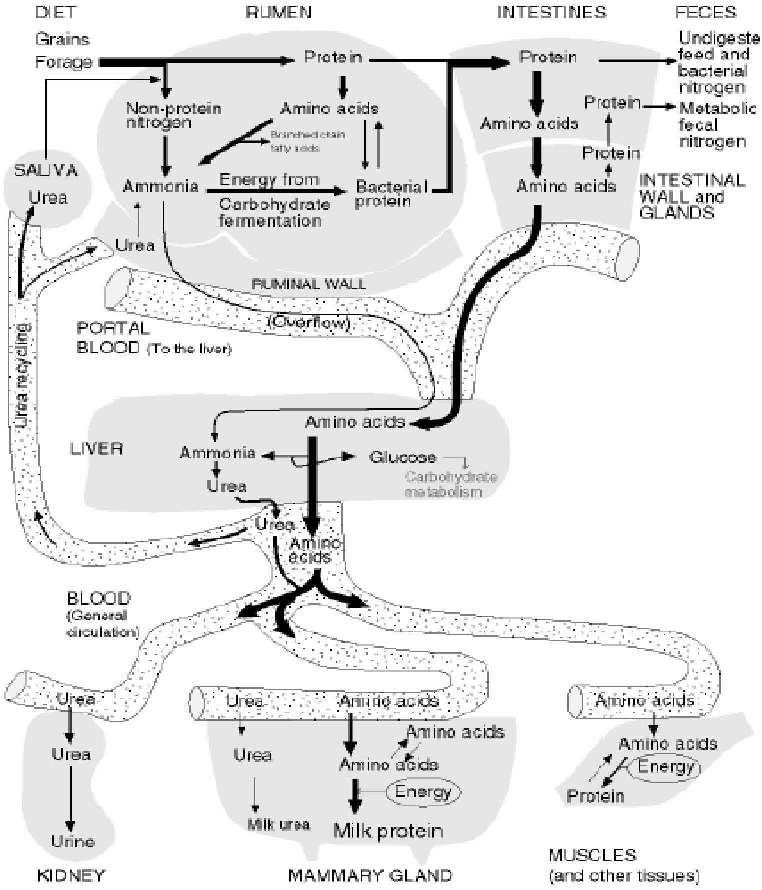

Figure 3. Dairy cows' nitrogen metabolism (Wattiaux 1998).

absorbed in the small intestine, forming metabolizable protein (MP). The AA and peptides in the MP are used for growth, production, maintenance, and reproduction in ruminants (NRC 2001). Rumen microbes cannot cause a change in the form or structure of RUP; therefore, they move into small intestine and get absorbed there, so they do not play a role in MCP synthesis, but are a great source of AA for the animal. Since MCP cannot meet the animal's requirements alone, RUP is used to complement AA profile of MCP so as to maximize the efficiency of N utilization and supply AA demands. Digestibility of RUP among feedstuffs differs from 50 to 100%, and it is considered that RUP is a completely true protein (NRC 2001). Flow of RUP toward the duodenum is affected by RUP concentration of the diet and the amount of N digestion.

Ratio of MCP and RUP in MP can be affected by N source and concentration in the diet. Blouin et al. (2002) assessed the MP availability in dairy cows from two isonitrogenous (16.3% CP) and isocaloric (1.62 Mcal NE_L/kg DM) diets. These two rations supplied 1,930 and 1,654 g/d of MCP due to variation in MP, RDP, and RUP supply with different sources of protein supplements. In another experiment, Doepel and Lapierre (2011) fed two isonitrogenous (17.5% CP) and isocaloric (1.45 Mcal NE_L/kg DM) rations at 26 kg DMI/d and observed 2,197 and 2,674 g/d MCP, which showed that there was no direct relationship between dietary CP concentration and MCP yield. Researchers also studied the MP availability from diets with different dietary protein concentrations. Raggio et al. (2004) estimated MP availability from three rations at similar NE_L values but varying levels of dietary CP concentration (12.7, 14.7, and 16.6% DM), and the supply of MP from these rations were 1,992, 2,264, and 2,501 g/d, respectively. Weiss and Wyatt (2006) estimated the supply of MP from rations having two CP levels (14.4 vs. 17.2%) by feeding conventional or brown midrib (BMR) corn silage in a 2 × 2 factorial design experiment. The supply of MP from low and high CP diet with BMR silage was 2,350 and 3,000 g/d, respectively, while the supply of MP with conventional corn silage diet was 2,370 and 2,970 g/d, respectively. This study indicated that MP supply was affected by dietary CP concentration but not main source of forage.

In addition, CT in the diet can influence the ratio of MCP and RUP in MP. Protein degradation in the rumen can be restrained by the action of CT (Barry and McNabb 1999; Min et al. 2003) through CT binding action with protein that preserves the bound protein from degradation by microbes in the rumen. However, sources and chemical composition of tannins (e.g., epigallocathecins and epicathecins; Patra and Saxena 2011) affect the relationship between N metabolism in rumen and CT which in turn influences total diet CP concentration and ruminal N digestibility (Min et al. 2003; Carulla et al. 2005; Waghorn 2008). For instance, a 28% decrease in the concentration of ruminal ammonia-N (NH_3-N) was observed when BFT hay was fed in a high-forage diet (2.1% CT) in continuous cultures (Williams et al. 2010a). On the other hand, this research showed in TMR diets containing CT at low (0.38%) and moderate (0.74%) levels derived from BFT did not change the concentration or flow of NH_3-N compared with alfalfa TMR. Likewise, John and Lancashire (1981) who fed two cultivars of BFT (Maitland and Empire) which were different in CT concentrations (1.45 and 0.25% DM, respectively) reported the high CT BFT attached more N and decreased concentration of NH_3-N in the rumen.

Fecal and Urinary Losses of N

An imbalance between energy and dietary protein concentration can increase N excretion in the urine by forage-based dairy cows (Dijkstra et al. 2013). This can become more severe in early lactation, as animals are in negative energy balance and more energy is needed to detoxify extra N derived from ruminal NH_3-N via ureagenesis (Lobley et al. 1995).

One of the major environmental issues in need of attention regarding N is the amount and excretion path of N. The excreted N through urine is more volatile than the one excreted in feces and is converted into ammonia quickly. Dietary protein affects the excretion of N to the environment. For example, in a recent study by Lee et al. (2014) cows fed rations containing 16.7% CP increased ammonia excretion while urinary N in the nitrate-N was a 100% more than fecal N compared to those fed a ration containing 14.8% CP. Urea is a soluble compound that can enter body fluids such as urine, milk, and blood. Increases in blood and milk urea are a result of feeding crude protein that exceeds the dietary requirement on the animal. Urea accumulates during an enzymatic transformation of excess N compounds that break down from diet N sources (Kauffman and St-Pierre 2001). It is assumed that in dairy cattle cases where more than 500 g/d of N is consumed, around 80% of it is secreted into urine (Castillo et al. 2001; Kebreab et al. 2001). If total CP consumption decreases, excretion of total and urinary N can be reduced. The efficiency of N utilization in dairy cows is low (VandeHaar and St-Pierre 2006). Therefore, research to improve the efficient use of N in dairy diets should continue while considering environmental effects of the reduced N load. In order to decrease diet CP to reduce environmental risks and optimize milk production in high producing dairy cows, precise ration balance is essential. Rotz (2004) showed that the major cause of excess N excretion is overfeeding RDP. The author also showed that less consumption of N through a decrease of CP concentration in the ration can lead to a decreased excretion of N, especially urinary N. In diets with 16.5% CP for dairy cows versus diets with higher CP levels (the dietary CP for dairy cows ranged from 13.5 to 19.4%), milk and its protein production was optimal while simultaneously less N was excreted (Colmenero and Broderick 2006a).

Other researchers have shown that using less CP in the diet is possible, but have stated that supplementation of essential amino acids (EAA) such as methionine, lysine, and histidine is crucial in the reduced protein dairy diets (Lee et al. 2012b; Giallongo et al. 2015). Lee et al. (2012a) argued that the amount of EAA tended to decrease from the amount necessary to keep production constant when the CP of the diet was reduced. Tamminga (1992) showed that the metabolic N (cells derived from the small intestine and fermented products from hind gut) and undigested RDP are excreted through feces. While on overfed CP diet, dairy cows produce more ammonia in the feces per cow compared to other livestock which reveals inefficient consumption of dietary CP in the rumen; this means a higher volume of urine excretion containing higher urinary N concentration. In a meta-analysis, Nennich et al. (2005) indicated that excretion estimates in dairy manure can be determined according to production levels and dietary factors for a given operation.

Elevated fecal N is more likely to be caused by higher feed intake and partially resulted from RUP than by of undigested feedstuff CP excretion (Flis and Wattiaux 2005). Huhtanen et al. (2008) studied the excreted fecal N in a meta-analysis and showed that it was more related to DMI than N intake. Furthermore, the authors demonstrated that the model had a better prediction when including both parameters. Since indigenous and metabolic N are critical determining factors for fecal N and are strongly-related to DMI, including N intake and DMI, in a model for predicting fecal N excretion seems essential (Van Soest 1994). Huhtanen et al. (2008) showed a 6-7 g fecal N excretion increase followed 1 kg increase in the DMI. On the opposite side, other researchers demonstrated that feeding diets with different CP levels ranging from 15.0 to 18.8% increased MUN concentration and fecal and urinary N excretions whereas having a small and inconsistent effect on DMI and milk protein yield (Groff and Wu 2005). Colmenero and Broderick (2006b) also found similar results of increased MUN concentration and urinary and fecal excretions and a quadratic effect on milk protein yield highest at 16.5% CP concentration when the dietary CP for dairy cows ranged from 13.5 to 19.4%. In this study, CP concentration did not have an effect on the DMI; however, the N intake and digestibility linearly increased with an increase in CP, suggesting that N excretion influenced N absorption and intake. In a recent study, Grosse Brinkhaus et al. (2016) fed three different diets containing sainfoin (SF; *Onobrychis viciifolia*), alfalfa hay, and BFT at about 20% DM. He found that fecal excretion and N intake of early lactation dairy cows were the same in all treatments. Nevertheless, when they reported the results as N intake percentage, fecal N showed a trend to be higher for SF treatment compared with BFT, but not with alfalfa hay. Also, total urinary excretion of N and percentage of N intake in SF cows were lower than in alfalfa hay-fed cows while no changes were observed in cows fed BFT. This different partitioning happened with simultaneous changes in urea N proportion and N excretion of urinary urea in absolute urinary N. Total urinary and fecal N excretion (represented as N intake percentage or absolute amounts) were not different among these rations (Grosse Brinkhaus et al. 2016).

Nitrogen Utilization Efficiency

One of the ways to reduce environmental risks of N is to increase protein utilization efficiency by dairy cows. This is achieved when the amount of excreted N to the environment reduces per kg of milk produced, resulting in higher economic benefits for the farms and reduced waste production (Huhtanen et al. 2008). Jonker et al. (2002) showed that increasing N intake 1 g more than the recommended amount, decreases efficiency of N utilization by 0.05%. Since feed N undergoes severe changes in the rumen, diet protein cannot be a reliable factor to assess the amount and composition of the protein which is finally offered to the cows. Jonker et al. (2002) also stated that increasing diet CP was related to the increase of N intake which corresponded to an increase in DMI and N excretion via milk, feces, and urine, a small portion of which can be available as a useful resource.

Aschemann et al. (2012) showed that feeding a diet with 12% CP did not affect milk yield, but the low CP diet decreased MCP yield and digestibility of nutrients in the rumen. Since milk production of the cows in this study was 29 kg/d which is considered low, and the DMI was restricted, protein effects on DMI could not be revealed. Studies with high-producing, lactating cows have demonstrated varying effects of low dietary protein or MCP on DMI. For instance, in studies with shortages of MP in the diet, DMI decreased along with a decrease in milk yield and N efficiency (Lee et al. 2011a, 2012a). In contrast, with no reduction of DMI and when fed diets with sufficient MP amounts, dairy cows did not experience a decrease in milk yield (Lee et al. 2012a; Giallongo et al. 2015).

In addition to manipulating CP amount, adding feedstuff to increase energy content of the diet leads to an improvement in N utilization efficiency. As an example, when a higher percentage of concentrate was added to the dairy cows' diets meaning an increase in the diet energy per unit milk production was elevated. In a recent study comparing high forage and high concentrate diets containing either flaxseed or flaxseed oil, Benchaar et al. (2014) observed that feeding HC diets to dairy cows elevated, DM (8%) and OM (9%) total tract digestibility while they did not detect any effect on ADF and CP digestibility. Moreover, absorption and digestibility from the diet increased or productivity was elevated by 100 g/kg N intake which resulted in improved energy utilization efficiency in dairy cows. Benchaar et al. (2014) showed that in many cases increasing the energy in dairy cow diets, leads to higher efficiency. Furthermore, their study demonstrated that increasing concentrate amount in the diet caused an improvement in N utilization which is determined by a decrease in ruminal NH_3-N concentration and MUN and also higher N consumption for milk protein synthesis. Such an increase in N utilization is probably caused by higher starch supply from diets containing more concentrate. Russell et al. (1992) demonstrated that availability of carbohydrate in rumen strongly affects NH_3-N utilization. Others detected that feeding fermentable carbohydrate through increased concentrate in dairy cows decreases the production of NH_3-N in rumen via decreased deamination process or increasing trapping AA released by rumen microbes or collecting available NH_3-N in the rumen by microbes (Hristov and Giallongo 2014). This finding was previously observed where the researchers showed that an increase in diet concentrate percentage reduced the concentration of NH_3-N in the rumen (Agle et al. 2010).

Diet protein content is the most outstanding parameter in determining efficiency of milk N, urinary N excretion reduction, and emission of ammonia from dairy cattle manure. Colmenero and Broderick (2006a) in a study showed that dairy cattle receiving 16% CP per day, produce 44 kg/d milk and do not have a problem in milk production or composition while another study has shown that diets with CP levels below 15% (MP deficiency at less than 12%) will probably cause milk yield reduction which might be partly caused by DMI reduction. Also, adding EAA might improve diets suffering metabolizable protein shortage which might be in part due to an increase in DM (Kalscheur et al. 1999).

Reduction of milk yield due to low diet CP should be assessed in more detail and advantages and disadvantages of rations with various CP levels should be studied especially when the diet of dairy herds needs to experience dramatic changes. For instance, it was determined in a study when diets fed with lower than required CP levels, dairy cows experience a reduction in N utilization efficiency and an increase in urinary N excretion amount (Kalscheur et al. 1999). Taking such studies into account, it is concluded that balancing diets to reach maximum milk yield may not support N excretion reduction to the environment in relation to the produced milk.

On the other hand, the most important limiting factor of MCP in dairy cows is metabolizable energy. Microbes in the rumen need some kind of metabolizable energy for growth and reproduction. The carbon derived from carbohydrates and ATP as energy are consumed for synthesis of protein and AA. Microbial growth rate and partial degradation of available energy as metabolizable in rumen determines the microbial yield (Nocek and Tamminga 1991). Moreover, there are some other parameters in ruminants which rely on energy and are harder to understand and evaluate such as lactation, pregnancy, maintenance, and growth. When discussing the efficiency of utilizing N, the most outstanding factor is the source of energy since it is needed to synchronize with N to produce an optimum amount of MCP. The National Research Council (2001) has determined and published the required metabolizable and net energy for cows in various conditions like production, reproduction, maintenance, and pregnancy even though they are sometimes not in coordination. For instance, the required maintenance energy for dairy cows is 0.54 MJ of ME/kg of $BW^{0.75}$ where the conversion rate of ME to NE is assumed to be 0.62 and the maintenance energy used also includes a 10% addition reserved for normal activities (NRC 2001). A study on energy requirements of non-lactating pregnant cows Mandok et al. (2013) showed their ME requirement to be 1.07 MJ of ME/kg of $BW^{0.75}$ (or 117 MJ of ME/d) which is higher than the NRCs (2001) estimation. The reason behind this difference is that the needed maintenance energy of selected cows for higher production has increased over time because pregnancy ME requirements in mid-late gestation have been previously underestimated. However, the present inconsistency among literature suggests the need for more study on energy consumption and requirements, and dairy cattle metabolism and its interaction with N which leads to N utilization efficiency.

There are many tools to improve N utilization. One possible strategy is including a forage containing CT in dairy cows' rations. McSweeney et al. (2001) showed CT are flavanol polymer units which are linked together by carbon- carbon bonds. These CT can form some complexes with pectin, cellulose and hemicellulose (McSweeney et al. 2001) and particularly with proteins in the rations (Hagerman and Butler, 1981). Waghorn (2008) demonstrated these complexes conserve against enzymatic degradations in the rumen, which can lead to a shift in excretion of N from urine to feces (Carulla et al. 2005).

The plants that grow in temperate climates are very few, one of which is BFT

(*Lotus corniculatus*). Studies on the use of BFT in sheep nutrition are abundant and are reviewed by Waghorn (2008), but the use of BFT in dairy cattle nutrition is limited. Hymes-Fecht et al. (2013) demonstrated replacing alfalfa silage with BFT silage in TMR diets improved N utilization efficiency and dairy cows' performance. They showed that N utilization efficiency increased from 21% in AH silage diet to 25% for BFT silage diets and MUN concentration, an index for inefficiency of ruminal NH_3-N and dietary N utilization, did not change between treatments. Other studies utilizing fresh BFT and lactating dairy cows which grazed pasture (Turner et al. 2005) or were fed with fresh ly harvested BFT (Woodward et al. 2000) demonstrated better performance than cows offered white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*) in each case, respectively.

Milk Urea Nitrogen

Production based on increased amount of forages (greater than 45% on a DM basis) in dairy cows is becoming more common and beneficial for farmers and the environment compared with production systems which use high concentrate rations (less than 45% on a DM basis;(Dillon et al. 2005). Moreover, feeding this type of ration which contains a high proportion of forage often has risks of high level RDP in the rumen which cannot be used by microbes. Excessive ammonia can enter into milk, blood and urine leading to elevated levels of NH_3-N (Spek et al. 2013; Grosse Brinkhaus et al. 2016).

In rations containing higher amounts of CP, specifically RDP, increased MUN excretion is observed. This might be associated with increased urinary and total N excretion. There is a positive correlation between increased blood urea concentration and higher concentrations of MUN. Milk urea-N is a result of inefficient and incomplete utilization of ammonia in the rumen (DePeters and Cant 1992). DePeters and Cant (1992) showed that nutritional parameters play an important role in MUN concentration and several factors exist that contribute to MUN concentration such as amount of diet carbohydrates, CP degradability and solubility, carbohydrate synchronization with N sources, and providing of substrates of protein accretion to microbes in the rumen. Rotz (2004) stated that MUN is an appropriate factor to evaluate the balance of the diet and determine the optimal efficiency of utilizing nutrients. Other researchers (Kalscheur et al. 2006) who have fed dairy cows diets with various levels of RDP, have observed that MUN increased from 9.5 to 16.4 mg/100 mL when the amount of RDP of the diet increased from 6.8 to 11.0%, respectively and milk production increased 2.1 kg/day when diet CP linearly increased 0.9 kg/day with an elevated diet RDP. At an RDP concentration of 11%, milk yield increased 2.1 kg/day when diet CP increased 0.09 kg/day which consecutively increased the RDP. They demonstrated cows fed diets with CP levels lower than suggested NRC (2001) requirements for RDP suffered lower milk yield and milk protein and fat because of lower growth of microbes resulting from lower RDP amounts. When RDP in dairy cow diets increased, MUN amounts increased linearly while conversion efficiency of feedstuff N to milk N reduced. Others observed a positive correlation between MUN and urinary N excretion (Kauffman and St-Pierre 2001; Nennich et al. 2006). In a study consisting of 16 previous works, Nennich et al. (2006) detected that MUN is an excellent predictive factor for urinary N excretion. Urinary N excretion determining factors include N intake, MUN, BW, DMI and DIM with N intake being the best. The correlation between urinary N excretion and MUN follows the formula below:

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

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.

Grosse Brinkhaus et al. (2016) showed when early lactation dairy cows were fed with three different diets containing SF, AH, and BFT (they replaced AH with SF or BFT about 20% DM) MUN concentration were lowest in SF diet, intermediate for AH and highest for BFT treatments. They reported that BUN was lower for SF treatment compared to cows fed BFT and AH. Simultaneously, the observed ruminal ammonia N concentration was lower for cows offered SF compared to those receiving BFT and intermediate levels for cows fed AH. Moreover, they demonstrated that excretion of N milk did not differ among these diets, both when represented as N intake percentages or absolute amounts.

Forage Nutrition for Dairy Cows

Alfalfa Hay

With the current rise in foodstuff prices, increasing forage feeding portion in dairy cows' rations is more important than before. Alfalfa which is named "queen of forages" is a high quality, cheap protein source and very palatable. Alfalfa (*Medicago sativa L.*) is a perennial legume with leaves which have high amounts of protein and fibrous stems. Yu et al. (2003) showed fiber digestibility and protein proportions in alfalfa change by increasing leaves to stems ratio and stem lignification which are all influenced by maturity.

Holt et al. (2010) showed in West, where high quality alfalfa grows very well, feeding cows with diets containing a higher level of AH is very popular. He declared in these areas AH supplies approximately 50-75% of the forage in the diets where total portion of the forage reaches 45-55% of dietary DM. Holt et al. (2013) showed it is common to fed dairy cows with high quality AH that supplies on average 21.3% CP and 38.3% NDF (DM basis). Although AH feeding supplies CP and adequate forage NDF to produce optimum milk yield, its CP is severely degradable and broken down by ruminal microbes. This leads to a higher amount of ammonia in the rumen which is beyond the capacity of microbes to use in synthesizing MCP; meaning more energy expenditure to convert NH_3-N to urea, hence excretion of N to environment (Elizalde et al. 1999a, 1999b).

For many years, alfalfa has been an undeniable source of forage and protein in lactating dairy cows ration. Based on the NRC (2001)10-20 % of alfalfa hay N is NPN. It seems the amount of protein which can be absorbed by rumen is the most important limiting factor when cows are fed with alfalfa. Since it is extremely degradable in rumen, reduced synthesis of MCP is predictable when feeding alfalfa. The amount of fermentable energy needed to couple with the N that can then be converted to metabolic and system-wide usable nutrients is one of the most important limiting parameters here as ruminal microbes cannot trap enough RDP derived from alfalfa to produce MCP (Elizalde et al. 1999a, 1999b).

Based on NRC (2001) recommendations, to avoid milk fat reduction in lactating dairy cows, inclusion of 25% of NDF in the diet is essential and 19% of that NDF should come from forage NDF. Thus, as milk fat production is a very important factor for farmers, feeding alfalfa is a suitable way to supply enough NDF in dairy cows' diet for milk fat yield. Also, when alfalfa hay portion in the diet is high, consideration of alfalfa particle size is important (Leonardi and Armentano 2003).

In the end, since alfalfa CP is very degradable and causes some costs to animal to excrete excessive ammonia produced in the rumen, protein part in the lactating dairy cows ration is very expensive for farmers, and also ammonia excretion has very harmful environmental impacts and has many costs for society and government, finding some strategies to avoid these costs is undeniable, so replacing AH with BFT can be one of such possible strategies to manage many issues currently engaging dairy farms.

Birdsfoot Trefoil Hay

Birdsfoot trefoil (*Lotus corniculatus*) belongs to a species which is spread all over the world and grows under very different climatological conditions and after white clover (*Trifolium repens*) and alfalfa (*Medicago sativa*. *L*) is categorized as one of the important forage legumes (Singh et al. 2007). Blumenthal and McGraw (1999) demonstrated that FT yield equals 50% of that of alfalfa (DM ha-1 year-1) and Cassida et al. (2000) demonstrated BFT yield cropped in lands ranges between 8000-10000 kg (DM ha-1 year-1). BFT is adapted to various weather conditions, has genetic variety and a high potential for an enhanced performance as well as genetic manipulation and improvement (Steiner 1999). BFT is cultivated less frequently compared to other legumes in many countries though it is spread across the globe (Blumenthal and McGraw 1999). Ahlgren (1956) stated that in early 1900s BFT was a major pasture source in the northern part of the US. Recent studies show that 1 million ha BFT is being cultivated in the US, the most being used in Midwest, Northeast and along Pacific coast (Beuselinck and Grant 1995) whereas 10-11 million ha alfalfa gets cultivated in the US annually. Conserved BFT hay as a crop is easier and cheaper to transport and store than BFT silage, thus, it provides a suitable way to handle additional forage from spring production to be used in upcoming months or to be sold.

In general, nutritional value of *Lotus* species which also includes BFT is considered similar to, or even better than, alfalfa and white clover. Crude protein in *Lotus* species ranges from 16 to 24% while ADF varies between 24 to 30% and DM digestibility is 72-78% (Blumenthal and McGraw 1999). Seaney and Henson (1970) stated that BFT can even be more productive than alfalfa hay in marginal sites. Barta (1980) demonstrated that BFT is stronger in water-logged and/or wet soils. Since there are many similarities between BFT and alfalfa hay species, professionals of the field are well-knowledged about them and much research has been done. Due to high adaptability and yield, BFT has replaced much of the clover which was previously grown with grass in North-East US. In New Zealand, BFT has gained attention where pasture-based ruminants' production is vast and basically depends on white clover (*Trifolium repens*) and perennial ray-grassed (*Lolium perenne*) (Woodward et al. 2002). BFT provides many benefits for pasture-based dairy cows because of improved digestibility, useful tannin content and anti-bloating characteristics. Birdsfoot trefoil is a forage legume which is nonbloating and very close to alfalfa in feeding value and has the ability to be fed as a main hay to cows since in some aspects it has better characteristics than alfalfa (Williams et al. 2011).

In recent years, there is more interest in feeding dairy cows with BFT or TMR as a pasture to increase ruminant yield efficiency on grazing condition or farms and to decrease harmful impacts on environment from extra N excretion. The studies which have been done to investigate BFT (hay and pasture) effects in dairy diets and their effects on milk yield and utilization of nutrients are very few (Christensen et al. 2015; Grosse Brinkhaus et al. 2016). Williams et al. (2010b, 2011) conducted in vitro studies of diets which contain BFT hay only or TMR, to show BFT hay improves dairy cows lactational performance, but there is minimal data regarding in vivo studies.

Another study by Woodward et al. (2000) showed cows fed with BFT had more milk yield compared to ryegrass diet (21.1 kg/d compared to 15.5 kg/d). It happened because of higher DMI, enhanced quality of forage resulting from higher CP amount (21.7% kg/d compared to 16.6% for BFT and ryegrass respectively) and reduced NDF (40.8% kg/d compared to 54.8% for BFT and ryegrass respectively) and CT action. Woodward et al. (2000) showed CT contributed to 40 to 50% of improvement in milk yield and also enhanced energy utilization efficiency by dairy cows (an elevation of 34 mL FCM/MJ ME vs. ryegrass). Hymes-Fecht et al. (2013) fed BFT in dairy cows as silage and Woodward et al. (2009) used fresh forage and both observed elevated N utilization, N excretion reduction in urine, and also a shift of N excretion to feces. But there are a few studies (Christensen et al. 2015; Grosse Brinkhaus et al. 2016) on BFT dry hay and its effects on dairy cows compared to BFT silage or fresh hay.

Cell Wall Structure and Condensed Tannins

Birdsfoot trefoil can meet dairy cows' nutritional requirements very well when compared to other forages. Birdsfoot trefoil has higher digestibility than grasses and even its digestibility compared to legumes is different. Forage NDF measuring shows concentration of lignin, cellulose, and hemicellulose which are cell walls structural carbohydrates in plants are determining factors in digestibility and degradability (Van Soest 1994). Rumen microbes have the ability to digest cellulose and hemicellulose, but lignin has a very complex structure which preserves it from digestion. It also has some crosslinks with other components in the cell wall which lead to reduced digestibility. In the plants with higher maturity, the lignification in stems exceeds the one in leaves, and legumes preserve a smaller stem to leaf ratio compared to grasses, resulting in higher digestibility for legumes than grasses with maturity (Waghorn et al. 1987a). Buxton and Russell (1988) showed concentration and chemical composition of lignin reveals the amount of negative effect it has on forage fiber digestion and rate. Wilson and Hatfield (1997) demonstrated lignification patterns in legumes are the most important reasons that contribute to different levels of digestibility in legumes. There are some documents which show lignification has a lower effect in legume family on BFT than alfalfa. Tomlin et al. (1965) showed despite similar lignin amounts in BFT and alfalfa, the BFT cellulose was digested faster than alfalfa cellulose. Others demonstrated higher stem digestibility for BFT than alfalfa (McGraw and Marten 1986). Mowat et al. (1969) showed a negative correlation between cell wall lignification and digestibility where cell wall lignification reduced digestibility significantly in alfalfa. However, higher digestibility of cell wall in BFT might result in elevated levels of DMI in ruminants. Van Soest (1965) measured the voluntary feed intake correlation with content of cell wall in birdsfoot trefoil, alfalfa, and a large number of grasses and considered Birdsfoot trefoil as a statistical outlier to evaluate correlations between voluntary feed intake and lignin or DM digestibility. In another study, the lambs that grazed BFT had higher intakes than lambs that grazed alfalfa or a mixture of alfalfa and BFT (Douglas et al. 1995). Nonetheless, in another research in limited feed allowance condition, lambs did not intake stems of birdsfoot trefoil due to lignification of stems (Douglas et al. 1999).

Feeney (1976) showed condensed tannin (CT) content is one of the most important advantages of some *Lotus* species which is also well-known as proanthocyanidins, and is located in stems and leaf vacuoles. In many dicotyledonous families and other plants in higher order, the secondary produced component is condensed tannin. These components are polyphenolic and water soluble which have a very wide range of chemical composition and structure while simultaneously showing protein binding characteristics (Feeney 1976; Rhoades and Cates 1976). The CT structure or content can be extremely different within species (Azuhnwi et al. 2011) and among species (Mueller-Harvey 2006) and also can vary in response to environmental conditions such as soil quality and microclimate (Tiemann et al. 2010). MacAdam and Griggs (2006) showed BFT is a productive, persistent, and hardy legume under irrigation in regions of the United States. Due to temperate seasons and altitude, the CT concentration in BFT produced in the mountain west region is commonly lower than BFT grown in other locations (18.7 vs. 24.2 g/kg for different regions of North America, (Grabber et al. 2013), while it is 23.6 g/kg, in New Zealand, based on Woodward et al. (2000) research and 21.6 g/kg, in New Zealand, based on Jacobs and Woodward et al. (2009).

In near neutral pH's, CT in BFT binds to proteins which leads to decreases in ruminal protein degradation and production of ammonia, whereas in abomasum with a low pH, this complex disassociate, releasing protein to be digested and absorbed in lower digestive tract of ruminants (Waghorn et al. 1987a).

Milk production elevation in dairy cattle (Turner et al. 2005; Woodward et al. 2009) as well as weight gain in beef cattle (Wen et al. 2002; MacAdam et al. 2011) and lambs (Douglas et al. 1999) follow distinctive epicatechins-based type (Waghorn 2008) and concentration (ranging from 20 to 120 g/kg; Aerts et al. 1999) characteristics of condensed tannins in BFT.

Sainfoin (SF; *Onobrychis viciifolia*) is one of the common CT-contained plants (Scharenberg et al. 2009) showed lower acceptance by feeding SF hay to lactating dairy cows as a supplement with other forages, also some experiments demonstrated SF had a higher palatability index than BFT (Scharenberg et al. 2007b), and in a pretest in nonlactating dairy cows, SF hay was more preferred than BFT hay (Scharenberg et al. 2009). These inconsistent results could be caused by different amounts of CT in the diets, structure of CT, or both (Hymes-Fecht et al. 2013). Grosse Brinkhaus et al. (2016) replaced AH with SF at 20% of basal ration which resulted in some effects. The activity and structure of the CT in the two tanniferous forage legumes were different, so different results were likely due to large amounts in CT content. The SF variety and growing conditions favored a very high CT content with a diet percentage of 3.6%, while in BFT diet the amount of CT was 0.5% which is likely too low to cause observation of much effect.

Effects of Condensed Tannins on N Metabolism and Productive Performance

There are important parameters that influence partitioning and excretion of forage type and source. A common source of dairy nutrition is corn and legume silage, where the former is rich in fermentable starch and can pair with RDP present in legume silage for use by rumen microbes following which N excretion to the environment may decrease. While total N intake can determine, N excreted through manure, carbohydrate type (e.g. sugars found in grass silage vs. starch from corn silage) and species of forages (e.g. BFT legumes vs. grasses) have the most important effect on N excretion path whether be urinary or fecal. In a recent study by (Halmemies-Beauchet-Filleau et al. 2014), two TMR rations containing red clover silage (RCS) and grass silage (GS) were compared. DMI and milk production tended to increase when GS and RCS were fed as a compound rather than fed alone. Polyphenol oxidase is a compound found in red clover which can bond to protein. N balance of whole body shows a tendency to reduce when RCS increases in the ration. Furthermore, dietary N proportion excreted in urine and feces and dietary N utilization for milk protein synthesis linearly increased. N partitioning in urine and feces increased when a reduction in N recovery in milk was observed (Halmemies-Beauchet-Filleau et al. 2014).

Some legume plants like BFT containing suitable amounts of CT are capable of reducing plant protein degradation into NH_3-N in the rumen which is different from the functioning of polyphenol oxidase existent in the clover since the CT content of the BFT can free proteins in abomasum resulting in feedstuff efficiency and N excretion waste decrease. Patra and Saxena (2011) showed that using BFT they can decrease urinary N excretion. Although another study using CT in the rations did not observe any effects on urinary or fecal N excretion, they found some improvement in milk yield efficiency (Hymes-Fecht et al. 2013). On the other hand, some positive responses in N utilization efficiency have been observed in some research done on BFT (Woodward et al. 2009). Grosse Brinkhaus et al. (2016) believed CT is a suitable tool which can reduce degradation of protein in the rumen significantly (via binding with proteins under ruminal pH) when fed early lactation dairy cows with three different diets containing SF, AH, and BFT (they replaced AH with SF or BFT about 20% DM), by referring to MUN, BUN and ruminal ammonia as evident, they observed reduced protein degradation feeding SF, but not with BFT. They presumed that lack of BFT effect in their study must have resulted from a lower CT content in the used BFT. Lower concentrations of BUN and ruminal ammonia have been frequently reported when fed SF (Scharenberg et al. 2007a; Arrigo and Dohme 2009; Azuhnwi et al. 2013). Whereas Hymes-Fecht et al. (2013) observed a urea reduction in MUN, BUN and urine of lactating dairy cows fed BFT.

At pH below 4, CT complexes are released (Mangan 1988), which is the case that happens in abomasum and thus the protected protein from microbes' degradation in the rumen can easily be digested in small intestine (Waghorn 2008). This protein can contribute in supplying metabolic protein. Beauchemin et al. (2007) demonstrated that when feeding CT, the N utilization process does not experience substantial improvement as CT- protein complex separation does not occur completely. Furthermore, Grosse Brinkhaus et al. (2016) reported a reduction in apparent total tract digestibility of N, and also observed no effects in the increase of N retention in milk or body by feeding plants containing high levels of CT such as SF. But by feeding BFT they observed a minor increase in retention of body N at the same excretion of N and a minor higher intake of N. It is then assumed that these animals may have received slightly more protein for gaining BW or maintenance; however, the authors declared that such an effect cannot be related to CT content of diets as SF content is several times higher than BFT content. Overall, they declared retention of body N were positive for all treatments while showing a trend to be lower for cows fed SF compared to cows fed BFT. Also, AH treatment effects were intermediate.

To explain these contradictory results, careful investigation of CT structure and content in two plants (SF = sainfoin; BFT = birdsfoot trefoil) which are sources of CT, but with different characteristics, is needed. Investigating SF and BFT gives us some clues that not all effects come from CT content differences. CT structure is a determining factor. There is some variation between these two forages in CT bound to fiber and protein which is 62 and 37% for BFT and SF, respectively, and soluble CT proportion is 38 and 63% for BFT and SF, respectively (Mueller-Harvey 2006). Moreover, the CT structure (procyanidins and prodelphinidins proportions) between BFT and SF is different. The procyanidins occurs frequently in BFT, while prodelphinidins is dominant in SF (Mueller-Harvey 2006). There are some findings which show if CT concentration in the plant is less than 90 g/kg of DM, inclusive, the CT can bind to proteins which are derived from the plant itself, while provided a higher CT content, it can play a free CT role and might interact with other ingredients (nutrients) in rations or microbes in the rumen (Barry and McNabb 1999). Grosse Brinkhaus et al. (2016) believed some of differences in their study between BFT and SF may have originated from these CT characteristics where CT content of SF was significantly higher than CT content of BFT. Altogether, in order to discover effects of diets containing legumes such as BFT on other parts of the diet and influences that BFT might have on N utilization efficiency and partitioning in dairy cattle, more studies need to be done.

In intensive dairy feeding systems, dairy lactating cows are fed with TMRs to have optimal production. In past centuries cows have been selected genetically to produce higher milk production on formulated rations, and grazing-based rations do not always supply the optimum requirements which are necessary for dairy cows with high levels of production and genetic potential (Kolver and Muller 1998). On pasture based feeding, there are some factors which tend to be limiting for a higher productivity of dairy cows such as DMI and utilized available protein and energy (Kolver 2003). Waghorn and Clark (2004) showed fresh forages contain high amounts of water and typically have 12-30% dry matter which is not comparable with TMRs with typical 35-60% dry matter (Robinson et al. 1990). Therefore, dairy cows need to intake higher amounts of fresh forages to fulfill their nutrient requirements as these forages tend to have lower available energy, high NDF and protein compared to TMRs (Bargo et al. 2003). High NDF content in forages means lower levels of digestibility, and thus lower DMI for cows (Allen 1996).

Hymes-Fecht et al. (2013) reported replacing alfalfa (*Medicago sativa*) silage with BFT silage, in TMR, enhanced N utilization and performance in lactating dairy cows. The dairy cows that offered fresh BFT in barn (Woodward et al. 2000) or fed BFT pasture (Turner et al. 2005) showed enhanced performance compared with cows offered white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*). When Scharenberg et al. (2009) fed SF hay to dairy cows as a supplement, they reported low acceptance, although the SF palatability index was higher than BFT in sheep (Scharenberg et al. 2007b). In a pretest in nonlactating dairy cows (Scharenberg et al. 2009), SF hay was more acceptable than BFT hay. These contradictory effects could have resulted from different dietary CT contents (Hymes-Fecht et al. 2013), the structure of CT, or both. The structure and content of CT can change significantly within species (Azuhnwi et al. 2011) or among species (Mueller-Harvey 2006) and can vary in interaction with different environmental conditions, such as soil quality and microclimate (Tiemann et al. 2010).

In a recent study by Grosse Brinkhaus et al. (2016) when fed three different diets containing SF, AH, and BFT, total yields of milk, milk fat, milk protein, intake and excretion of N in milk of dairy cows were similar in all treatments. In another study conducted by Christensen et al. (2015) cows were fed three diets containing BT, AHT, and ABT, where milk production tended to increase for cows consuming ABT or BT diets compared to AHT, while DMI and CP were similar among all treatments. Also ABT and BT diets resulted in decreased fiber intake compared with AHT.

Milk production elevation in dairy cattle (Turner et al. 2005; Woodward et al. 2009) as well as weight gain in beef cattle (Wen et al. 2002; MacAdam et al. 2011) and lambs (Douglas et al. 1999) follows distinctive epicatechins-based type (Waghorn 2008) and concentration (ranging from 20 to 120 g/kg; Aerts et al. 1999) characteristics of condensed tannins in BFT.

Reinhardt et al. (2012) showed average daily gain (ADG) for cattle in common feedlot feeding approximately from 0.79 to 2.43 kg/d for a period from 90-120 days. Wen et al. (2002) demonstrated cattle ADG spring gains feeding BFT were 1.26 to 1.53 kg/d in pure BFT stands in Missouri and others in Mountain West (i.e., Idaho, Utah, Montana, Wyoming, and parts of Nevada and Arizona) observed those gains to be 1.30 to 1.64 kg/d in pure BFT stands in 61-77 days (MacAdam et al. 2011). Such gains and production amounts are uncommon in pasture-based productions and can be explained by CT in BFT. For example, Waghorn et al. (1987) showed proteins which bound to BFT were not utilized as an energy source in the rumen but presented more in abomasum and digested and absorbed more than other proteins in other species which bound to CT. This leads to elevated levels of feed efficiency and AA absorption in the ruminants fed BFT.

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 (Logan). The study was conducted at the Caine Dairy Research Center (Wellsville, UT), Utah State University from September to November 2014.

Experimental Layout and Design

The experiment was conducted as a crossover design with two groups of eight cows, two treatments, and two 16-d periods. The first 14 d of each period were used to gradually transition animals to the next treatment, and the last 2 d were used for measurements.

The experiment consisted of two 16-d periods arranged in a crossover design. Cows were adjusted to the diets from day 1 to 14, milk production and composition measured, and data on total N balance collected on 2 d from d 15 to 16. The crossover periods were separated by a 7-d washout period.

Hays, Cows, and Experimental Diets

The source for the alfalfa hay-containing diets was a third cutting from a mature stand, and was cut the year before the experiment started. Forage was cut at pre-bloom stage with a conventional mower-conditioner (Model 830, John Deere, Moline, IL). The cut forage was allowed to sun-cure for 2 d in the field, turned once with a rake to reduce leaf loss, and then baled later that day with a conventional square baler at between 85 and 87% DM. The hay was stored in metal hay barns until the time of the trial. Hay was bright green, fine stemmed, and mold-free. The birdsfoot trefoil hay used in this experiment was Norcen variety and planted in fall 2013 on a private ranch (Garland, UT). It was also third cutting and harvested the year previous to the experiment by mower-conditioner (Model 830) and allowed to sun-cure for 5 d before baling. Chemical composition of the hays and corn silage used to construct experimental diets is reported in Table 2. Eight multiparous Holstein dairy cows in mid-lactation (days-in-milk = 150 ± 22.3) were paired by days-in-milk, previous 305-day mature equivalent milk production, and body weight (BW; mean = 721 ± 56.3 kg at the beginning of trial), and the cows were randomly assigned into one of two dietary treatments.

Cows were housed individually in tie-stalls fitted with rubber mattresses covered with straw, and allowed free access to water. Cows were individually fed twice daily for ad libitum intake at 110% of expected daily intake, with 70% of allotted feed fed at 0600 h and 30% fed at 1500 h. Feed offered and refused was recorded daily, and samples were taken during the sampling week to determine DMI. Cows were milked twice daily at

Table 2. Chemical composition (means ± SD) of forages (n = 3)

	Forage		
Item, % of DM	Alfalfa hay	Birdsfoot trefoil hay	Corn silage
DM	93.1 ± 0.25	89.4 ± 3.47	35.9 ± 0.67
OM	89.3 ± 0.76	88.1 ± 0.37	92.4 ± 0.21
CP	20.4 ± 0.93	15.9 ± 0.25	8.50 ± 0.001
NDF	31.6 ± 4.28	35.7 ± 4.59	43.4 ± 1.12
ADF	25.1 ± 0.79	30.8 ± 0.93	26.5 ± 0.67
NFC[a]	31.9 ± 1.14	29.7 ± 0.19	43.1 ± 2.15
Condensed tannins	ND[b]	2.80 ± 0.21	-

[a]NFC = 100 – CP – NDF – ether extract – ash.
[b]Not detected.

0400 and 1600 h, and milk production was recorded throughout the entire experiment During total collection period, milk yield was measured at each milking using a Perfection 3000 Boumatic weigh meter system (Boumatic, Madison, WI), and milk samples were taken by proportional sampler during the morning and afternoon milkings on d 15 and 16 of each experimental period. Milk samples were stored at 4°C and preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA). Individual milk samples were analyzed by Rocky Mountain DHIA Laboratory (Wellsville, UT) for fat, true protein, lactose, and MUN. Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Milk fat and protein yields were calculated by multiplying milk yield from the respective day by fat and true protein concentration of the milk from an 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, where milk true protein and MUN were expressed as kilograms per day. After completion of each total collection period at d 16, experimental apparatus was removed from cows and they were returned to a normal herd for a 7-d washout period. Experimental procedures were repeated for the second period after 2-wk adaptation period to new experimental diets. Two treatment diets were formulated with a forage-to-concentrate ratio of 68:32, differing only in a hay source used. High-forage diets are typically fed in the Intermountain West USA (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada), a relatively greater legume such as alfalfa hay compared with the Midwestern USA; baled alfalfa hay commonly provides 50 to 75% of the dietary forage with total forage levels averaging 45 to 55% of dietary DM (Holt et al. 2010). Dietary treatments consisted of either alfalfa hay-based TMR (AHT; 40% alfalfa hay, 20% corn silage, and 40% concentrates in DM basis) or BFT-based TMR (BFTT; 40% BFT hay, 20% corn silage, and 40% concentrates in DM basis) (Table 3). The diets were formulated based on NRC (2001) recommendations to provide sufficient NE_L, metabolizable protein, vitamins, and minerals to produce 30 kg of milk/d with 3.5% fat and 3.0% true protein with 23 kg/d of DMI and 750 kg of body weight (BCS = 3.0). The diets were mixed for 15 min in a TMR wagon (Model 455, Roto-Mix, Dodge City, KS).

Data Collection and Sampling

During the total collection, offered and refused feed fed to cows was weighed and sampled to determine the daily DMI. Samples of alfalfa hay, BFT hay, and corn silage were sampled weekly to determine DM concentration, and dietary concentrations of forages and concentrates were adjusted every week on an as-fed basis to reflect changes in the concentrations of nutrients due to change in DM. Sampled feeds were composited by period for chemical analysis. Samples of the TMR and orts for each cow were taken on a daily basis during the sampling week, composited by period, and stored frozen at −20°C until analyzed.

Total urine collections were made from all cows on d 15 to 16 using indwelling Foley catheters (26 French, 75-cc balloon; C. R. Bard, Inc., Covington, GA). Catheters were inserted in the bladder of each cow using local anesthesia under direction of the farm veterinarian on d 14 of each experimental period. Urine output was measured

every 6 h for 2 d. Clean 25-L plastic containers with 480 mL of 4 N H2SO4, included to acidify the urine as it entered the containers, were attached to catheters

Table 3: Ingredients and chemical composition (means± SD) of experimental diets fed to lactating dairy cows.

	Experimental diet[a]	
Item	AHT	BFTT
Alfalfa hay	46.0	-
Birdsfoot trefoil hay	-	49.4
Oat hay	3.59	-
Corn silage	18.5	18.0
Corn grain, flaked	16.2	15.5
Soybean meal	6.33	7.13
Canola meal	6.33	7.13
Calcium carbonate	1.01	1.01
Salt	0.31	0.31
Urea	0.70	0.70
Magnesium oxide	0.18	0.18
Sodium bicarbonate	0.70	0.70
Vitamins and minerals[b]	0.14	0.14
Chemical composition (% DM ± SD)[c]		
DM (%)	57.5 ± 2.19	56.3 ± 0.89
OM	90.9 ± 0.41	91.5 ± 1.08
CP	15.6 ± 0.17	15.1 ± 1.01
RDP (% of CP)[c]	63.7	62.7
RUP (% of CP)[c]	36.3	37.3
NDF	33.4 ± 0.37	35.7 ± 0.45
ADF	21.2 ± 2.49	22.6 ± 0.66
NFC	44.2	44.6
NE_L, (Mcal kg^{-1})[d]	1.61	1.63
Condensed tannins	0.06 ± 0.030	1.36 ± 0.154

[a] AHT= alfalfa-based TMR; BFTT= birdsfoot trefoil-based TMR.

[b] 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 on 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).

[c] Values shown with standard deviation of means (n = 3). DM, dry matter; OM, organic matter; CP, crude protein; RDP, rumen-degradable protein; RUP, rumen-undegradable protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; NFC, non-fiber carbohydrates =100 – (NDF, % + CP, % + EE, % + ash, %).

at 0200 and were emptied at 0800, 1500 and 2100 h to obtain daily composite volume and samples (final pH < 3). After the weight of the acidified urine was recorded, 2 sets of 5-mL aliquots were taken at each collection time point, combined for each day, and the pH confirmed to be < 3 and stored at −20°C until the analysis of Urinary N concentration.

Total fecal collections were taken on all cows on d 15 to 16 using a portable fecal collection box. Immediately after a cow defecated, the feces were placed in a sealed plastic container. Feces were weighed and thoroughly mixed daily, then subsampled. Fecal samples (approximately 400 g) were dried in a forced air oven at 60°C, ground in a Wiley mill (2-mm screen), and stored in airtight containers.

Ruminal fluid samples were obtained using a Geishauser probe 4 h after the morning feeding on d 15 and 16. The fluid was collected with a solid, tube-like probe with rows of small holes on the end (Geishauser 1993). The first 100 mL of ruminal fluid was discharged to avoid contamination from saliva, and then 400 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). Five milliliters of the ruminal fluid was mixed with 1 mL of 1% sulfuric acid and stored frozen (−40°C) for ammonia-N (NH3-N) analysis. Another 5 mL of the ruminal fluid was retained and mixed with 1 mL of 25% metaphosphoric acid, and then stored at −40°C for VFA content determination.

Chemical Analyses

Concentration of DM of pooled hays, diets, and orts was determined by drying at

55°C for 48 h in a forced air oven. Dried samples were ground using a Wiley mill (Standard Model 4; Arthur H. Thomas Co., Swedesboro, NJ) fitted with a 1-mm screen and stored at −20°C for chemical analyses. The DM concentrations of the samples were used to calculate intakes of DM and nutrients. Analytical DM concentration of samples was determined by oven-drying overnight at 105°C, and OM was determined by ashing in a 550°C muffle furnace oven for 5 h (AOAC 2000; Method 942.05). Crude protein concentration was determined by combustion methodology (Flash 2000 Automatic Elemental Analyzer, Thermo Fisher Scientific; (AOAC 2000; Method 990.03). 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 manufacturer, based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination, and samples were pretreated with heat-stable amylase (Type XI-A from Bacillus subtilis; Sigma-Aldrich Corporation, St. Louis, MO). Nutrient compositions of fecal samples were determined using the same procedures used for feed sample analyses.

Condensed tannins of alfalfa hay and birdsfoot trefoil hay were isolated from ground samples of the hay (0.5 mm) using the modified HCl-butanol-acetone assay (Grabber et al. 2013), and a spectrophotometer (Bio-Mate 3, Thermo Fisher Scientific, Madison, WI) was used to quantify the CT. Purified CT from birdsfoot trefoil was used as a standard. Diet concentrations of CT were 0.06 ± 0.030 and 1.36 ± 0.154% in AH and BFT hay, respectively (Table 3).

Urine samples were thawed and composited per cow by period. Urinary urea-N was analyzed using a commercial kit (Stanbio Urea-N Kit 580, Stanbio Laboratory Inc., San Antonio, TX). Urinary N was calculated based on Wattiaux and Karg (2004) equation.

Apparent total-tract digestibility of DM and nutrients were measured using two different methods: acid-insoluble ash (AIA) as an internal marker (Van Keulen and Young 1977) vs. total fecal collection. Fecal samples (approximately 200 g, wet weight) were collected for each cow from the fecal collection. 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 in −20°C for chemical analysis.

Concentration of NH_3-N in the ruminal contents was determined as described by Rhine et al. (1998), using a plate reader (MRXe; Dynex Technologies Inc., Chantilly, VA). Ruminal VFA were separated and quantified using a GLC (Model 6890 Series II; Hewlett-Packard Co., Avondale, PA) with a capillary column (30 m × 0.32-mm i.d., 1-μm phase thickness, Zebron ZB-FAAP; Phenomenex Inc., Torrance, CA) 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).

Statistical Analysis

Data were analyzed with the mixed model procedure of SAS (SAS Institute 2013). Individual cow was the experimental unit for all variables. The model for all parameters reported in the current study included the fixed effect of dietary treatments, group, and their interaction; cow and period were considered random effects. Degrees of freedom were adjusted using the Kenward-Roger option. Least squares means are reported throughout. Treatment effects were declared significant at $P \leq 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Characteristics of Experimental Diets

Concentration of CP was 20.4 and 15.9% for alfalfa hay and BFT hay, respectively (Table 2). Concentrations of NDF and ADF were greater in BFT hay compared with alfalfa hay, whereas non-fiber carbohydrate concentration was greater in alfalfa hay. Birdsfoot trefoil hay contained 2.8% CT, while alfalfa hay did not contain CT. Nutritive value of *Lotus* species including BFT is often considered similar to or even better than alfalfa and white clover. Concentration of CP in *Lotus* species ranges from 16 to 24%, while its NDF concentration varies between 28 and 36% and DM digestibility is 72-78% (Blumenthal and McGraw 1999). Therefore, the BFT hay used in the present study had reduced quality of nutrients while the alfalfa hay was of greater quality; according to Holt et al. (2013), alfalfa hay containing 20.7% CP was considered of a high quality. Due to high adaptability and yield, Ahlgren (1956) stated that in the early 1900's BFT was a major pasture source in the northern part of the US. The use of BFT as a harvested feedstuff was less common as alfalfa cultivars became more available. Due to many similarities between BFT and alfalfa hay species, a comparison between the two can be a remarkable means of evaluating BFT and alfalfa, where the most common difference is that BFT contains tannins and alfalfa usually does not.

The forage to concentrate ratio of the diets offered to cows was 68:32. To keep this ratio equal in both treatments, oat hay was added to the AH diet as alfalfa hay had less NDF and more CP. To supply enough CP, soybean and canola meals was used in both diets but was included at a slightly higher percentage in the BFT diet. Diets were approximately isonitrogenous across treatments, averaging 15.0% CP, and isocaloric (1.60 Mcal/kg) as shown in Table 3. Diets were formulated to meet NRC (2001) recommendations for RDP, RUP, NDF, ADF, NE_L, minerals, and vitamins of a mid-lactation dairy cow.

Intake and Digestibility of DM and Nutrients

In the present study, intakes of DM, NDF ADF did not differ between treatments (Table 4). However, CP intake tended to decrease by feeding BFTT ($P = 0.09$). Although BFT hay in the current study was very mature and contained more NDF than alfalfa hay (Table 2), feeding BFTT did not result in decreased NDF intake compared with AHT. Our findings are in agreement with Christensen et al. (2015) who reported no difference on DMI between AHT and BFTT. Previous studies on the effects of CT extracts or feeding CT-containing forages on feed intake in ruminants have resulted in contradictory 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 reported due to feeding BFT silage-based diets (Woodward et al. 2001; 2.59% CT), whereas Baah et al. (2007) did not report any effect of supplementing CT extract on DMI in either Jersey heifers (with 0.60% quebracho CT extract) or lactating dairy cows (Benchaar et al. 2008; with 0.45% CT). Conversely, decreased DMI with CT has been reported in sheep in a study Priolo et al. (2000) with 2.5% CT from carob pulp or dairy cows by Dschaak et al. (2011) with 2.3% CT from quebracho CT extract and Barry and McNabb (1999) with 7.5 to 10.0% CT from BFT pasture. In the current study, our results suggest that the CT in the BFT hay diets did not negatively affect DMI. The CT concentration of BFTT (1.36%) in the present study is similar to the one used by Hymes-Fecht et al. (2013) who reported no differences in DMI due to different CT concentrations.

In both studies, CT concentration was less than 5.0% of DM which is the threshold reported to suppress intake (Patra and Saxena 2011). Whereas DMI was similar between dietary treatments in the current study, in a comparative study of commonly cultivated forages, voluntary feed intake relative to cell wall content considerably

Table 4. Intake and digestibility of DM and nutrients in lactating dairy cows fed different legume hay-based diets

	Dietary treatment[a]			
Item	AHT	BFTT	SEM	*P*
Intake (kg d^{-1})				
DM	21.4	20.7	0.83	0.46
CP	3.41	3.16	0.143	0.09
NDF	7.22	7.70	0.321	0.18
ADF	4.57	4.75	0.256	0.49
Digestibility based on marker (%)				
DM	67.8	70.0	1.40	0.03
OM	73.5	73.2	1.16	0.65
CP	64.8	69.1	1.36	< 0.01
NDF	55.2	56.0	2.84	0.65
ADF	53.9	48.8	2.63	0.07
Digestibility based on total fecal collection (%)				
DM	68.0	64.8	1.46	0.20
OM	75.6	71.1	1.02	< 0.01
CP	69.1	60.7	1.71	< 0.01
NDF	55.6	51.7	1.90	0.18
ADF	52.4	46.1	2.89	0.21
Digested NDF intake (kg d^{-1})	4.00	4.30	0.312	0.75
Undigested NDF intake (kg d^{-1})	3.24	3.37	0.214	0.87
BW (kg)	721	721	29.5	0.86

[a] AHT = alfalfa hay-based TMR; BFTT = birdsfoot trefoil hay-based TMR.

increased for BFT hay compared with alfalfa hay (Van Soest 1965).

Using AIA, digestibilities of DM and CP increased by feeding BFTT, while ADF digestibility tended to decrease by feeding BFTT (P = 0.07; Table 4). In contrast, based on total collection of feces, digestibilities of DM, NDF, and ADF were similar between treatments, but OM and CP digestibilities decreased by feeding BFTT. Generally, marker-based digestibility results are inconsistent with those of total collection. Therefore, outputs in this area should be interpreted with more attention when applying the AIA approach. For example, Van Keulen and Young (1977) showed that DM digestibility estimations by total fecal collection were similar to AIA method. Block et al. (1981) reported correlation coefficients for digestibility between total collection and AIA approaches in wethers fed hay (orchard grass and alfalfa), corn plants (whole plant or stover corn plant diets stored fresh and frozen or ensiled), and cows fed different hays (orchard grass and alfalfa) which were 0.40, 0.96 and 0.95, respectively. The lower value (0.40) for wethers fed hay was due to high quantity of orts containing variable AIA concentrations. The author claimed that AIA, as an internal marker, was suitable for estimating digestibility compared with total collection when the experiment is well-organized. For example, when the number of animals is high enough, the offered diet is ad libitum, well mixed to avoid sorting and selection, and feed and orts are sampled while intake is determined. Feed intake is typically measured for multiple days (for example, 1 wk in a Latin square design), and fecal samples are taken simultaneously with the measurement of the feed intake. Thus, apparent differences in digestibilities between AIA and total fecal collection reported in the present study should be originated from a short fecal collection period (2 d) for AIA method. In fact, it is not uncommon in a dairy experiment to collect fecal samples for 5 d to estimate nutrient digestibilities (Eun et al. 2014). In a very recent study, de Souza et al. (2015) compared markers with total collection in a cross-over design with 10-d adaptation and 4-d sampling. They reported apparent digestibility measured using TiO_2 (titanium dioxide) as an external marker, was more similar to total collection results in dairy cows relative to Cr_2O_3 (chromic oxide). There was greater precision, lower error, and greater accuracy in estimating forage intake, fecal excretion, and digestibility. The primary drawback of Cr_2O_3 was its movement through the animal digestive tract independent of undigested diet, and consequently fecal Cr_2O_3 concentrations exhibit diurnal variation (Lippke 2002). The authors tried to increase the number of Cr_2O_3 administration per day (through cannula) to solve this problem, but were not successful. Glindemann et al. (2009) reported a similar problem with TiO_2, but when they administrated (through cannula) TiO_2 twice per day they were successful in eliminating this disadvantage. These studies (Lippke 2002; Glindemann et al. 2009) indicate that markers have some limits compared with total collection such as length of sampling for marker estimation which is an important key to have an accurate comparison between marker and total collection methods.

In a recent study, Grosse Brinkhaus et al. (2016) reported no changes in OM digestibility when cows were fed three different concentrations of CT-containing diets (alfalfa, SF, and BFT diets). The effects of CT on feed intake have been inconsistent among studies in ruminants. Baah et al. (2007) reported no change in DMI in heifers containing 0.6% of quebracho trees CT extract (containing 70% of CT) in a high concentrate diet. Beauchemin et al. (2007) also reported that DMI did not change in beef cattle fed higher levels of CT extract (1 and 2% of DMI, containing 90% CT) in a high forage diet. In contrast, other studies indicated increased DMI in sheep (Carulla et al. 2005) fed Acacia mearnsii extract (4.1% of DMI, containing 61.5% CT) and in dairy cows (Woodward et al. 2001) fed ensiled BFT (2.6% CT). Barry and McNabb (1999) observed DMI was not decreased in sheep fed diets containing 3 to 4% CT. However, greater concentrations of CT (7.5 to 10%) substantially reduced DMI in sheep. These results show CT have negative effects on DMI in ruminants when fed high concentrations and the impacts may differ based on CT source. Supplementation of CT may decrease fermentation of carbohydrate (Carulla et al. 2005) due to formation of complexes between cellulose, hemicellulose, and pectin with CT (McSweeney et al. 2001). Consequently, leading to reduction in fiber digestibility due to feeding CT-containing legumes (Scharenberg et al. 2007a) or adding CT extracts (Carulla et al. 2005). Apparent total tract digestibilities of DM, OM, and CP were not influenced in lactating dairy cows fed different CT mixture (Benchaar et al. 2006, 2007). Benchaar et al. (2007) observed no changes in fiber and starch apparent total tract digestibilities in cows fed a CT mixture at 43 mg/kg of DMI. However, digestibilities of these nutrients were elevated when a greater dose (87 mg/kg of DMI) of mixture (Benchaar et al. 2006) was used, implying that may be a dose response. The different digestibilities in this study, may be because BTF was more mature than alfalfa due to higher NDF and ADF.

In this study, CP digestibility was lower in BFTT than AHT diet based on total collection (60.7% vs. 69.1, respectively). Christensen et al. (2015) and Grosse Brinkhaus et al. (2016) reported higher CP digestibility for BFT compared with alfalfa, which is inconsistent with our results. This may be from the use of a more mature and poorer quality BFT in this study compared with the BFT used in the other two studies.

Christensen et al. (2015) fed second crop and earlier harvested BFT hay, while the cows in this study were fed a very mature BFT hay with a higher stem-to-leaf ratio.

Milk Production and Efficiency

Milk yield averaged 28.8 kg/d, and was not different between dietary treatments (Table 5). In addition, ECM and FCM yields were similar between AHT and BFTT. Dietary treatments did not affect milk components and their yields. Feeding either AHT or BFTT resulted in similar feed efficiencies. The result for milk yields are in agreement with Christensen et al. (2015) and Grosse Brinkhaus et al. (2016) where the authors did not report any effect on milk production by feeding BFT compared to alfalfa. In contrast, Woodward et al. (2000) and Hymes-Fecht et al. (2013) reported increased milk yield in cows fed BFT. The concentration of CT in the BFTT of the current study was approximately similar to both studies: 1.9% CT by Woodward et al. (2000) and 1.6% CT by Hymes-Fecht et al. (2013). Other factors than CT may influence milk production by dairy cows fed BFT. In the current study, CP concentration in alfalfa was 4.5 percentage units higher than that in BFT. Woodward et al. (2000) reported higher milk yield and DMI due to higher pasture quality with greater CP concentration.

Woodward et al. (1999; 2000) and Hymes-Fecht et al. (2013) also reported that digestibility of total diet fiber varied in response to CT concentration, suggesting that the relationship between concentration of CT and nutrient digestibility can have varying effects on milk yield; on average the authors reported up to 40% milk yield increment between cows fed *Lotus* compared with ryegrass. However, there were no effects on digestibility in the current study comparing alfalfa to *Lotus*, which strongly suggests that lower BFT hay quality (protein concentration) in the BFTT may not have contributed to increased nutrient utilization for milk production. This reduction in hay protein was adjusted for by adding more protein supplements which may have increased the overall diet digestibility (Bargo et al. 2003), reduced the potentially beneficial effects of CT on N metabolism (Baah et al. 2007), and created an associative effect on forage digestion (Niderkorn and Baumont 2009) that could not have been elucidated in the present study parameters. Feeding CT-containing diets to dairy cows has been shown in other studies to affect milk protein composition (Christensen et al. 2015; Grosse Brinkhaus et al.

Table 5. Milk yield and composition of lactating dairy cows fed different legume hay-based diets

	Dietary treatment[a]			
Item	AHT	BFTT	SEM	*P*
Lactational performance				
Milk (kg d^{-1})	29.4	28.1	2.64	0.47
ECM (kg d^{-1})[b]	28.9	27.2	2.47	0.31
3.5% FCM (kg d^{-1})[c]	27.8	26.6	2.34	0.38
Milk composition (%)				
Fat	3.20	3.21	0.162	0.67
True protein	3.20	3.16	0.100	0.35
Lactose	4.56	4.54	0.118	0.80
Milk component yield (kg d^{-1})				
Fat	0.93	0.89	0.082	0.40
True protein	0.95	0.86	0.080	0.13
Lactose	1.36	1.29	0.145	0.39
Solids-not-fat	2.57	2.40	0.250	0.30
Efficiency				
Milk yield/DMI	1.39	1.35	0.106	0.59
3.5% FCM/ DMI	1.35	1.26	0.134	0.31
ECM yield/DMI	1.35	1.30	0.106	0.49

[a] AHT = alfalfa hay-based TMR; BFTT = birdsfoot trefoil hay-based TMR.
[b] ECM = (0.327 × milk yield) + (12.95 × milk fat yield) + (7.20 × milk protein yield).
[c] 3.5% FCM = (0.432 × milk yield) + (16.2 × milk fat yield).

2016).

In the present study, milk protein concentration did not change due to feeding BFT hay, but it increased in other studies feeding BFT silage with similar CT concentrations (Hymes-Fecht et al. 2013) and BFT hay (Christensen et al. 2015; Grosse Brinkhaus et al. 2016). In the aforementioned studies, cows fed diets with the highest CT concentration (1.6, 0.50, and 3.0% DM) also had the highest milk protein concentration. This supports the theory of CT-dietary N complexes that form in the rumen and can lead to higher dietary protein absorption in the small intestine which is then converted to milk protein. However, in the current work, we did not observe this pattern by feeding BFT hay compared to alfalfa, perhaps due to a reduced CT concentration in the hay and reduced overall diet CT as well as lower BFT hay quality.

Ruminal Fermentation Characteristics

Mean ruminal pH averaged 6.32 in both treatments (Table 6), which is normal for cows fed high-forage diets. Dietary NDF concentration for all treatments in the current study were sufficient to maintain the optimal ruminal pH. Dietary treatments did not influence ruminal pH. Similarly, Hymes-Fecht et al. (2013) reported minor effects on ruminal pH by feeding a BFT silage-based diet compared with a alfalfa silage-based diet. Christensen et al. (2015) also observed a similar pattern for rumen pH when cows were fed BFT compared to alfalfa hay. Although feeding BFTT tended to decrease total VFA concentration ($P = 0.07$), it had little impact on ruminal fermentation, because feeding BFTT reduced the total VFA concertation at only 2% compared with feeding AHT. If the total VFA concentration should have been a consequence of feeding BFTT, it may have resulted from CT effects on fiber digestion and alteration of rumen microbes' species. In literature, there have been inconsistent results reported on the effect of feeding CT-containing forages on ruminal VFA concentration. Carulla et al. (2005) reported that VFA concentrations did not change in sheep that were fed diets supplemented with CT-containing black wattle (*Acacia mearnsii*). Grosse Brinkhaus et al. (2016) demonstrated that diets containing SF (2.2% CT) had the lowest VFA concentration but not BFT-containing diets (3.0% CT). Additionally, Hymes-Fecht et al. (2013) observed low VFA concentration in the diet with 1.6% DM CT. Waghorn and Shelton (1997) reported a diet containing 1.8% of CT (from *Lotus pedunculatus*) resulted in a noticeable decrease in ruminal VFA and CP digestibility, whereas the same amount of CT from *Lotus corniculatus* had lesser effects. The tendency for decreased total VFA concentration due to feeding BFT in the current study suggests lower fermentability by feeding BFT hay, which could be explained by higher NDF and ADF contents, and consequently lower OM and CP digestibilities in BFTT diet compared to AHT diet. Feeding BFTT decreased molar proportions of isobutyrate and isovalerate compared with AHT. The decreased proportions of the branched-chain VFA may support decreased NDF digestibility, as the branched-chain VFA functions support growth factors for fibrolytic bacteria (Bryant 1973).

Concentration of NH_3-N, a representative of dietary protein degradation (Broderick 1995), was different between two diets (9.90 vs. 5.50 mg 100 mL^{-1} for AHT and BFTT, respectively, Table 6). Tannin-protein complexes inhibit the fermentation of forage protein to NH_3-N 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. Concentration of NH_3-N in the current study indicates that CT concentration may have been enough to form substantial tannin-protein complexes, one of the most important effects of CT on ruminal fermentation. Lower ruminal NH_3-N concentration has been frequently reported when feeding CT-containing forages such as SF (Scharenberg et al. 2007a; Arrigo and Dohme 2009; Azuhnwi et al. 2013). Under typical cattle-feeding conditions manipulation of ruminal protein degradation, or 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 increased, NH3-N accumulation in the fermenters decreased (R2 = 0.78). Thus, feeding BFT hay can

Table 6. Ruminal fermentation characteristics of lactating dairy cows fed different legume hay-based diets

	Dietary treatment[a]			
Item	AHT	BFTT	SEM	*P*
Mean pH	6.27	6.37	0.09	0.45
Total VFA (mmol)	112.7	110.3	5.76	0.07
Individual VFA (mol 100 mol $^{-1}$)				
Acetate (A)	66.7	66.3	0.61	0.48
Propionate (P)	20.2	20.4	0.60	0.75
Butyrate (B)	9.42	10.2	0.26	0.15
Isobutyrate	0.79	0.63	0.032	< 0.01
Isovaleric	1.24	1.01	0.109	< 0.01
Valeric	1.32	1.31	0.050	0.74
Caproic	0.295	0.345	0.0382	0.38
A:P	3.37	3.32	0.11	0.73
(A+B): P	3.85	3.83	0.13	0.92
NH_3-N (mg 100 mL^{-1})	9.90	5.50	0.579	< 0.01

[a] AHT = alfalfa hay-based TMR; BFTT = birdsfoot trefoil hay-based TMR.

contribute to improving utilization of dietary N in ruminal fermentation and reducing N excretion. In addition, NH3-N reduction in the rumen could have resulted from the combined effects of decreased dietary protein degradation, decreased protein solubility oran inhibition of proteolytic bacteria or proteolytic enzymatic activity (Noviandi et al. 2014). However, in the present study, CT is probably the main force on reduced ruminal NH_3-N concentration.

Utilization of N

There was a trend for lower N intake in BFTT compared to AHT diet (*P* = 0.09; Table 7). Since DMI was the same, this lower N intake can be explained by lower CP concentration in BFTT than AHT diets. Concentration of MUN, which reflects dietary N utilization and ruminal NH_3-N (Nousiainen et al. 2004), was lower in BFTT treatment compared to AHT. Hymes-Fecht et al. (2013) reported the lowest MUN in cows fed the highest amount of CT (1.6% DM) which is comparable with the CT amount in our study. Grosse Brinkhaus et al. (2016) observed the lowest MUN concentration in the SF diet which had intermediate CT content (2.2% DM). One way to reduce ruminal protein degradation is by using CT-containing feedstuff. The CT can bind to protein (Hagerman and Butler 1981) under ruminal pH conditions and reduce excess RDP. This is due to microbes' inability to degrade CT or CT-protein complexes (McSweeney et al. 2001). In the present study, BFTT reduced ruminal ammonia, and MUN. Lower ruminal ammonia concentrations have been repeatedly observed when feeding forages containing CT such as SF (Scharenberg et al. 2007a; Arrigo and Dohme 2009; Azuhnwi et al. 2013). In addition, Benchaar et al. (2008), and Christensen et al. (2015) observed no effect of

Table 7. Nitrogen partitioning of lactating dairy cows fed different legume hay-based diets

	Dietary treatment[a]			
Item	AHT	BFTT	SEM	*P*
N intake (g d^{-1})	548	505	23.1	0.09
Milk protein output (g d^{-1})	947	864	80.4	0.13
Milk N output (g d^{-1})	158	145	13.4	0.13
MUN (mg dL^{-1})	13.3	11.9	0.37	< 0.01
Milk NPN output (g d^{-1})	5.95	5.67	0.548	0.41
Total urine output, wet (kg d^{-1})	26.0	20.4	1.24	< 0.01
Urinary N output (g d^{-1})[b]	272	227	14.1	< 0.01
UUN (mg dL^{-1})	680	754	56.2	0.31
UUN output (g d^{-1})	169	156	11.9	0.39
Total fecal output, wet (kg d^{-1})	47.3	50.1	2.01	0.13
Fecal output, dry (kg d^{-1})	6.89	7.17	0.27	0.41
Fecal N, wet (%)	2.47	2.66	0.04	< 0.01
Fecal N output (g d^{-1})	168	194	6.18	< 0.01
N output of feces and urine (g d^{-1})	437	422	18.0	0.24
Total N output (milk + urine + feces; g d^{-1})	588	561	22.6	0.82
Milk N:N intake[c]	0.28	0.29	0.032	0.39
UN:FN[d]	1.00	0.83	0.061	0.03
MkN:MaN[e]	0.46	0.42	0.044	0.82

[a] AHT = alfalfa hay-based TMR; BFTT = birdsfoot trefoil hay-based TMR.
[b] Predicted using the following equation: 0.026 × MUN (mg)/100 mL × BW (kg) (Wattiaux and Karg 2004).
[c] Efficiency of use of feed N to milk N.
[d] UN:FN = ratio of urinary N to fecal N, where urinary N and fecal N are expressed in g/d.
[e] MkN:MaN = ratio of milk N to manure N, where milk N and manure N are expressed in g/d.

CT supplementation on MUN concentration.

Urinary N excretion was higher (P < 0.01) for AHT compared to BFTT diet (272 vs. 227 g/d, respectively). Grosse Brinkhaus et al. (2016) observed that urinary excretion of N and its percentage of N intake were lower in SF than in alfalfa, but not in BFT cows. Moreover, Hymes-Fecht et al. (2013) reported lowest N excretion and N components via urine in the diets which had the highest amount of BFT.

Conversely, Christensen et al. (2015) demonstrated highest N dietary excretion in the diet containing BFT compared to alfalfa. These results suggest there are some inconsistency about urea N excretion in animals fed CT-containing forages. Our findings show lower ruminal NH3-N in BFT compared with AHT diet. This implies lower degradable protein in the rumen, due to CT-protein complexes in the rumen in BFT treatment.

Fecal N excretion was lower in AHT compared to BFTT diet-based on total collection (168 vs. 194 g/d, respectively) and equations (107 vs. 147 g/d, respectively). Several studies have shown that BFT fed to dairy cows as a fresh forage (Woodward et al. 2000), preserved silage (Hymes-Fecht et al. 2013) and hay (Grosse Brinkhaus et al. 2016) shifted excretion of N to feces. Hymes-Fecht et al. (2013) indicated that CT in BFT silage diverted excretion of N from urine to feces, which led to decreased ammonia emissions from manure. In a recent study authors showed fecal N excretion was highest for the diet containing SF which had the moderate CT concentration (Grosse Brinkhaus et al. 2016; 2.2% CT). Some studies have indicated there is a full compensation in the lowered N excretion in urine, which shifts to fecal N, and has been reported repeatedly when SF was fed to sheep (Scharenberg et al. 2007a; Azuhnwi et al. 2013). One explanation for higher fecal N excretion in BFTT as compared to AHT diet is that CT-protein complex leading to higher N escapes from rumen degradation into the small intestine, and a higher N percentage is evacuated through feces.

The sum of urinary and fecal N excretion amounts (manure) did not differ between treatments (437 vs. 422 g/d, respectively, alfalfa and BFTT diets). This finding is in agreement with Grosse Brinkhaus et al. (2016) who reported no difference in manure N excretion in feeding diets containing alfalfa, SF and BFT.

CONCLUSIONS

Feeding BFT hay in high-forage lactation diets did not influence digestibilities for DM, NDF, and ADF compared to AH. However, feeding BFT hay decreased CP and OM digestibilities compared with AH. Although forage nutritive values of BFT hay were less than AH, feeding BFT hay did not change milk yield, milk components, and ruminal VFA production. Cows fed BFT hay reduced urinary N, MUN, and ruminal NH_3-N concentrations compared to those fed AH. Feeding BFT hay resulted in changing N excretion pathway from urine to feces, which can lower volatile N compounds into the environment. All these results imply that lower protein degradation in the rumen resulted from the functional effects of CT in the BFT hay by binding dietary N and reducing ruminal N degradation. Therefore, our results on changes in N partitioning support our hypothesis of the effects of CT in BFT. However, the shift in N partition between urine and feces was not associated with lactational performance and N utilization efficiency due mainly to poor nutritive values of BFT hay in the current study. We have not determined how the shift in N excretions by feeding BFT hay influence whole farm N management, which will guide comprehensive benefits of feeding BFT hay toward sustainable dairy production. It is concluded that replacing AH in dairy rations with BFT hay can have a positive environmental effect while maintaining similar lactational performance.

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