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Serkan Ates

This study investigated the effects of diversification strategy through the inclusion of forb- and legume-based pastures in the feedbase of dairy cows on annual forage production, botanical composition, spring milk yield, N partitioning and methane emissions from dairy cows in Western Oregon. Using a randomized complete block design, 3 plots served as blocks. Those were subdivided into three pasture types resulting in 9 grazing plots, which served as the experiment unit. The pasture production component examined the productivity and species composition of three types of specialized pasture: grass-, forb- and legume-based pastures (Chapter 3). The second component of the study involved grazing dairy cattle on the three pasture types over two grazing periods and the feed intake, milk production, milk components, nitrogen partitioning and enteric methane production were measured (Chapter 4). A total of 27 Jersey dairy cows were randomly assigned to 9 grazing plots in 29 April. Cows were allocated approximately 16 kg/d of pasture with a post-grazing residual of 1300 kg of DM/ha in both periods. DMI, milk yield, composition, urine and fecal nitrogen output was taken on d 15, 18, and 21 and was repeated in the second period.

Methane emissions were collected using the SF_6 tracer method from d 16 to d 21 in the first grazing period. The total annual DM production of pastures were comparable (P=0.28). Grass based pastures had substantially higher ($P < 0.05$) early spring production that exceeded 3700 kg DM/ha. Whereas forb-based pastures had the highest ($P < 0.05$) summer DM yield at 4500 kg DM/ha with legume-based pastures around 4300 kg DM/ha. Herbage DMI was highest in cows that grazed legume-based pastures at 15 kg DM/cow/day and lowest in cows grazing grass-based pastures ($P < 0.05$). Legume-based pastures were the highest nutritive value with the exception of NDF which forb-based pastures had the lowest ($\mathrm{P} < 0.01$). Legume-based pastures yielded between 22.7 – 23.1 L/d of milk, highest of the three treatments ($P < 0.01$). Milk fat content tended to be higher in legume-based pastures ($P = 0.07$) while milk protein content remained unaffected by pasture type ($P = 0.31$). Forb-based pasture diet decreased N (%) of urine substantially ($P < 0.01$) and increased fecal N (%) ($P < 0.01$). Microbial protein supply remained unaffected by treatment ($P = 0.14$). Methane emission tended to be decreased by forb-based pastures ($P = 0.07$). These studies indicate a potential of legume- and forb-based pastures to fulfill nutritional deficiencies in late spring in pasture based dairy production as well as a potential to reduce the environmental impact of pasture-based dairy production.

Milk Production, Pasture Performance, and Environmental Sustainability of Specialized Pastures

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Randi Wilson, Author

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LIST OF ABBREVIATIONS

Abbreviation	Description	Units
ADF	Acid detergent fiber	%
ADG	Average daily gain	g of gain/d
ARS	Agricultural Research Service	
ATP	Adenosine triphosphate	
BCS	Body condition score	
BCT	Bound condensed tannins	mg/g
BW	Body weight	kg
CF	Crude fiber	%
CT	Condensed tannin	mg/g
CH_4	Methane	g/d/cow
CO_2	Carbon dioxide	
CP	Crude protein	%
CT	Condensed tannins	
DDG	Dried distiller's grain	
DM	Dry matter	%
DMD	Dry matter digestibility	%
DMI	Dry matter intake	kg/cow/d
FCM	Fat-corrected milk	Lt/cow/d
GHG	Greenhouse gases	
H_2	Metabolic hydrogen	
HAB	Hyper ammonia-producing bacteria	
ME	Metabolizable Energy	
MUN	Milk urea nitrogen	
N	Nitrogen	
N_2O	Nitrogen oxide	
NDF	Neutral detergent fiber	
PD	Purine derivatives	
PM	Rising plate meter	½ cm
PSM	Plant secondary metabolites	
RBP	Rumen bypass protein	
SCC	Somatic cell counts	
SF6	Sulfur hexaflouride	
SNF	Solid non-fat	%
TP	Total phenolic	
UCT	Unbound condensed tannins	mg/g
TMR	Total mixed ration	
USDA	United States Department of Agriculture	
VFA	Volatile Fatty Acids	

CHAPTER 1
INTRODUCTION

1.1 General Introduction

In Oregon, dairy production is the 4th leading commodity and valued at over 500 million dollars (USDA FASS, 2014). Over 40% of Oregon dairy operations statewide are pasture-based feeding systems where majority of the source of feed comes from home-grown forages and pastures from March to November (T. Kerr, personal communication, Feb. 22, 2018). About 15% of Oregon acreage is utilized for forage growth; 80% of which is utilized as livestock feed either as hay, silage, or pasture livestock grazing (Shewmaker *et al*., 2015). Most pasture-based dairy operations are located off the northern coast in Tillamook County and in the Willamette Valley (T. Kerr, personal communication, Feb. 22, 2018) where the temperate climate allows for the abundant growth of forages.

The main reason dairy farmers in Western Oregon must practice partial confinement in early spring and late fall is due to climate and soil type. Grazing during that time risks damage to pasture through compaction and pugging of the soil; subsequently decreasing forage yield. The practice of partial confinement increases the cost of feed and labor during that time, while also reducing the quality of pasture regrowth in late summer. Low-quality forages later in the grazing season require producers to supplement to meet their cattle's nutritional requirements, which subsequently increases production costs. In the dairy production industry, operational decisions are largely dependent on cost, especially with the saturated market driving down the price of milk. The number one cost to Oregon dairy production is feed with grazed pasture being the cheapest available feed to producers (USDA ERS). Though dairy cattle produce less on pasture, the decrease in cost for labor and feed makes up the difference as well as increases the health and longevity of the cows (White *et al*., 2002).

The agro-ecological conditions of Oregon are highly conducive to grow a wide range of forages. However, management of pastures in Western Oregon is challenging due to environmental restrictions and implications (Downing, 2018). Oregon's Mediterranean climate is optimal for pasture growth and can produce over 22,000 kg DM/ha per year of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture (Downing, 2009), but physically grazing when grass-clover pasture production and quality is optimal for dairy production is difficult due to the high risk of pasture damage in the heavy, clay-type soil. Intensive grazing can result in soil pugging and compaction, severely damaging plant regrowth over time. In Oregon, deferred grazing during times of high rainfall is the preferred method used to decrease soil and plant damage. This practice, though beneficial to safeguarding soil and pasture, can increase dairy production cost through increases in labor, manure management, and use of supplemental feeds (Laurenson *et al*., 2015). Deferment of grazing also leads to a decrease in nutritive value of grass-clover pasture from maturity when it is finally able to graze. This will ultimately result in decreased milk production and increase in production cost from supplemental feeds. However, novel, summer-active forages consisting of forbs and legumes have been shown to help improve milk production in summer as the quantity and quality of the grass-clover pastures go down (Waghorn and Clark, 2004).

These plants maintain feeding value through summer and possess chemical compounds that have been found to increase milk yield and animal health, as well as decrease both enteric methane emissions and nitrogen emissions (Waghorn and Clark, 2004). Feeding condensed tannins through birdsfoot trefoil at 13.6 g/kg of DM increased milk yield in dairy cattle up to 27% when compared to perennial ryegrass only diet (Woodward *et al*., 2000). Feeding of birdsfoot trefoil hay as a replacement for alfalfa indicates a shift in nitrogen output from urine to feces, significantly decreasing urinary N losses (Ghelichkhan *et al*., 2018). Both birdsfoot trefoil and the forb, chicory, decrease enteric methane emissions by including in the diet (Ramírez-Restrepo and Barry, 2005). These results show that condensed tannin producing forage forbs and legumes are invaluable tools for improving the production and sustainability of dairy production systems.

Most research studying the potential of specialized forage crops in dairy productions have taken place in New Zealand, Southern Australia, and Ireland, but have yet to be heavily explored in Western Oregon despite having similar Mediterranean-type climates. This thesis intends to cover and research the use of specialized pastures in Western Oregon dairy production and their effect on milk production as well as the sustainability of these systems.

1.2 Aims and Research Objectives

The aims of this research are (i) to compare pasture production and animal performance of diverse grass-, forb-, and legume-based dairy cow pasture and (ii) to assess the sustainability of these grazing systems using methane and nitrogen emission measurements. The research addressed three different specific objectives:

1. Determine annual and seasonal production and botanical composition of forage in grass-, forb- and legume-based pastures.
2. Assess the effects of including forb and legume pasture in the feedbase of dairy cows on production of milk and components.

3. Assess the effects of grass, legume and forb based pastures on urinary, milk, and fecal N losses and methane emissions.

CHAPTER 2
LITERATURE REVIEW

2.1. Agroecological Conditions and Challenges for Pasture Management

Pastures provide an inexpensive source of high-quality feed for livestock, but grazing does not come without challenges. Climate is the main governing factor affecting the pasture growth and the producer's grazing plans. In Oregon, the climate varies across the state. Due to the rain shadow effect of the Cascades, Eastern Oregon possesses an arid climate in contrast to Coastal Oregon where the state has high rainfall, but mild and generally uniform temperatures throughout the year (Shewmaker *et al*., 2015). In Western Oregon, between the Coastal Range and Cascades, the temperature changes vary throughout the year, but unlike Eastern Oregon has high rainfall in the fall, winter, and early spring, reaching around 1000 mm annually (PRISM Climate Group). This climate zone is well-suited to grow the forage needed to meet the nutritional demands of lactating dairy cattle during spring and early summer.

However, the amount of rainfall combined with soil type can make grazing challenging when the forages are at optimal grazing quality. In order to avoid damaging their pasture, most grazing dairies in Oregon house their cattle indoors from November to April and supplement hay and/or silage. The practice of partial confinement is largely due to the excessive rainfall during the cool-season and heavy clay soils which cause waterlogging and inevitably leads to the pugging and compaction of the soil if grazed by cattle too early in spring (Shewmaker *et al*., 2015). This practice, despite saving the forage and soil from physical damage, negatively affects the forage nutritional quality and future forage regrowth (L'huillier *et al*., 1987). Without grazing or mechanical removal, the forage begins to lignify, becoming more fibrous and of decreasing feed value. Low quality forages cannot satisfy energy requirements of lactating dairy cows.

Soil type and soil health are also a huge factor in both the planning of grazing and the production of forages. In Oregon, soil types of agricultural areas vary from the poorly-drained Brenner silt clay loam of Tillamook County in the Northwest to well drained Actem cobbly loam of Harney County in the Southeast (Soil Survey Staff). In Western Oregon silty clay loam soils are the most prevalent in agricultural locations, particularly in the Willamette Valley. Clay soils are a great medium of growth for forage species but are not great for grazing in early spring due to waterlogging problems. These soils tend to have high water-holding capacities and low pH (Soil Survey Staff). The latter can be a major limiting factor for the persistence and yield for all pasture species in Western Oregon.

Low soil pH (pH < 6) can adversely affect the yield of forage crops that are not tolerant to soil acidity. Legumes show decreased persistence and low establishment due the sensitivity of the symbiotic bacteria in root nodules due to soil acidity (Begrenji *et al*., 2017). Yield of plants can also be inhibited by aluminium toxicity, which is directly related to soil acidity and negatively affects root elongation (Haling *et al*., 2011). Low pH is also implicated in reducing soil aggregate stability subsequently exacerbating compaction issues and reducing water and air accessibility and movement through soil (Karki and Goodman, 2011). These responses encourage management strategies like using forage crops that are resistant to low pH or applying lime to increase soil pH. Doing so improves forage growth and quality as well as stabilizes soil aggregates to improve soil quality (Karki and Goodman, 2011). The combination of these two aspects, soil and climate, contribute to challenges such as loss of forage production and feeding value leading to an increase cost of production.

Traditionally, producers of pasture-based dairies rely heavily on perennial ryegrass and white clover mixtures in their pastures. These pastures provide an abundance of high-quality feed in spring. However, in late spring and summer, these pastures decrease in quantity and quality, forcing producers to either supplement with hay or silage to support the nutrient needs of their high-producing dairy cattle. To decrease costs from supplementation and increase pasture quality in spring and summer grazing for Western Oregon dairy farms, a method of utilizing alternative, drought-tolerant and summer active forages in pastures is proposed. These forages include chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), birdsfoot trefoil (*Lotus corniculatus*), red clover (*Trifolium pratense*), berseem clover (*Trifolium alexandrinum*) and balansa clover (*Trifolium michelianum)* and have been shown to improve pasture production and feed availability in late spring and summer without negatively affecting cattle health, milk production, and overall production costs in New Zealand, Southern Australia, and other locations with similar climate and grazing restrictions as Western Oregon.

2.2 Pasture Production

Temperate climatic conditions are highly conducive to growing forages and are one of the reasons Oregon is leading the United States in grass seed production (USDA FASS, 2014). The most popular pasture mixture for permanent milk cattle pastures contain perennial ryegrass and white clover. This combination of perennial ryegrass and white clover is easy to establish and maintain, and they persist for 10 to 15 years (Rohweder and Albrecht, 1995). Seasonal and annual herbage yield of these pastures can vary according to grazing practices, climatic conditions and input management. Downing (2018) reported that in Tillamook County, Oregon, under intensive rotational grazing, grass-clover pastures have shown to yield between 12 and 24 t DM/ha annually with a daily DM growth rate between 47 and 56 kg/ha per day depending upon the annual precipitation and pasture management. In New Zealand, a country with comparable climate to Western Oregon, Waghorn and Clark (2004) reported that the total annual DM production of perennial irrigated dairy pastures ranged between 12 and 25 t DM/ha under intensive rotational grazing.

Perennial ryegrass is particularly susceptible to high temperatures and lack of soil moisture. Similarly, white clover often fails to persist in areas receiving less than 400 mm rainfall due to its shallow root system (Brock, 2006). Even with irrigation, perennial ryegrass will not continue to produce as much forage due to its physiological dormancy under high summer temperatures (Volarie and Norton, 2006). Furthermore, this pasture combination does not tolerate waterlogging as well, a problem prevalent in Western Oregon due to its heavy clay soils and high precipitation in the fall, winter, and spring. McFarlane *et al*. (2003) determined perennial ryegrass yield will decrease around 25% with as little as 4h of flooding. Forages in waterlogging areas tend not to persist and allow undesirable weeds to grow. Often these are unpalatable weeds to livestock and not beneficial to production.

2.2.1 Specialized Pastures

A proposed method of improving pasture growth and quality under the pressure of waterlogging and its reverse, drought, is to employ specialized forage species which have improved yield and nutritive value in summer where perennial ryegrass and white clover may be lacking. These plants include herbaceous species like chicory and plantain and niche legumes like birdsfoot trefoil and balansa clover. These forage species have been growing in popularity for use in grazing livestock production in areas like New Zealand and Southern Australia but have not been heavily studied for their use in dairy production in Western Oregon. These species have been shown in those areas to cause substantial improvement in lamb, beef, and dairy production, especially during summer when the production of perennial ryegrass and white clover pastures is of low quality both in yield and nutritive value. The forage legume that leads these crops in feeding value is alfalfa, a high yielding and fast-growing perennial legume. However, it is not grazed due to causing frothy bloat in ruminants and it is nearly impossible to grow in Western Oregon because of its need for well-drained, neutral pH soils (Orloff, 2007).

The combination of limitations previously stated has led Western Oregon grazers to search for other forage alternatives. Chicory is a high yielding forage in summer drought due to having deep taproots that allow them to access ground water. Similarly, birdsfoot trefoil also has a deep taproot to allow water access during drought, but also persists well in waterlogged areas where perennial ryegrass does not. Table 1 presents attributes of these and other similar crops. The total annual herbage yields of these crops have not been studied thoroughly in Western Oregon. In New Zealand, Minneé *et al*. (2015) determined chicory pastures can yield between 18 to 22 t DM/ha annually, with the latter being under irrigation. In dryland situations, Minneé *et al.* (2015). also determined plantain to yield between 14 and 19 t DM/ha annually. These plants are generally easy to establish but tend not to persist more than two years under intensive grazing pressure by dairy cattle (Nie *et al*., 2008; Minneé *et al*., 2015).

Table 1. Attributes of perennial forage crops with deep taproots

Species	**Productive Lifetime**	**Establishment**	**Soil Requirements**
Alfalfa	3-8 years	– Moderately slow – Sow at 6-10 kg/ha	– High soil fertility – pH > 5.8 – free-draining soil
Red Clover	2-3 years	– relatively fast – tetraploids: sow at 5-8 kg/ha – diploids: sow at 4-6 kg/ha	– high soil fertility – acid soils – tolerates most soil types
Birdsfoot trefoil	2-5 years	– slow, good weed control vital – sow at 8-12 kg/ha – specific *Rhizobium*	– low to moderate soil fertility – acid soils – free-draining soil, but flood tolerant
Sulla	1-2 years	– relatively fast – sow at 10-15 kg/ha – specific *Rhizobium*	– moderate soil fertility – pH > 6.0 – free-draining soils
Chicory	3-5 years	– relatively fast – sow at 5 kg/ha with white clover	– high soil fertility – acid soils – tolerates most soil types

Hodgson and White, 1999

On the other hand, birdsfoot trefoil tends to be hard to establish. It is sensitive to weed pressure and should be seeded with a nurse crop to ensure establishment (Hall and Cherney, 1993). In New Zealand, Armstrong (1974) studied the yield of different birdsfoot trefoil varieties. The birdsfoot trefoil variety, ‘Maku’, yielded nearly 6350 kg/ha annually under intensive grazing management, where another variety, ‘4701’, yielded around 4536 kg/ha annually under the same conditions. In a more recent study conducted by Ramírez-Restrepo *et al.* (2006) in New Zealand, over a three-year period birdsfoot trefoil yielded between 4808 kg/ha and 9616 kg/ha annually under rotational grazing by sheep, with the second year having the highest yield and the third year having the least. In North America, Hunt *et al.* (2016) showed that ‘Norcen’, a variety of birdsfoot trefoil developed in the US, can produce up to 7500 kg/ha in total dry matter production in an organic system depending upon the year. Conversely, Grabber *et al*. (2014) showed ‘Norcen’ only producing up to 2620 kg/ha despite not being an organic crop. Though birdsfoot trefoil yield can be variable, it seems to depend heavily on environment and management strategies.

2.2.2 Feeding Value of Pastures

Feeding value is the combined effects of forage nutritive value and animal feed intake. Feeding value of pastures are highly variable depending on the climate, soil conditions, pasture fertility, plant species and varieties. Feeding value of pastures declines with increasing plant maturity. For cool-season grasses such as perennial ryegrass and orchardgrass, the reduction in nutritive quality can be drastic. The increase in fiber content of pastures decreases animal intake due to the reduction of digestibility, the effect of physically filling the animal, and slowing digesta passage rate. Reduction of animal intake, along with the reduction of energy and protein in the forages, negatively influences production factors such as weight gain and milk yield. If forages mature, the cost of production increases with the need to supplement appropriate energy and protein to support production.

The feeding value of legumes also decrease with increasing maturity, but not to the extent of grasses. While grasses will mature, go to seed, and finally go dormant, legumes tend to remain more digestible and retain nutritive value even when they begin to flower and go to seed. Legumes also tend to be more active at higher temperatures then grasses and quite a few species survive well in drought-like environments (Waghorn *et al*., 1998).

Herbaceous forages, such as chicory and plantain, follow a similar trend as the legumes in retaining high nutritive value compared to grasses later in the grazing season. Chicory and plantain are summer-active and maintain green and leafy characteristics through summer due to a deep taproot that allows them to access water deeper in the soil (Nie *et al*. 2008). They tend to also be highly digestible due their high water soluble carbohydrate (WSC) contents. These easily digestible sugars contribute to the energy needed in the production animal's diet that grasses cannot meet in the summer. However, high WSC does indicate a need to supplement a fiber source in the form of hay or silage to prevent rumen acidosis.

2.2.3 Secondary Metabolites

The majority of these forage species, especially chicory and birdsfoot trefoil, produce plant secondary metabolites (PSM) as a defense against insects and microorganisms. PSMs include tannins, saponins, and polyphenolic compounds. These compounds have been reported to act as natural anthelminthics, antibiotics, and antioxidants in ruminant livestock production (Min *et al*., 2003; Piluzza *et al*., 2014; Harlow *et al*., 2017; Nwafor *et al*., 2017; Naumann *et al*., 2017). Indirectly, PSMs may help improve yield of animal products by improving animal health. These compounds also have potential for reducing methane and nitrogen emissions. Compounds in plantain like acubin, sorbital, and mannitol have been shown to act as a diuretic, causing the cow to urinate more often, thus diluting the urine of ruminants and reducing nitrogen leaching. These compounds have been observed to have antimicrobial affects as well (Rumball *et al*. 1997).

One PSM, condensed tannins (CT), can be produced by many forages including chicory and birdsfoot trefoil. CT-containing forages can be used as supplements to the normal diet to increase the efficiency of protein utilization by the ruminant (Nwafor *et al*., 2017). CT both bind proteins, protecting them from rumen degradation, and inhibit protein-metabolizing microorganisms. This process increases rumen bypass protein which the animal processes in the liver to be utilized as amino acids for milk synthesis and tissue generation. Since utilization of nitrogen in the form of amino acids is increased by the animal, nitrates being released into the environment through urine are decreased. It will also help increase rumen bypass protein (RBP) as well. However, if fed at higher than 4-5% of DM, CT and other PSMs can act as anti-nutritional factors, causing decreased feed intake and negatively affecting ruminant metabolism (Nwafor *et al*., 2017; Naumann *et al*., 2017).

Red clover, which will also be included in this study as a part of the legume component with birdsfoot trefoil, also has the potential to improve production via PSMs. Red clover produces isoflavanoids, called formononetic and biocanin A, which act similar to ionophores and inhibit hyper ammonia-producing bacteria (HAB) to help improve production (Flythe and Kagan, 2010). Harlow *et al*. (2017) determined that by including biocanin A (6.3 g/d) in the diet of grazing steers being supplemented 1.4 kg dried distiller's grain (DDG) improved the average daily gain (ADG) by nearly 23% from steers receiving no supplement and nearly 12% more from steers supplemented only with 1.4 kg DDG.

2.2 Environmental Sustainability of Milk production from Pastures

With the onset of global warming, there has been a growing consumer concern and research interest in the environmental impacts of agricultural systems throughout the world. With consumer push for the shift from confinement to pasture-based systems to decrease cost and meet consumer demand for improved animal welfare and healthier dairy products, sustainability of these systems has come into question. The sustainability of grazing systems is highly depenpent on management practices. Unsustainable grazing systems cause significant soil compaction, water pollution through nitrate leaching, and contribute to greenhouse gas (GHG) emissions. Dairy farms produce methane (CH_4), nitrogen oxide (N_2O), and anthropogenic carbon dioxide (CO_2) through the use of fossil fuels, manure storage and application, as well as by the cattle themselves (Saggar *et al.*, 2004). Grazing systems are also currently understood to have higher enteric methane emissions and nitrogen leaching issues than confinement systems (O'Brien *et al.*, 2014). Higher emissions in grazing systems are mostly due to a lack of waste handling and storage. This section will focus on the environmental effects of dairy cattle on soil health, nitrogen leaching problems, and methane emissions, also referred to as enteric emissions, produced by the cattle through rumen methanogenesis.

2.2.1 Grazing Impact on Water and Soil Health

Grazing pastures in Western Oregon in early spring can risk causing pugging and compaction damage, which will kill or hinder the future growth of forages and decrease the health of the soils in those areas. McFarlane *et al.* (2003) found that pugging damage can decrease overall pasture yield by 40% the next spring as well as decrease the tiller density of perennial ryegrass between 39-54%. Compaction from animals or machinery can physically prevent the elongation and growth of roots as well (Haling *et al.*, 2011). Therefore, often producers in Western Oregon utilize a deferred grazing method to avoid damaging their pastures in early spring (Laurenson *et al.*, 2016). Deferred grazing increases the cost of supplementation in the barn and promotes the decrease in forage quality later in the grazing season as the forages, primarily grasses, mature (Waghorn *et al.*, 1998).

Another environmental impact of grazing livestock is nitrogen leaching. Protein is an integral nutrient to producing both milk and meat, which is usually metabolized by rumen microorganisms into ammonia. The protein not utilized by the bacteria and the animal is transformed into urea and released as urine into the environment, which contributes to environmental nitrogen leaching issues. Nitrogen leaching by ruminant livestock has been linked to eutrophication of water sources, acidification of soils, and the indirect production of N_2O, a greenhouse gas with 310 times the global warming potential of CO_2 (Saggar *et al*., 2004). According to de Klein and Monaghan (2011) about 75-90% of all nitrogen ingested by intensively grazed livestock is lost in the form of urea in urine. De Klein *et al*. (2010) also determined that 95% of all nitrogen leached in a grazing system is from nitrogen deposited in urine patches, while the remaining 5% is from fertilizer and manure application. For this reason, mitigation of cow-level nitrogen leaching is pertinent to improving the sustainability of grazing systems. One strategy to decrease nitrogen leaching is by grazing forages containing PSM, which improve nitrogen utilization in the cow as well as by acting as a diuretic and diluting the urine.

Perennial ryegrass and white clover pastures tend to exceed ruminant protein requirements, thus exacerbating nitrogen leaching issues. Diversifying pastures with forbs and legumes have been proven to decrease nitrogen leaching in New Zealand and other geographical locations with similar climates to Western Oregon. Totty *et al*. (2013) showed that the inclusion of chicory, plantain, and big trefoil (L*otus pedunculatus*) in perennial ryegrass and white clover pastures significantly decreased urinary N output (g/d/cow) of Jersey/Holstein cross dairy cattle nearly 20% from cattle grazing pure perennial ryegrass and white clover swards. Another study by Cheng *et al*. (2017) followed up grazing Jersey/Holstein cross heifers on pure swards of chicory and plantain and their mixtures with perennial ryegrass and white clover. They measured urinary N output (g/d/cow) during fall and spring grazing periods. Though urinary N output was not significantly changed in fall, grazing pure chicory and plantain swards in spring significantly decreased urinary

N output nearly 27% from perennial ryegrass and white clover pastures. The diverse mixtures also decreased from perennial ryegrass, but only between 3-12% with the mixtures containing plantain having the greatest decrease.

Birdsfoot trefoil has also been examined for its value in reducing N output. However, grazing pure swards and even mixtures of birdsfoot trefoil and their effect on urinary N output with dairy has not been heavily studied. Despite that, many indoor feeding studies with birdsfoot trefoil hay and silage have been attempted. Ghelichkhan *et al*. (2018) showed feeding birdsfoot trefoil hay to Holstein dairy cattle significantly decreased urinary N and overall urine output when compared with feeding alfalfa hay, which is comparable to birdsfoot trefoil in feeding value. However, fecal N output was significantly increased. Ghelichkhan *et al*. (2018) hypothesized this was due to the protein-binding affect of the condensed tannins in the birdsfoot trefoil protecting the proteins from degradation throughout the digestive tract and being deposited in the feces. A similar, indoor feeding experiment with Holsteins by Christenson *et al*. (2015) compared birdsfoot trefoil hay and alfalfa hay based total mixed ration (TMR) and showed no significant difference in urinary N output. Hymes-Fecht *et al*. (2013) compared urinary N output of lactating Holsteins fed red clover, alfalfa, and birdfoot trefoil silages. Though there was not a significant difference between treatments, birdfoot trefoil silages made from cultivars of normal and high condensed tannin concentrations had a tendency to decrease urinary N output.

2.2.2 Grazing Impact on Air Quality

About 11% of global emissions are attributed to agriculture with about 8.4% of that being from the United States (Rotz, 2018). In the United States, beef and dairy industries have been implicated in producing 3.4% of total GHG emissions. Dairy cattle in particular produce less than half of those emissions at 1.3% (Rotz, 2018). According to the USDA ARS's Integrated Farm System Model (2015), grazing operations produce 15% less total emissions than confinement systems annually. Despite that, grazing dairy farms maintain a 10 to 20% higher carbon footprint (kg CO_2 equivalent) than confinement dairies due to greater enteric emissions from pasture grazing (Rotz, 2018). For this reason, livestock agriculture has come under a lot of public scrutiny for its environmental impact. As a result, there has been a spike in cattle methane emission research to better understand the phenomenon and also to decrease emissions in order to improve the consumer image and sustainability of dairy production.

Like many processes in the rumen, methanogenesis is performed by the rumen symbiotic microorganisms. These anaerobic microorganisms are aptly named methanogens and belong to the Domain Archaea. One of the major end products of rumen fermentation is metabolic hydrogen (H_2) (Boadi *et al*. 2017). It is utilized by methanogens in the rumen to produce CH_4, which releases energy in the form of adenosine triphosphate (ATP) that the microorganisms can use to power other processes (McSweeney *et al*., 2016) and prevents the build-up of excess H_2 (Patra *et al*. 2017). The generation of CH_4 also results in a 2-12% loss of energy from feed ingested by the cattle (Patra *et al*., 2017). There are quite a few different methanogens in the rumen and they all utilize different pathways, but the result is the same. After the methanogen produces the CH_4, it will rise to the top of the rumen until the cow eructates, or belches, out the gas into the atmosphere. Once in the atmosphere, methane reacts with hydroxyl radicals (-OH) to form carbon monoxide, nitrogen oxides, ozone, formaldehyde, and water vapor which exacerbates the effects of global warming (McSweeney *et al*., 2016).

Diet can significantly affect the methanogenesis processes in the rumen. The microbes that ferment cellulose to produce acetate and butyric acid, two of the three major volatile fatty acids (VFAs), produce the most H_2 for use by methanogens. Fermentation of starch from grain-based diets results in propionate, the third VFA, and water, leading to the suppression of methanogenic activity and subsequently reducing enteric emissions (Boadhi *et al*., 2017). However, a diet solely based on grain is harmful to rumen microbiota and subsequently the cow, so increasing grain in the diet is not a viable option for reducing methane emissions.

Enriquez-Hidalgo *et al*. (2014) compared the enteric methane emissions of dairy cows grazing perennial ryegrass pastures and pastures with 50% perennial ryegrass and 50% white clover. There was no significant difference with cows producing between 350 – 360 g/cow of methane per day. Alternatives to reducing enteric methane emissions have been found in supplemented natural, plant-derived compounds called secondary metabolites as discussed in a previous section. Condensed tannins are a secondary metabolite produced in both chicory and birdsfoot trefoil and have been shown to decrease methane emissions and increase ME intake through supplementation by grazing or feeding as a hay, silage, or even extract of the pure compound added to the feed (Naumann *et al*., 2017).

2.3 Milk Yield and Composition from Pastures

Milk production in pasture-based systems can vary, but generally is lower than in indoor-housing systems. Pasture-based systems in the United States will often supplement cows with concentrate during milking to meet the energy needs of high producing dairy cows. In comparison, dairies in New Zealand rarely supplements cows with concentrates. Table 2 documents milk yields of various grazing studies conducted in temperate systems similar to Western Oregon including

Ireland, New Zealand, and Southern Australia. Based upon the sward type, concentrate supplementation, breed of cattle, and stage of lactation, milk yield can be highly variable. Generally, milk yield tends to increase with increasing herbage allowance and concentrate supplementation.

Table 2. Summary of studies accessing milk yield of dairy cattle grazing differing sward types, herbage allowance, and concentrate supplementation

Sward Type	Herbage Allowance (kg/d/cow)	Concentrate Supplementation (kg/d of DM)	Cattle Breed	Average Milk Yield (kg/d)	Reference
PR	20	0	H	23.8	Curran *et al*., 2010[1]
PR	30	6	H	27.9	Muir *et al*., 2014
PR	16.9	1	H	18.3	Prendiville *et al*., 2009
PR	14.7	1	J	13.8	Prendiville *et al*., 2009
PR	16.2	1	X	16.7	Prendiville *et al*., 2009
CG/WC	-	5.5	J	24.8	White *et al*., 2001
PR/WC	14.3	5.4	J	19.0	van Wygaard *et al*., 2019
PR/WC	17	1	H, HR, NR	19.8	Enriquez-Hidalgo *et al*., 2014
PR/WC	16	0	X	15.2	Totty *et al*., 2013
CH	30	6	H	27.8	Muir *et al*., 2014
PR/CH	30	6	H	26.9	Muir *et al*., 2014
PR/WC/CH[2]	17	0	H, X	12.6	Minneé *et al*., 2017
PR/WC/PL[3]	17	0	H, X	12.2	Minneé *et al*., 2017
Diverse Mixture[4]	16	0	X	16.9	Totty *et al*., 2013
Diverse Mixture[5]	17	0	X	20.1	Bryant *et al*., 2016
Diverse Mixture[6]	35	0	X	18.5	Bryant *et al*., 2018
BFT[7]	60	0	H	16.5	Harris *et al*., 1998
BFT[8]	17.1	0	H	21.2	Woodward *et al*., 2000
SC	19	4	H	26.7	Wales & Doyle, 2003

Abbreviations: **PR** - Perennial ryegrass; **CG** – Crabgrass; **WC** - - White clover; **CH** - Chicory; **PL** - Plantain; **BFT** - Birdsfoot trefoil; **SC** - Subterranean clover **H** - Holstein; **J** – Jersey; **HR** - Holstein x Norwegian Red; **NR** - Norwegian Red; **X** - Holstein x Jersey

[1] Fed 4 kg/d concentrate and gradually weaned off prior to experimental period
[2] Chicory at 40% daily DMI
[3] Plantain at 40% daily DMI
[4] High-sugar ryegrass, White clover, Chicory, Plantain, & Birdsfoot trefoil
[5] Perennial ryegrass, Prairie grass, White clover, Red clover, Plantain, & Chicory
[6] Perennial ryegrass, Italian ryegrass, White clover, Chicory, Plantain, & Lucerne
[7] Birdsfoot trefoil at 73% DM of diet, also included White clover
[8] Fed fresh cut indoors

Prendiville *et al*. (2009) reported that Jersey cows that grazed perennial ryegrass produced milk with 5.3% fat, 4.1% protein, 4.5% lactose contents. Milk composition can be altered by type of feed or pasture grazed. White *et al*. (2001) showed that Jersey cattle being fed a TMR in confinement generally have a higher fat yield than Jerseys on grass-clover pasture. Jersey cows that fed in confinement had 4.1% fat while Jersey cows on pasture had a decreased fat percent of 3.7%. However, protein (%), SNF (%), and lactose (%) did not significantly differ.

Not much research of Jersey cattle grazing forbs and legumes and the effect on their milk yield and composition has been conducted, but Jersey × Holstein crosses have been heavily used in New Zealand for research concerning grazing with forbs and legumes. Though they are similar in size, Jersey × Holsteins tend to have lower yields in fat, protein, but have higher lactose and overall milk yields when compared to Jerseys on pasture (Prendiville *et al*. 2009). Totty *et al*. (2013) showed that Jersey × Holsteins crossbred cows grazing perennial ryegrass and white clover produced 6.1% fat, 4.1% protein, and 4.8% lactose in fall season. Bryant *et al*. (2018) reported that crossbreds grazing forb mixtures had 5.15% fat, 4.30% protein, and 4.89% lactose.

CHAPTER 3

Dry Matter Production of Grass-clover and Specialized Forb and Legume Pastures in Dryland Conditions

3.1 Introduction

Pastoral farming in Western Oregon is greatly constrained by the excessive waterlogging problems in winter to early spring and dry conditions in summer characterized with high evapotranspiration. Destocking to avoid pugging damages typically leads to low pasture utilization and accumulation of lower quality forages in the late spring–summer period resulting in poor quality pastures with low legume contents. This in turn causes significant reduction in grazing days and milk production while increasing the need for supplementary feed. A significant distinction between grasses and legumes is that reduction in feeding values of legumes with maturation is less profound than grasses (Waghorn *et al*., 1998). Pasture forbs like chicory and plantain also have high digestible organic matter in the summer and fall when compared to that of grass-clover pasture mixes (Box *et al*., 2017; Cheng *et al*., 2017). Diversifying pastures through specialized forb- and legume-based pastures help maintaining high growth rates and feeding quality (nutritive value + feed intake) towards summer and provide superior animal performance (Muir *et al*., 2014).

Legume species, such as birdsfoot trefoil (*Lotus corniculatus*), are highly tolerant to both waterlogging and summer dry conditions. In addition, birdsfoot trefoil contains highly active plant secondary metabolites (PSM) such as condensed tannins (CT). Birdsfoot trefoil requires less lime than white clover for nodulation in acidic soils, although it is typically slow to establish from the seedling stage to a mature plant (Ramírez-Restrepo *et al*., 2006). Despite their agro-ecological suitability and advantages in improving milk quality (increased n-3 and n-6 fatty acids), legumes containing condensed tannins do not have a major role in pastoral farming in Oregon because appropriate agronomic and grazing management practices have not been developed yet. One of the important features of this study will be the development of agronomically successful legume-based pastures through incorporating balansa clover (*Trifolium balansae*), a highly waterlogging tolerant, self-regenerating annual legume into the mixtures. It is thought the slow establishment and low forage production of birdsfoot trefoil in the year of establishment will be offset by balansa clover and their seasonal production will complement each other for an extended production period (Ates *et al*., 2010). Thus, this trial assessed the production capabilities such as seasonal DM yield, seasonal growth rates, and botanical composition of forb-based and legume-based pastures compared to grass-clover.

3.2 Materials and Method

3.2.1 Site, Establishment and Experimental Design

The study was conducted between 2018 and 2019 at the Oregon State University Dairy Research Farm in Corvallis, Oregon (44° 34’ N, 123° 18’ W 78 m a.sl.). The soil type is a combination of Amity silt loam, Holcomb silt loam, and Bashaw silty clay loam. Soil tests indicated the site had an organic matter content of 5.8%, 113 kg ha^{-1} available P 113 kg ha^{-1} (Bray), 1772 kg ha^{-1} Ca and 230 kg ha^{-1} K, 298 kg ha^{-1} Mg, 0.17 dS/m soluble salt, and that soil pH was 5.9.

The pastures were sown in a randomized complete block design with three replicates on 20 May 2018. A 5.85 ha paddock was divided into three, 1.95-ha blocks to serve as replicates for the experiment. Each block was divided into 3 subplots (0.65 ha), which were randomly allocated to a combination of grass- (1), forb- (2) or legume-based pastures (3), giving a total of 9 grazing plots. The pasture plants and their seeding rates were presented in Table 3.4. The three pasture mixtures were sown with 15-cm row spacing and were fertilized with 56 kg/ha N as urea at seeding. The pastures were irrigated to ensure successful establishment until August 2018 and then no irrigation was applied at other times. A mixture of balansa and berseem clover both at the seeding rate of 3 kg/ha were overdrilled in legume pastures on 18 September 2018. Pastures were rotationally grazed from early November to mid-December 2018 by weaned lambs to decrease the accumulated herbage mass in all plots to 1000 kg DM/ha. A total of 209 kg/ha of dairy effluent (liquid manure) was applied in October 2018.

Table 3. Pasture composition and seeding rates (kg/ha)

Species	**Common name**	**Forb**	**Legume**	**Grass**
X Festulolium	Festulolium			10
Festuca arundinacea	Tall fescue			10
Dactylis glomerata	O. grass			3
Trifolium repens	White clover	4		4
Cichorium intybus	Chicory	6		
Plantago lanceolata	Plantain	5		
Lotus corniculatus	Birdsfoot trefoil		10	
Trifolium pratense	Red clover		2	
Trifolium alexandrinum	Berseem clover		3	
Trifolium balansae	Balansa clover		3	

3.3 Meteorological Conditions

Monthly mean low and high air temperature from April 1, 2018 through October 30, 2019 are presented in Figure 3.1, Monthly mean high temperature for both 2018 and 2019 followed the same trend of long-term means of high air temperatures in most cases, whereas monthly lows seemed to diverge from the long-term means. Summer low temperatures tended to be higher and winter lows tended to be lower than the long-term means.

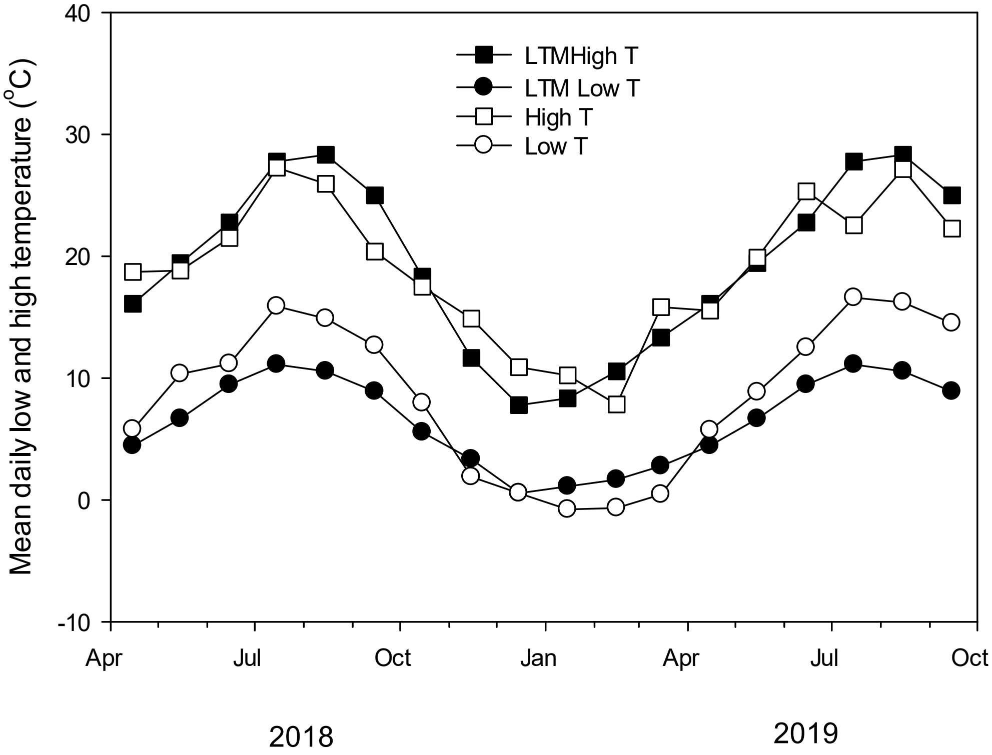

Figure 1. Monthly mean low (○) and high air temperature (□) from 1 April 2018 to 30 October 2019. Long-term means of low (●) and high (■) air temperature are for the period 1980-2010.

Monthly precipitation and long-term means of precipitation are presented in Figure 3.2. Monthly precipitation also diverged from the long-term means of precipitation. Most months received up to 50% lower precipitation rates then long term means with the exception of April of both years and February 2019, where it was nearly double from the long-term means.

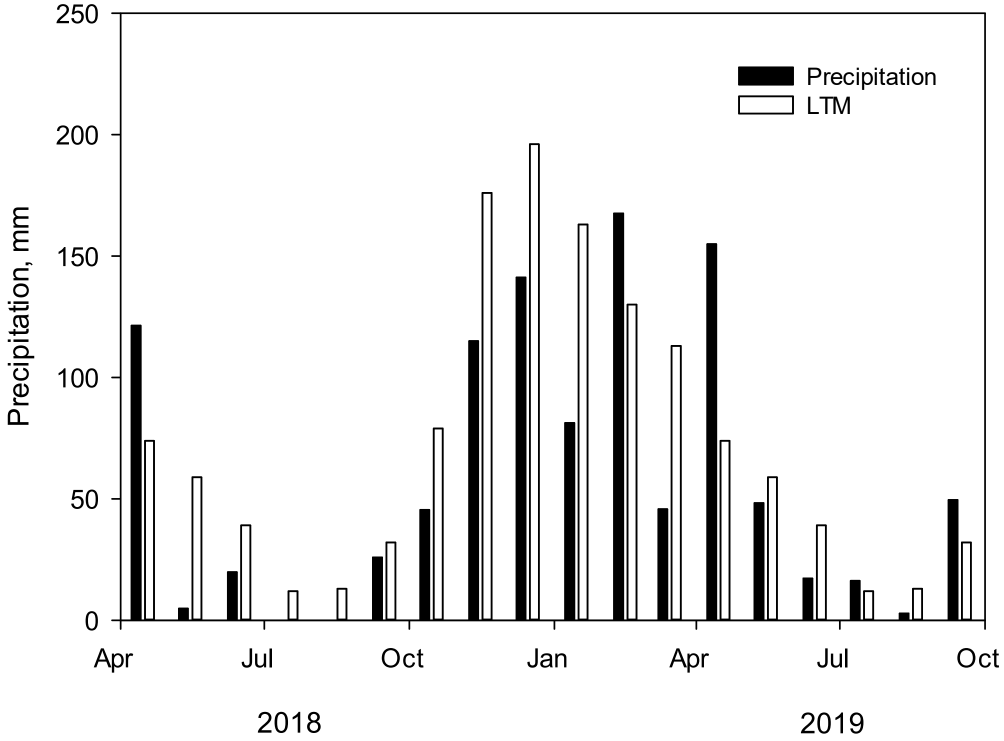

Figure 2. Monthly precipitation (■) from 1 April 2018 to 30 October 2019. Long-term means of precipitation (□) is for the period 1980-2010.

3.4 Measurements and calculations

3.4.1 Pasture dry matter production

Treatments were observed over the 2018-2019 growing season from 21 November 2018 to October 2019. Dry matter (DM) production (kg/ha) of pastures was measured inside 1m^2 grazing exclosure cages during active growth in spring, summer, and autumn. Plots were harvested roughly at monthly intervals during active growth and two-month intervals during fall and winter. Herbage growth was measured from a 0.25 m^2 quadrat by cutting with electric shears to a stubble height of approximately 6.0 cm. Exclosure cages were placed over a new representative area pre–trimmed to 6.0 cm stubble height at the start of each new growth period. After cutting, cages were relocated to new pre–trimmed sites in each pasture treatment. All herbage from the quadrat cuts were dried in an oven (65 °C) until constant weight.

3.4.2 Botanical Composition and Pasture Growth Rates

Quadrat cuts were sub–sampled for sorting into botanical fractions (grass, legume, forb, weed and dead material) before they were dried at 65 °C. Herbage growth rates (kg/ha/d) were calculated at each harvest by dividing total DM production by the number of elapsed days since the previous harvest.

3.4.3 Statistical analyses

Herbage DM yield and daily growth rates were analyzed for each regrowth cycle by ANOVA with three replicates. Botanical composition of the pasture treatments was not compared as the species that were used in each mixture were different. The computations were carried out using GENSTAT statistical software. Means were separated by Fishers protected L.S.D ($P<0.05$) when ANOVA was significant.

3.5 Results

3.5.1 Dry Matter Production

Seasonal DM yields (kg/ha) are presented in Table 4. At the beginning of establishment in fall 2018, Forb-based pastures had the greatest seasonal dry matter yield of the treatments at 1765 kg/ha, which was 20% more than grass pastures and 32% more than legume pastures ($P<0.01$). In the following spring, Grass pastures had the superior seasonal dry matter in April with 3736 kg/ha, which was 34% more than forb-based pastures and 24% more than legume pastures ($P<0.01$). In May 2019, Forb-based pastures again have the highest seasonal yield 3844 kg/ha ($P<0.01$). Grass-based pastures yielded the second highest in May at 2696 kg/ha and legume-based pastures had the lowest yield at 2096 kg/ha (*P*<0.01). June 2019 was the highest yielding month for all treatments with forb-based pastures at 4481 kg/ha, legume-based pastures at 4261 kg/ha, and grass-based pastures at 4012 kg/ha (*P*<0.05). In July 2019, the seasonal DM yield decreased dramatically with legume- and forb-based pastures having the highest at 2591 and 2316 kg/ha, respectively (P<0.01). Grass-based pastures had the lowest seasonal DM yield for July 2019 at 1551 kg/ha, 34-41% lower than the other treatments (*P*<0.01). Total annual DM yield (kg/ha) did not differ between treatments and ranged from 13.034 to 14.881 kg/ha/year (*P*=0.28).

Table 4. Seasonal dry matter yields (kg/ha) of grass-, forb-, and legume-based pastures in 2018/2019 growing season

Harvest dates	Grass	Forb	Legume	SE	P values
11 November 2018	1415b	1765a	1212b	108.8	0.01
9 April 2019	3736a	2475b	2874b	259.3	0.01
6 May 2019	2696b	3844a	2096c	199.4	0.01
18 June 2019	4012	4481	4261	279.2	0.50
22 July 2019	1551b	2316a	2591a	145.5	0.01
Total annual DM yield (kg/ha/year)	13.410	14.881	13.034	740.9	0.28

3.5.2 Mean Daily Growth Rates

Mean daily growth rates are presented in Table 5. In November 2018, forb-based pastures had the fastest herbage growth rate at 22 kg/ha/d, which was 23% faster than grass-based pasture and 32% faster than legume-based pasture herbage growth rates (*P*<0.01). In April 2019, Grass pastures had the fastest herbage growth rate at 27 kg/ha/d (*P*<0.01). Forb- and legume-based pastures had similar growth rates at 18 and 21 kg/ha/d, respectively. May 2019 showed the highest growth rates for forb-based pastures at 142 kg/ha/d and was 30% faster than grass-based pastures and 45% faster than legume-based pastures (*P*<0.01). In June 2019, forb-based again had the highest growth rates at 104 kg/ha/d, with legume-based pastures having intermediary rates at 99 kg/ha/d and grass-based pastures with the lowest at 93 kg/ha/d (*P*<0.05). In July, the growth rates for all pastures decreased with legume-based pastures having the highest at 76 kg/ha/d and growing 11% faster than forb-based pastures and 39% faster than grass-based pastures (*P*<0.01).

Table 5. Herbage growth rates (kg/ha/d) of grass-, forb- and legume-based pastures in 2018/2019 growing season

Periods	Grass	Forb	Legume	SE	P values
11 November 2018	17b	22a	15b	1.3	0.01
9 April 2019	27a	18b	21b	1.9	0.01
6 May 2019	100b	142a	78c	7.4	0.01
18 June 2019	93	104	99	6.5	0.50
22 July 2019	46c	68b	76a	4.3	0.01

3.5.3 Botanical Composition

Botanical composition for grass-, forb-, and legume-based pastures in the 2018 and 2019 growing season are presented in Figure 3. With the exception of November 2018 where clover and grass were nearly at a 50:50 ratio, grass dominated the composition of the grass-based pastures. Clover decreased dramatically, dropping nearly 32% from November 2018 to April 2019. By July 2019, clover was less than 1% of the botanical composition. The forb-based pastures were largely dominated by chicory with secondary levels of plantain and white clover. In the establishment year, chicory dominated at 69% with plantain at 12% and the remaining 19% including white clover, weeds, dead material, and annual ryegrass at nearly equal inclusions. In April 2019, chicory dominated even more at 87% with plantain at 8%, annual ryegrass at 3% and white clover at 0.9%.

In the establishment year, chicory dominated at 69% with plantain at 12% and the remaining 19% including white clover, weeds, dead material, and annual ryegrass at nearly equal inclusions. In April 2019, chicory dominated even more at 87% with plantain at 8%, annual ryegrass at 3% and white clover at 0.9%. In May 2019, chicory decreased to 42%, plantain increased to 23%, and clover increased to 14%. The remaining composition included 17% annual ryegrass, 3% weeds, and less than 1% of dead material. In June 2019, plantain decreased only 3%, while chicory increased to 66% of the composition. White clover inclusion decreased again to 3% while the number of weeds decreased to less than 1% and annual ryegrass decreased to 6%. Dead material increased to 4%. In July 2019, chicory and plantain continued to dominate at 59 and 27%, respectively, despite a 2% increase in weed inclusion. White clover also increased to 5%, while dead material increased by 1% and annual ryegrass decreased by 4%.

In legume-based pastures, red clover dominated for the entirety of the 2018 and 2019 growing season. Red clover remained between 32 and 47% throughout the 2018 and 2019 growing season with the exception of June 2019 where it was 50% of the botanical composition. Birdsfoot trefoil was at very low inclusion (4-11%) for the majority of the 2018 to 2019 growing season, until July 2019 where it jumped to 27%. Annual legumes, which included berseem and balansa clovers, persisted around 22-28% of inclusion with the exception of April 2019 and July 2019 where the inclusion of annual legumes dropped to 11 and 16% respectively. Weeds were heavily prevalent in the establishment year and the beginning of 2019 between 17 to 30% of inclusion but decreased late spring and summer to between 5 and 7%. Dead material was low between 0-7% in the 2019 growing season and was highest in the establishment year at 15%. Annual grass remained relatively low between 1-9% with the exception of May 2019 where it jumped briefly to 20%.

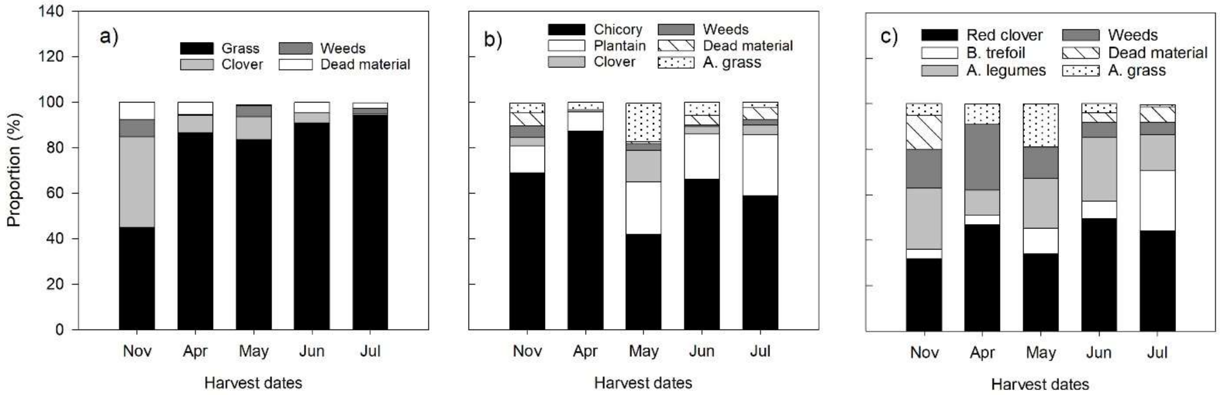

Figure 3. Botanical composition of grass- (a), forb- (b) and legume-based (c) pastures in 2018-2019 growing season.

3.6 Discussion

This part of the experiment evaluated the seasonal dry matter production and botanical composition of diverse grass-clover, forb-based, and legume-based pastures in Western Oregon in rainfed conditions. The study supported the hypothesis that grass-clover pasture would provide the highest yield in early spring, but legume-based and forb-based pastures would outperform grass-clover pastures in late spring and summer. In early spring, grass-clover pastures produced 1261 kg/ha more than forb-based pastures and 862 kg/ha more than legume pastures. Though the results from early spring of this experiment aligned with the hypothesis, they were also much lower than previously reported. Labreveux *et al*. (2004) reported orchardgrass cultivars producing between 3500 to 3600 kg/ha in early spring which agreed with the results of this experiment. However, the chicory and plantain cultivars produced between 5400 and 5800 kg/ha whereas this experiment only produced around 2500 kg/ha in early spring. In contrast, Cheng *et al*. (2017) reported chicory mixtures producing nearly 2600 kg/ha in spring, which aligns more with our results. This result could be due to the uncharacteristically low rainfall in fall of the establishment year and early April when compared to the long-term means. Drought tolerance and faster establishment of the grass species in the grass-clover pastures may have given them an advantage over the slower growing summer-active forbs and legumes as well.

Forb- and legume-based pastures outperformed grass-based pastures in summer. Due to a near doubling of precipitation in May compared to the long-term means, grass-based pastures were able to maintain production in June with around 4000 kg DM/ha but was surpassed by forb and legume pastures at 4500 and 4300 kg DM/ha, respectively. Muir *et al*. (2015) reported much lower yields of chicory and grass pastures during summer with chicory pastures only producing 1600 kg DM/ha and perennial ryegrass only producing 2900 kg DM/ha.

Forb-based pastures were heavily dominated by chicory and plantain in the summer. Due to the elevated production and superior nutritive quality compared to grass-based pastures in the summer, dairy producers utilize strategies of grazing chicory as a "break crop". In other words, they graze chicory pastures for a few hours a day to supplement the low nutrition of summer grass-clover crops (Barry, 1998; Muir *et al*., 2015; Sanderson *et al*., 2003; Waugh *et al*, 1998). Chicory can also be utilized as a weed suppressing, rotation crop when renovating a pasture. Chicory establishes relatively fast and, depending on the cultivar, produces a large canopy which can help with weed suppression during the pasture renovation process (Li *et al*., 2014). The forb's deep taproot also helps with improving water infiltration into the soil by breaking up any compaction (Kumar *et al*., 2018).

DM yield of grass-based pastures dropped 60% from June to July. This is likely due to grass drought avoidance strategies like summer dormancy (Nie *et al*., 2009). Forb- and legume-based pastures also dropped in production in July but maintained higher production than grass-clover production. These production values are reflected by seasonal growth rates. Forb pastures grew 142 kg DM/ha/day in May 2019 and 104 kg DM/ha/day June 2019, far surpassing both grass- and legume-based pastures. Forb-based pasture growth rates in this trial also surpass most extension/reference guides available to producers which normally report a summer growth rate between 60-70 kg DM/ha/day (Dairy NZ; https://www.dairynz.co.nz/feed/crops/chicory/). However, other reports show summer growth rates can be as high as 150 kg/ha/day (Li and Kemp, 2005; Moloney and Milne, 1993).

In the establishment year, grass-based pastures had about a 50:50 ratio of grass to clover with minor inclusions of weeds and dead material. However, in the following spring, grass was largely dominating with legume content only around 8% in April. The legumes were not able to recover. This could be due to the grazing management over the fall and winter. Dairy heifers were allowed to graze the grass-based pastures in order to clean up with fall and winter growth with the hopes of improving production in the spring. The heifers could continuously graze the area, which indicates they may have selected the legumes over grasses, severely depleting the legume content and reducing their regrowth capability.

Legume-based pastures had a low birdsfoot trefoil content during the establishment year at only 4%, which is carried through to the next year. By July, after multiple grazings, the proportion of birdsfoot trefoil increased to nearly 30%. This is due to the slow establishment of birdsfoot trefoil making it unable to compete with other plants and invasive weeds (Chapman *et al*., 2008). For this reason, the decision to plant them alongside annual clovers as companion crops at lower seeding rates was made. The intension of this decision was that the fast growth of red, balansa, and berseem clovers would offset the slow growth of the birdsfoot trefoil, allowing it to establish and persist. The fast-annual growth would also potentially smother any weeds which may outcompete the sensitive birdsfoot trefoil. While a number of studies investigated the effect of cereals as companion crops with birdsfoot trefoil (Hunt *et al.,* 2016), the potential for these annual legumes to assist birdsfoot trefoil as nurse crops is unknown and research on the topic is suggested.

3.7 Conclusions

Based on the results obtained in one growing cycle, all pasture types had similar total annual production potentials under unirrigated conditions. Both forb and legume pastures compensated for their lower early spring forage production later in the season as compared to grass-clover pastures. The alternative specialized pastures showed a great potential to be included in the feedbase of dairy cows. It is of note that both legume and forb pastures contained mainly short-live perennial species and therefore they would be less persistent than grass clover pastures in the long-run. Therefore, economic implication of incorporating these pastures into the feedbase should be investigated.

CHAPTER 4

Milk Production, Nitrogen Utilization, and Methane Emissions of Dairy Cows grazing Grass-, Forb-, and Legume-based Pastures

4.1 Introduction

Perennial ryegrass-based pastures form the main feedbase for dairy cows in temperate agro-ecologies (Lee *et al*., 2016). In well-managed dairy farming systems, perennial ryegrass can persist under intensive grazing and, in combination with white clover provides high animal performance (Dineen *et al*., 2018). However, grass-clover pastures alone cannot maintain high milk yields as cool season grasses suffer from low growth rates in high temperatures, leading to seasonal feed deficits. The reduction in pasture supply as associated with summer dormancy requires supplementation of grazing cows to prevent reductions in milk yields and farm income. Diversification of the forage base through inclusion of summer active forages in grazing rotations is often adopted as an alternative management strategy. Specialized legume and forb pastures provide higher production of superior quality forage, during the late spring-summer period and compliment grass growth (Chapman *et al*., 2008).

Specialized pastures can be grazed as supplementary to grass based pastures or be part of seasonal sequence-grazing programs where grazing animals switch from grass dominated pastures in spring to legume or forb pastures in summer to maintain high milk yields and improve seasonal productivity (Moore *et al*., 2004). Furthermore, management of seasonally wet soils is a further challenge for sustainable production and persistence of pastures in the Pacific Northwest. Grazing in wet conditions often causes soil consolidation due to pugging in saturated heavy clay soils, leading to deterioration of soil physical conditions (Drewry, 2006). Deferment of grazing grass-clover pastures in poorly drained soils leads to low forage utilization and accumulation of poor quality pastures in the late spring-summer period. Alternative feed profiling strategies through planting summer active pasture species that are tolerant to poorly drained and acidic soils may improve soil health conditions.

Furthermore, plant species with high bioactive compounds like chicory, plantain and birdsfoot trefoil can also help to mitigate the environmental impact of dairy farming. In particular, greenhouse gas (GHG) emissions and nitrate leaching by ruminant livestock on pasture is a growing concern. Dairy production has specifically come under some scrutiny despite only contributing to 1.3% of total GHG emissions (Rotz, 2018). Research has shown that pasture-based dairies decrease net GHG emissions via feeding less concentrate and stored silage (Arsenault *et al*., 2009), but increase nitrate leaching issues from deposition of urine patches (Rotz *et al*., 2009).

While it is not possible to alternate the forage base with specialized pastures containing forbs or legumes completely, incorporating alternative forage species as crops could improve animal and herbage production, and profitability of the farm systems. Hence, this study investigated the effect of feeding value of grass- (control), forb- and legume-based pasture mixtures on milk yield, milk components, nitrogen partitioning and methane yields from individual cows. It is hypothesized that the forb- and legume-based pastures maintain their high nutritive quality as compared to grass-clover pastures during late-spring and summer and therefore they have greater milk production and overall farm profitability potentials with less environmental pollution problems.

4.2 Materials and Methods

A grazing experiment was carried out at the Oregon State University Research Dairy in Corvallis, OR (44° 34' N, 123° 18' W 78 m. a.sl.) to test the effect of different pasture types (grass-clover vs. specialized legume or forb pastures) on milk production, nitrogen partitioning and methane (CH_4) emissions from dairy cows. All procedures were approved by the Institutional Animal Care and Use Committee (ACUP# 5026) prior to the commencement of the experiment. Twenty-seven Jersey multiparous and primiparous cows in mid-lactation were used in a randomized complete block design with 9 cows in each treatment. Cows were randomly allocated to their treatments based on age (mean ± s.d.; 3.2 ± 1.5 years), live weight (mean ± s.d.; 480 ± 46 kg), milk production (mean ± s.d.; 24.8 ± 6.2 L/cow per day) and days in milk (mean ± s.d.; 157 ± 64 d). Each herd of cows contained 2 multiparous and a primiparous cows. Prior to the commencement of the experiment, all the cows had grazed a diverse pasture mixture together as one herd.

4.2.1 Experimental Design and Grazing Management

A 5.85 ha was used to conduct a 39-day grazing experiment between 29 April and 6 June 2019. The grazing experiment was split into two periods the first being 21 days and the second being 18 days. Each consisted of an acclimation period (first 14 days in period 1 and first 11 days in period 2) and an experimental period (final 7 days of each period). Cows were offered a dietary treatment of (1) a grass-based pasture (**Control**) that consisted of festulolium (X *Festulolium braunii*), soft-leaf tall fescue (*Festuca arundinacea*), orchardgrass (*Dactylis glomerata*), white clover (*Trifolium repens*); (2) chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), white clover (**Forb-based pastures**), or (3) red clover (*Trifolium pretense*), birdsfoot trefoil (*Lotus corniculatus*), berseem clover (*Trifolium alexandrinum*), balansa clover (*Trifolium balansae*) (**Legume-based pastures**). The pastures were sown in nine 0.65 ha (62 × 105 m) plots in a randomized complete block design with three replicates. Temporary electric fences were used to separate the pastures and to separate daily pasture allocations. A “put and take” grazing management was applied to match the seasonal forage growth to animal intake (Bransby, 1989). Each treatment had a core group of nine cows (testers) with three spare cows (regulators). Cows were strip grazed and allocated an estimated 16 kg of DM/cow per day with a post-grazing residual of 1300 kg of DM/ha in both periods. Water troughs were moved as needed to ensure ad libitum access to water. The cows were milked twice daily (approximately 0500 and 1800 h) and offered a new pasture allowance after each afternoon milking. All cows received 2 kg DM of rolled grain mix (corn and barley mix 50:50) and 91 g/d/cow mineral mix that was offered in two equal portions right after the morning and afternoon milkings throughout the grazing experiment (acclimation and trial periods). The grain mix contained an average of 9% of crude protein (CP), 12.4% of the neutral detergent fiber (NDF), and 2.3% of ash. Mineral mix was consisted of 17-21% calcium, 7% phosphorus, 8% magnesium, 1.65% sulfur, 20-24 ppm selenium, and 200 IU/lb vitamin A. Cows were scored weekly by two trained, independent evaluators using a five-point BCS scale (1 = thin; 5 = fat). Grass-based pastures were grazed with a group of heifers in mid-March to prevent accumulation of low quality herbage material.

4.2.2 Pasture Measurements

Group herbage DM intake was estimated by determining pre- and post-grazing pasture mass with a rising plate meter (PM; Jenquip, Feilding, New Zealand) by collecting 50 measurements in each daily allocation of pasture during the experimental period (last 7 days). The PM was calibrated by regression against pasture masses by collecting 18 quadrats (each 0.25 m^2, 9 pre-grazing and 9 post-grazing quadrats) per pasture. Quadrats were cut to 30 mm residual height with electric hand shears. Apparent group DM intake of cows was calculated from herbage disappearance between pre- and post-grazing herbage and area allocated. Calibration was repeated in period 2. Calibration and intake estimation were successfully accomplished except for the forb pastures in period 1. The reproductive stalks of chicory plants prevented accurate measurements of pasture mass with PM. Therefore, intake in the forb pastures was calculated by taking 30 pre-grazing and 30 post-grazing quadrat cuts on two occasions during the experimental period. The regrowth of the forb pastures in period 2 did not hinder the PM measurements. Calibration curves for each treatment were generated by fitting a single line through all the data. The calibration curves used were:

Period 1:

Grass-based pastures (kg of DM/ha) = 87.7 PM – 305.5; $R^2 = 0.64$

Legume-based pastures (kg of DM/ha) = 110.3 PM – 405.7; $R^2 = 0.81$

Period 2:

Grass-based pastures (kg of DM/ha) = 65.1 PM – 32.9; $R^2 = 0.72$

Legume-based pastures (kg of DM/ha) = 61.0 PM – 79.2; $R^2 = 0.81$

Forb-based pastures (kg of DM/ha) = 79.1 PM – 403.5; $R^2 = 0.84$

Random pluck samples were collected from pre-grazing allocations of each pasture to determine nutritive value and botanical composition of forage on offer. A total of 50–75 pluck samples, representative of herbage eaten by cows, were collected by hand randomly across pasture (with a “zigzag” pattern) in each plot at 2 day intervals during the experiment period (last 7 days). Samples were collected within each plot before animals were turned onto fresh pastures. Sub samples were sorted into botanical components then dried at 65°C for 48 h. Percentage botanical composition of samples on a dry weight basis was then calculated. A well-mixed bulk sample was ground in a Wiley mill with a 1-mm stainless steel sieve (Thomas/Wiley, Swedesboro, NJ) for chemical analyses. Samples were analyzed for DM (method 2001.12; AOAC, 2003), ash (method 942.05; AOAC, 2003), and ether extract (method 920.39; AOAC, 2003). The CP concentration of all samples was determined by the Kjeldahl method according to the Association of Official Analytical Chemists (1990; LECO FP828, MI, USA). Neutral detergent fibre and ADF were assayed according to the methods described by Van Soest *et al*. (1991) using an Ankom$^{200/220}$ Fiber Analyzer (ANKOM Technology Corp., Macedon, NY). Samples were also analysed for their total phenolic and condensed tannins contents. Digestible dry matter content (DMD) was calculated using the following formula DMD=88.9-(0.779×ADF). Total N intake was calculated using the N content (%) of the pasture on offer and the average daily intake (kg) of the 3 treatment groups.

4.2.3 Milk Measurements

Daily individual milk yield was automatically recorded by the AfiMilk system (Kibbutz Afikim, Israel). Two milk subsamples were collected from each cow after AM and PM milkings on d 0 (baseline), 15, 18, and 21 for period 1 and d 12, 15, and 18 for period 2 to determine milk composition. Samples were analyzed commercially (Willamette DHIA Laboratory in Salem, OR) for fat, protein, lactose, somatic cell counts (SCC) and milk urea nitrogen (MUN) by near-infrared spectrophotometry (NIRS). Milk N output was calculated by dividing the milk protein content (%) by 6.38 to give N (%). This was then multiplied by the milk yield (kg/d) to give the total N output in milk.

4.2.4 Blood, Urine, and Fecal Measurements

Immediately after the morning and afternoon milkings on d 0 (baseline), 15, 18, and 21 in period 1 and d 12, 15, and 18 in period 2, the cows were taken into the OSU Dairy free stall barns and restrained for sample collection. Urine samples were collected midstream after manual stimulation of the vulva, acidified below a pH of 3.0 with sulfuric acid to prevent nitrogen volatization, and then stored at -4°C until analysis. Feces were collected via manual stimulation or as they defecated and frozen at -4°C until analysis. Blood samples (approximately 20 mL) were collected from the jugular vein into evacuated tubes (Becton Dickinson Vacutainer Systems; Becton Dickinson and Co., Franklin Lakes, NJ) containing lithium heparin or empty for plasma and serum isolation. After blood collection, tubes with lithium heparin were placed on ice and empty tubes were kept at 21°C until centrifugation (~30 min). Serum and plasma were obtained by centrifugation at 1.900 × g for 15 min. Aliquots of serum and plasma were frozen (−20°C) until further analysis for creatinine and urea N concentration. Samples were analysed as reported by Calamari *et al*. (2016) at the laboratory of the Istituto di Zootecnica, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy.

Fecal samples were thawed, weighed and dried in an oven at 55°C for 72 h to determine DM. Dry fecal samples were ground to 1 mm and analyzed for DM, ash and N contents. N contents of feces plasma, and urine samples were determined by using an N analyser (LECO FP828, MI, USA). Subsamples of urine collected after the morning and afternoon milking from each cow on d 0 and 21 in period 1 and d 18 in period 2 were analyzed for concentration of purine derivatives and urea by using HPLC (Agilent 1260 Infinity, Agilent Technologies, Waldbronn, Germany) fitted with a Luna® 5 µm C18(2) 100 Å, LC Column 250 x 4.6 mm (00G-4252-E0, Phenomenex, Torrance, CA) and a SecurityGuard™ cartridges for C18 HPLC columns with 3.2 to 8.0mm internal diameters (cat#AJ0-4287, Phenomenex). Urine samples were diluted 10-fold with double distilled water and filtered using syringe filters and 1ml disposable luer lock syringe (57022-N04-

C and 58901-S, MicroSolv Technology Corporation, Leland, NC). Filtrated diluted samples were inserted into a 1 mL transparent HPLC vials (82028-402, VWR, Radnor, PA, USA). Urea was determined by fluorescence detection after derivatization using xanthydrol (90-46-0, Alfa Aesar, Tewksbury, MA, USA) and following the gradient III and the automatic HPLC autosampler program of the method of Clark *et al*. (2007) with modifications. Briefly, the run was 7 min with a full run (up to 12 min) every 10 runs using a blank to clean the column. The column was kept at room temperature (instead of 35ºC). The injection volume after derivatization was 8 µl (instead of 40.5 µl). Furthermore, though xanthydrol was solubilized in 1-isopropanol as indicated by Clark *et al*. (2007), xanthydrol separated quickly decreasing the derivatization of urea. To address that issue, we ran the second point of the standard curve every 10 runs plus we used 3 samples that were added into the sequence every 10 samples and used the data to adjust for the final urea concentration. Quantitation of urea was determined by a 5-point standard curve (4-fold dilution) of purified urea (BDH4602-500G, VWR) prepared in 2.4 pH double-distilled water to match the acidified urine.

Creatinine, uric acid, and allantoin concentration were performed using the same column as for the urea following the method described by George and collaborators (2006). A standard curve constituted of 480 µg/mL of allantoin (97-59-6, Spectrum, New Brunswick, NJ, USA), 120 µg/mL of creatinine (60-27-5, TCI, Portland, OR, USA), and 108 µg/mL of uric acid (69-93-2, Alfa Aesar) diluted in 5-concentrations of 4-fold dilution was used for final quantification.

4.2.5 Estimation of Microbial N Supply and Urinary N Excretion

Microbial N supply was estimated by using equations previously described (Totty *et al*, 2013; Chen, 1989; Verbic *et al*. 1990; Chen *et al*., 1995; Joint FAO/IAEA Division, 2003). The microbial

N supply was estimated by the urinary excretion of purine derivatives (PD), allantoin, uric acid, and creatinine and expressed as the following:

PD index = {[total PD (mmol/L)]/creatinine (mmol/L)} x $BW^{0.75}$.

The PD index was based on the total PD [allantoin (mmol/L) + uric acid (mmol/L)]. Creatinine excretion (mmol/kg of $BW^{0.75}$) was determined by using the estimated daily urinary volume (L) calculated from the equation by Pacheco *et al*. (2009). The estimated urinary creatinine excretion (0.9 mmol/kg of $BW^{0.75}$) was included in the following equation to estimate the daily PD excretion (mmol/kg of $BW^{0.75}$):

Urinary N excretion (g/d) was estimated using the equation urinary g of N/d = 21.9 (mg/kg) × BW (kg) × [1/ urinary creatinine (mg/kg)] × urine N (g/kg), as described by Pacheco *et al*. (2009). The estimated urinary creatinine excretion (0.9 mmol/kg of $BW^{0.75}$) was included in the following equation to estimate the daily PD excretion (mmol/kg of $BW^{0.75}$):

Daily excretion of PD (dPD; mmol/kg of $BW^{0.75}$) = PD index × 0.9

The amount of purine absorbed daily was estimated by:

Daily absorbed purine (daP) = [dPD (mmol/kg of $BW^{0.75}$) – 0.385 × $BW^{0.75}$] + 0.85;

Microbial N (g of N/d) supply was calculated with the following equation:

Microbial N (g of N/d) = (daP x 70)/(0.116 × 0.83 × 1000).

4.2.6 Methane (CH_4) Emission Measurement

CH_4 emission of individual cows was determined using the SF_6 tracer method (Johnson *et al*., 2007). A brass permeation tube about 1 cm in diameter and about 4 cm long containing compressed SF_6 gas was targeted to the rumen or reticulum and administered with a bolus gun in cows at the beginning of the trial. The release rate from the permeation tubes was about 1200 ng/min or 2 mg/d. The perm tube was loaded with 600 mg of SF_6 and the release rate was measured gravimetrically for 6 weeks before the perm tube was placed in the cows. A halter containing a collection system comprised of a filtered intake tube, capillary tubing and an evacuated PVC collection canister was fitted to the animal, and the intake tube was positioned near the mouth and nose of the animal. The evacuated canister (< 0.5 mb) had a negative pressure, which drew air continuously for a 24-hour periods through the filter. After the samples were collected, the canister was removed and pressurized with high purity nitrogen gas (N_2). The collected gas was sampled and assayed using a gas chromatograph to determine the concentrations of CH_4 and SF_6. The emission rate of the permeation tube and the ratio of SF_6 to CH_4 in the collection canister were used to calculate the enteric emission rate of CH_4 from the animal (Johnson *et al*., 2007). Samples were collected from six replications per treatment (only from two cows in each grazing plot) for six consecutive days (on day 16 to 21) during period 1. For the same six days, two ambient air controls were collected in canisters located in different paddocks.

4.2.7 Statistical Analysis

All parameters were analyzed by analysis of variance (ANOVA) based on a 3× 2 factorial model that accounted for the main effects of pasture types and period in a complete randomized design. The exception was individual methane emissions that were analysed by pasture type as it was only performed during period 1. Treatment means for urine, feces, milk and blood parameters were determined using data collected from individual cows during the experimental periods (AM and PM of d 15, 18, and 21 in period 1 and on d 12, 15 and 18 in period 2). Averages across the 3 cows in each plot were used as the experimental unit (pasture plots) rather than individual cows. Herbage and total DMI intakes were estimated as means for the treatment group as cows grazed pastures as small herds (3 cows) together. Baseline data collected from individual animals were not included in the statistical analyses as treatment effects were not significant. The computations were carried out using GENSTAT statistical software version 18 (VSN International Ltd., Rothampstead, UK) by one-way ANOVA, with pasture treatment (3 levels) and cow block (9 levels) as factors (Payne *et al*., 2009). Significant differences among treatment means were compared by Fisher's protected least significant difference at $P < 0.05$.

4.3 Results

4.3.1 DMI and Pasture Quality

Dry matter intake (DMI) and pasture chemical and botanical composition are presented in Tables 6, 7, and 8, respectively. A treatment × period interaction was detected for the pre-grazing pasture mass (kg of DM/ha, $P < 0.01$; Table 6). Pre-grazing pasture mass of legume and forb pastures was greater than the grass-clover pasture by 600-1000 kg DM/ha in the first period. While the pre-grazing pasture mass of forb- and grass-based pasture was similar in the second period, grass-clover pastures had approximately 500 kg DM/ha more pasture mass. Herbage feed intake was significantly different between treatments (kg/cow/day, $P < 0.05$; Table 6) with the cows that grazed legume pastures having the greatest herbage DMI. The cows that grazed grass-clover pastures had 1.4 kg lower herbage DMI (kg/cow/day) than those grazed on legume pasture in both periods. The herbage DMI intake of cows that grazed forb-based pastures was intermediary but did not differ from either grass clover or legume pastures. Overall cows had similar herbage DMI in both periods ($P = 0.29$). Total (pasture + concentrate) herbage DMI (kg/cow/day, $P < 0.05$; Table 6), herbage DMI per kg of body weight (BW) (g/kg, $P < 0.01$; Table 6), and total herbage DMI per kg of BW (g/kg) followed the same pattern as herbage DMI. Herbage DMI/kg of BW (g/kg) of cows that grazed grass clover pastures was 2.8 and this was lower than those grazing legume and forb pastures (*P*< 0.01).

Total DMI/kg of BW (g/kg) of cows that grazed grass, forb and legume pastures in the first period were 3.2, 3.5 and 3.6, respectively (*P*<0.01) and was similar in the second period (*P*=0.57). Body condition score (BCS) of the cows ranged from 2.9 to 3.1 but the difference was not significant in either period (*P*= 0.49).

Table 6. Effect of pasture type on feed intake and feed efficiency of grazing dairy cows in Period 1 (29 Apr-19 May) and Period 2 (19 May-7 June)

Yield	Period 1 (29 Apr-19 May)			Period 2 (19 May-7 June)				P values		
	Grass	Forb	Legume	Grass	Forb	Legume	SE	Pas[1]	Per[2]	P× P
PreGPM (kg of DM/ha)	3537b	4547a	4184a	2605c	2251cd	2096d	122.7	0.05	0.01	0.01
Herbage DMI (kg/cow/day)	13.9	14.4	15.3	13.3	14.5	14.7	0.42	0.05	0.29	0.63
Total DMI (kg/cow/day)	15.9	16.4	17.3	15.3	16.5	16.7	0.42	0.05	0.29	0.63
Herbage DMI (% BW)	2.8	3.0	3.2	2.7	3.0	3.1	0.09	0.01	0.51	0.76
Total DMI (% BW)	3.2	3.4	3.6	3.1	3.5	3.6	0.09	0.01	0.57	0.79
BCS	2.9	3.1	3.1	2.8	3.0	2.9	0.15	0.49	0.17	0.93

[1]Pas = Pasture; [2]Per = Period

The nutritive value was significantly different among pastures for all measured parameters with greater quality forage in the second than in the first period (Table 7). Overall, forb pastures had the highest ash content (11.3%) while the grass-clover pastures had the lowest ash content (9.3%) (*P*<0.01). The ash content of legume pastures was higher than grasses but lower than forb pastures. The average DMD of the legume pastures was 71.1% and this was 1.2-2.0% higher than forb and grass-clover pastures, respectively (*P*<0.01). There was a significant interaction between pastures and periods for the crude protein (CP) contents (*P*<0.05). Legume pasture had a sharp increase in CP from 20.6% in the first period to 25.3% in the second period (*P*<0.05). CP content of grass pasture also sharply increased from 15.8% in period 1 to 20.0% in the second period. CP in forb pasture remained generally stable. The ADF content of the legume pasture was lower than both grass-clover and forb pastures (*P*=0.01). The grass-clover pastures had higher NDF content than forb and legume pastures by approximately 9%. The NDF content of pastures had a tendency to be higher in the first period than the second period (*P*=0.06). There was a significant interaction between pastures and periods for the ether extract (EE) (P<0.01). Overall, the EE contents of grass-clover (1.8%) and legume (2.1%) pastures remained stable in both periods. The EE content of forb pastures, however had a sharp decrease from 2.2% in the first period to 1.6% in the second period.

Total phenolic compounds (TP) and condensed tannin (CT) concentration, both unbound (UCT) and bound (BCT) of the three pasture treatments are also reported in Table 7. TP, CT, UCT, and BCT were all highest in legume-based pastures at an average of 148.1 mg/g, 359.7 mg/g, 51.6 mg/g, and 308.1 mg/g, respectively (P < 0.01). Forb-based pastures were second highest at an average of 6.5 mg/g (TP), 57.1 mg/g (CT), 4.5 mg/g (UCT), and 52.7 mg/g (BCT) (P< 0.01).

Table 7. Nutritive value (% of DM) of grass-clover, forb and legume-based pastures in Period 1 (29 Apr-19 May) and Period 2 (19 May-7 June).

Items	Period 1 (29 Apr-19 May)			Period 2 (19 May-7 June)			SE	P values		
	Grass	Forb	Legume	Grass	Forb	Legume		Pas	Per	P× P
Ash, %	8.4	10.2	8.9	10.1	12.4	10.8	0.22	0.01	0.01	0.59
DMD, %	68.5	69.7	70.2	69.8	69.7	72.0	0.47	0.01	0.01	0.17
CP, %	15.8d	18.3c	20.6c	20.0bc	19.2bc	25.3a	0.79	0.01	0.01	0.05
ADF, %	26.2	24.6	23.9	24.5	24.6	21.7	0.60	0.01	0.01	0.17
NDF, %	47.1	35.6	37.0	43.8	35.7	36.2	0.87	0.01	0.06	0.15
EE,%	1.8bc	2.2a	2.1abc	1.8bcd	1.6d	2.1ab	0.10	0.05	0.05	0.01
TP, mg/g	0	6.2	104.9	0	6.7	86.4	3.80	0.01	0.08	0.06
CT, mg/g	0	65.8	324.2	0	48.4	395.2	32.39	0.01	0.51	0.38
UCT, mg/g	0	4.4	59.1	0	4.5	44.1	3.22	0.01	0.09	0.06

BCT, mg/g	0	61.4	265.1	0	43.9	351.1	32.44	0.01	0.40	0.27

DMD: digestible dry matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, EE: ether extract, TP: total phenolic, CT: condensed tannins, UCT: Unbound condensed tannins, BCT: Bound condensed tannins

Botanical composition of pastures is presented in Table 8. Grass-clover pastures had over 90% grass component in period 1. This decreased to 80% in the second period. In reverse, white clover increased from 7% to 20%.

For the forb pasture, chicory, plantain, and white clover were the prominent sown species. Volunteer annual ryegrass was also a significant component of forb pastures, exceeding 20% of the composition. Chicory decreased 11% from period 1 to period 2 while plantain remained the same between periods at 23% of the total DM. In reverse, both white clover and volunteer annual ryegrass increased in the second period at 6% and 4 % respectively. Weeds were observed at 3% of the total DM in period 1 but not in period 2. Dead material was not observed in period 1 but was observed at 2% of the total DM in period 2.

The legume pastures were predominantly composed of red clover, birdsfoot trefoil, and volunteer annual ryegrass, with lower inclusions of berseem and balansa clovers. The average legume content of the pastures was 82% in period 1. Despite a 7% increase in the proportion of the annual clovers (balansa and berseem clover) in the second period, overall the average legume content decreased to 72% as the volunteer annual ryegrass increased by 12% from period 1 to period 2. Weeds were observed at 3% of the total DM in period 1 but not in period 2. Dead material was not observed in period 1 but was observed in period 2 at 1% of total DM.

Table 8. Botanical composition (% of total DM) of the grass, forb and legume-based pastures in Period 1 (29 Apr-19 May) and Period 2 (19 May-7 June)

	Period 1 (29 Apr-19 May)			Period 2 (19 May-7 June)		
Component	Grass	Forb	Legume	Grass	Forb	Legume
Sown grasses	81	-	-	69	-	-
A. ryegrass (volunteer)	12	22	15	11	26	27
White clover	7	9	-	20	15	-
Chicory	-	44	-	-	33	-
Plantain	-	23	-	-	23	-
Red clover	-	-	56	-	-	40
Birdsfoot trefoil	-	-	14	-	-	13
Berseem clover	-	-	4	-	-	10
Balansa clover	-	-	8	-	-	9
Dead material	-	-	0	-	3	1
Weeds	0	2	3	0	0	0

4.3.2 Milk Production and Composition

Milk production and composition are presented in Table 9. Milk yield (L/d) of the cows was significantly different among pastures ($P < 0.01$) but it remained similar in both periods (P =0.43). The cows that grazed legume and forb pastures had greater milk yield than the cows that grazed grass clover pastures ($P < 0.01$). Averaged across the treatments, milk yields of the cows were 22.9, 22.0 and 20.4 L/d for legume, forb and grass-clover pastures, respectively. Similarly, legume and forb pastures provided greater 4% FCM (L/d) as compared to grass clover pastures ($P < 0.01$). There tended to also be a slight decrease in 4% FCM between the first and second period (P=0.06). Milk solids (kg/d) followed the same trend with milk and 4% FCM yields. Cows from legume and forb pastures had the highest milk solid production that exceeded 2.0 kg/d while cows from grass-clover pastures produced 1.8 kg/d. The average milk solid production of the cows was stable in both periods.

The average milk fat (g/d) production of the cows in grass-clover pastures was 902 g/d. This was 125 and 173 g/d lower than the production of the cows from forb and legume pastures, respectively. The average milk fat (g/d) production of the cows decreased in all treatments from 1051 in period 1 to 952 g/d in period 2 (*P* <0.01). Forb and legume pastures also provided higher milk protein production (g/d) than grass pastures (*P* < 0.01). The average milk protein production (g/d) of the cows on legume, forb and grass-clover pastures were 777, 744 and 697 g/d, respectively.

For milk components, milk fat content appeared to be greater from the cows that grazed legume (4.9%) and forb pastures (4.7%) as compared to grass based (4.5%) pastures (*P*= 0.07). Milk fat content decreased from 4.9% in the first period to 4.5% in the second period (*P*< 0.05). While the milk protein content did not differ among pasture treatments (*P*=0.31), it increased from 3.4% in the first period to 3.5% in the second period (*P* < 0.05). Milk solid non-fat (SNF) and lactose contents were higher in the milk of cows that grazed forb pasture than they were in cows that grazed legume or grass-clover pastures (*P*< 0.05). The SCC (cells/ml) of the cows did not differ among pasture mixtures (*P*=0.45) in either period (*P*=0.12).

Table 9. Milk yield and components of dairy cows grazing grass, forb, and legume-based pastures in Period 1 (29 Apr-19 May) and Period 2 (19 May-7 June)

Yield	Period 1 (29 Apr-19 May)			Period 2 (19 May-7 June)				P values		
	Grass	Forb	Legume	Grass	Forb	Legume	SE	Pas	Per	P× P
Milk Yield (L/d)	21.1	21.9	23.1	19.8	22.2	22.7	0.63	0.01	0.43	0.47
4% FCM (L/d)	23.0	24.6	26.1	20.4	23.8	24.5	0.97	0.01	0.06	0.66
Milk solids (kg/d)	1.9	2.0	2.1	1.8	2.0	2.1	0.06	0.01	0.60	0.50
Milk fat (g/d)	972	1058	1121	832	995	1028	50.0	0.05	0.01	0.74
Milk protein (g/d)	712	746	777	696	777	778	20.7	0.01	0.77	0.53
Components										
Milk fat %	4.7	4.9	5.0	4.2	4.5	4.7	0.15	0.07	0.05	0.73
Milk protein %	3.4	3.5	3.4	3.5	3.5	3.5	0.07	0.31	0.05	0.54

SNF, %	9.0	9.2	9.1	9.1	9.3	9.1	0.06	0.05	0.27	0.93
Lactose, %	4.6	4.8	4.7	4.6	4.8	4.7	0.05	0.01	0.58	0.68
SCC, 10^3 cells/ml	158	45	80	85	34	31	1.1	0.05	0.05	0.23

4.3.3 Measurements of N in Urine, Feces, Milk, and Plasma

Measurements of N in urine, feces, milk, and plasma are presented in Table 10. Total N intake of cows ranged from 385 (g N/d) to 622 (g N/d). Averaged across the periods, cows that grazed legume pastures had 111-158 g N/d more N intake than forb and grass-clover pastures, respectively ($P< 0.01$). Cow that grazed grass based pastures had also substantially lower N intake than those cows grazed forb pastures. The N intake of cows was 57 g N/d higher in the second than first grazing period ($P< 0.01$).

Percentage of N in urine of the cows was lower in forb pastures than grass and legume-based pastures which had similar urine N contents ($P<0.01$). Averaged across the periods, urine N content of the cows were 0.24%, 0.33% and 0.40% for forb, grass and legume-based pastures, respectively. The urine N content of the cows was 26% greater in the second than first period. Similarly, cows that grazed forb pastures the lower urine NH_3 content than those grazed grass pastures ($P<0.05$). NH_3 in legume-based pastures did not significantly differ from grass- or forb-based pastures. There was a significant interaction between treatment and period for the urea from urine ($P<0.05$). Period did not affect urea in urine of cows of grass- and forb-based pastures, but urea in urine of cows in legume-based pastures significantly decreased in the second period by 25%. Forb-based pastures had the lowest urea of the treatments, averaging 30.9 mmol/L. Urea in grass-based pastures was on average 47% higher than forb-based pastures while legume-based pastures ranged from 56 – 75% higher than forb-based pastures.

An interaction was detected between period and treatment for the urine creatinine values (*P*<0.01). Creatinine values of urine from cows that grazed forb-based pastures were the lowest of all the treatments in the first period at 1.8 mmol/L, but nearly double in the second period at 3.0 mmol/L. In the second period there was only a 0.1 and 0.3 mmol/L difference between forb-based pastures and grass-clover and legume-based pastures, respectively (*P*<0.01). In the first period, there was a 1.1 mmol/L decrease between grass-clover and forb pastures and a 0.06 mmol/L decrease between forb pastures and legume pastures (*P*<0.01).

Table 10. Nitrogen partitioning of dairy cows grazing grass, forb, and legume-based pastures in Period 1 (29 Apr-19 May) and Period 2 (19 May-7 June)

Yield	Period 1 (29 Apr-19 May)			Period 2 (19 May-7 June)			SE	P values		
	Grass	Forb	Legume	Grass	Forb	Legume		Pas	Per	P× P
Intake (g N/d)	385	461	534	455	473	622	20.9	0.01	0.01	0.21
Urine										
N (%)	0.28	0.18	0.36	0.38	0.29	0.43	0.028	0.01	0.01	0.80
NH_3 (mmol/L)	4.7	3.5	3.9	5.9	5.4	5.9	0.25	0.05	0.01	0.23
Urea (mmol/L)	55.2c	26.8d	106.6a	61.7bc	34.9cd	79.6b	6.60	0.01	0.45	0.05
Creatinine (mmol/L)	2.9ab	1.8d	2.4c	3.1a	3.0ab	2.7bc	0.12	0.01	0.01	0.01
N output (g/d)	103.5	107.6	154.2	129.7	96.7	161.3	12.28	0.01	0.47	0.35
Feces										
N (%)	2.2	2.9	2.7	2.0	2.7	2.6	0.05	0.01	0.01	0.39
Ash (%)	20.5	21.1	18.0	19.0	20.0	18.1	0.66	0.01	0.14	0.49
DM (%)	10.5bc	10.4c	12.1a	10.6bc	11.2b	11.1b	0.24	0.01	0.88	0.01
Milk										
Urea N (mg/dl)	9.6	5.9	16.9	13.3	9.1	17.9	0.56	0.01	0.01	0.28
N output (g/d)	112.3	118.5	123.8	110.0	123.0	122.9	2.94	0.01	0.84	0.49
Plasma Urea (mmol/L)	3.9	2.3	7.0	5.0	3.3	6.8	0.32	0.01	0.05	0.12

DM content of the fecal material from cows ranged from 10.4 to 12.1%. The fecal N content of cows that grazed grass clover pastures was lower than those cows that grazed legume and forb pastures that had similar fecal N contents (*P*< 0.01). The mean fecal N content of the cows were 2.1, 2.7 and 2.8% for the cows that grazed grass-clover, legume and forb pastures, respectively. Fecal ash content was significantly different between pastures, but not between periods ($P< 0.01$; $P=0.14$). Cows that grazed forb-based pastures had the highest fecal ash content of 21-20% ($P<0.01$). Fecal ash content was intermediate in cows that grazed grass-clover pastures and cows grazing legumes had the lowest fecal ash content ($P<0.01$).

Milk urea nitrogen (MUN) content ranged from 5.9 to 17.9 (mg/dl). There was an increase in MUN from 10.8 mg/dl in the first period to 13.4 mg/dl in the second period ($P< 0.01$). In both periods, cows from the legume pastures had the highest MUN content in their milk at 17.4 mg/dl while the cows that grazed forb pastures had the lowest MUN with 7.5 mg/dl. MUN content in cows that grazed grass pasture-clover pastures was intermediate with 11.5 mg/dl. Milk N output was greater with the cows that grazed forb and legume pastures than those that grazed grass-clover pastures ($P< 0.01$). However, the excretion of N through milk was not affected by the grazing period ($P=0.84$). Plasma urea concentration of cows that grazed legume pastures was greater than those grazed on grass-clover or forb pastures ($P< 0.01$). The plasma urea concentration of cows that had forb pastures was the lowest ($P< 0.01$). Cows had significantly greater urea concentrations in their plasma in the second compared to the first period ($P<0.05$).

4.3.4 Microbial Protein Supply

Urinary concentration of purine derivatives (PD) of dairy cows grazing grass, forb, and legume-based pastures are presented in Table 11. There was a significant interaction between periods and pastures for allantoin concentration in urine ($P<0.05$). Allantoin ranged from 6.8 to 10.4 mmol/L. Urine from cows grazing forb-based pastures had the lowest allantoin concentration at 6.8 mmol/L, but the highest in the second period at 10.0 mmol/L ($P<0.05$). Urine from cows grazing grass-clover pastures had 10.4 mmol/L of allantoin and decreased by 1.0 mmol/L in the second period ($P<0.05$). Allantoin concentrations remained between 8.1 and 8.5 mmol/L in the urine of cows grazing legume-based pastures in both periods ($P<0.05$).

Uric acid concentrations in urine (mmol/L) significantly differed between pasture treatments ($P<0.01$) and tended to have an interaction between pasture and period ($P<0.06$). The uric acid concentration in urine ranged from 1.4 to 2.4 mmol/L. Cows grazing grass-clover pastures had the highest uric acid concentration in both periods at 2.4 and 2.0 mmol/L respectively ($P<0.01$). Urine from cows that grazed forb-based pastures had the lowest uric acid concentration at 1.4 mmol/L and second lowest at 1.8 mmol/L. Urine from cows that grazed legume-based pastures had uric concentration of 1.6 mmol/L in both periods.

Total purine derivatives (PD) concentration of urine showed an interaction effect between pastures and grazing period ($P<0.05$). The PD concentrations of urine ranged from 8.2 to 12.8 mmol/L. Urine from cows grazing forb-based pastures had the lowest total PD concentration at 8.2 mmol/L in the first period, which jumped to 11.8 mmol/L in the second period. Cows in grass-clover pastures had the highest urine total PD concentration at 12.8 mmol/L.

The allantoin:creatinine ratio was significantly different between pasture treatments, but not periods ($P<0.01$; $P<0.65$). There also tended to be an interaction between treatment and period ($P=0.06$). The allantoin:creatinine ratio of urine from cows on grass-clover pastures was the highest at 2.0-2.4. Both allantoin:creatinine ratios of urine from cows grazing both forb- and legume-based pastures ranged from 5.0-5.8.

Total PD:creatinine was not different among pastures, but significantly increased from the first to the second period (*P*<0.01). In the first period, total PD:creatinine ranged from 4.5 – 5.1 and increased to a range of 4.7 – 5.8.

The PD index of urine significantly decreased from the first to the second period (*P*<0.01). Overall, PD index decreased 73.5 across treatments from the first period to the second. The PD index was not different among cows grazing different pastures. Similarly, microbial protein supply (g of N/d) estimated from urine also significantly decreased from the first to the second period (*P*<0.01). Microbial protein supply decreased 61.7 g of N/d across treatments from the first to the second period.

Table 11. Urinary concentrations of purine derivatives (PD) and microbial N of dairy cows that grazed grass, forb, and legume-based pastures in Period 1 (29 Apr-19 May) and Period 2 (19 May-7 June)

Yield	Period 1 (29 Apr-19 May)			Period 2 (19 May-7 June)			SE	P values		
	Grass	Forb	Legume	Grass	Forb	Legume		Pas	Per	P× P
Allantoin (mmol/L)	10.4a	6.8c	8.1b	9.4ab	10.0ab	8.5b	0.63	0.05	0.08	0.05
Uric acid (mmol/L)	2.4	1.4	1.6	2.0	1.8	1.6	0.12	0.01	0.65	0.06
Total PD (mmol/L)	12.8a	8.2c	9.7bc	11.5ab	11.8ab	10.1bc	0.72	0.05	0.11	0.05
Allantoin:creatinine	4.5	5.0	5.1	4.7	5.8	5.5	0.27	0.05	0.08	0.52
Total PD:creatinine	4.8	5.0	4.2	3.8	4.0	3.9	0.24	0.25	0.01	0.46
PD index	492.3	505.2	424.8	397.9	408.8	387.1	24.89	0.13	0.01	0.45
Microbial N (g /d)	369.3	380.3	315.8	292.7	302.4	285.1	20.12	0.14	0.01	0.45

PD: purine derivates

4.3.5 Methane Emissions

Methane emission data from individual cows and their relationship to animal productivity are presented in Table 12. Methane emission (g/kg) in relationship to animal productivity parameters (DMI, milk yield, FCM, milk protein yield, and milk fat yield) among pasture types did not differ (*P*>0.05). However, total daily methane production of the cows (g/d/cow) in the forb pastures tended to be lower than grass-clover and legume pastures by 14 and 20%, respectively (*P*=0.07).

Table 12. The effect of pasture type on methane emissions and their relationship to animal productivity during Period 1 (29 Apr-19 May)

Item	Grass	Forb	Legume	SED	P values
CH_4 (g/d)	325	278	348	29.6	0.07
CH_4 (g/kg of DMI)	20.7	17.4	20.2	1.81	0.13
CH_4 (g kg of milk)	14.9	14.7	14.7	2.0	0.92
CH_4 (g/kg of FCM)	14.2	13.1	13.1	1.5	0.60
CH_4 (g/kg of milk protein)	458	412	435	42.9	0.42
CH_4 (g/kg of milk fat)	349	307	304	37.1	0.35

4.4 Discussion

4.4.1 Dry Matter Intake (DMI) and Milk Yield

The present study investigated the production and environmental efficiency of alternative forage-base for dairy cows in mid-late spring period by comparing specialized legume and forb-based pastures with traditional grass-clover pastures in seasonally wet and acidic soils. The basic premise was that deferment of grazing grass-clover pastures in early spring due to excessive wet conditions in poorly drained pastures would penalize forage utilization, quality and thereby cow performance. The reduction in nutritive value of legume and forb pastures, however are of lesser concern as they tend to retain their quality with advanced maturity or forage mass accumulation (Brown and Moot, 2004). Specifically, legumes maintain higher crude protein, higher ME, and lower structural carbohydrates, which ultimately improve intake and milk yield (Harris *et al.*, 1997; Harris *et al.*, 1998; Waghorn *et al.*, 2004). Furthermore, most legumes and forb species have slower growth rates than grasses in early spring and thus the commencement of spring grazing of those pastures is naturally delayed (Mills *et al.*, 2015).

The milk yield obtained in the current study is aligned with the hypothesis and was highly related to the feeding value of the forages that defines their capacity to produce high-value animal products. Although grass-clover pastures were grazed by light stock in early spring to prevent excess amount of low quality forage accumulation, both legume and forb pastures provided higher quality forages than grass-clover pastures as evidenced by lower NDF (<37%) and higher crude protein contents (>18%) as compared to grass clover pastures that had 47.1% NDF and 15.8% CP contents. The high feeding value legumes have shown to improve DMI and animal production with different class of animals and in various grazing systems (Steinshamn, 2009; Waghorn and Clark, 2004; Dewhurst, 2013). Thus, increased DM intake of high quality forages is the primary objective of pasture-based feeding programs for maximizing the animal production outputs. In the present study, averaged across the periods, cows that grazed legume pastures had 1.4 kg higher herbage DMI than those grazed grass-clover pastures. This finding is in line with the results of Harris *et al*. (1997) who reported that inclusion of white clover at 50% of the cow's diet increased the DMI up to 13% and milk production up to 35% when compared to grass only pastures. In the present study, legume-based pastures had the highest DMD, lowest ADF, intermediary NDF, and lowest overall EE of the treatments. The lower ADF and NDF improves feed intake and increases digestibility, thus contributing to the increased observed milk yield in comparison to the other treatments. Overall, the increased DM intake of legumes can be associated to their higher rate of rumen fermentation, physical breakdown and passage rates through the rumen (Waghorn & Clark, 2004; Dewhurst, 2013).

In the present study, forb-based pastures that mostly consisted of chicory and plantain (67% in period 1 and 56% in period 2) provided intermediary DMI and milk yield in both periods. The production of milk solids of the cows grazed forb pastures was 10% greater than those grazed grass-clover pastures. Similar to the findings of the current study, Minnee *et al*. (2012) reported higher DMI and production of milk solids by 6% and 17%, respectively when chicory or plantain were incorporated from 20 to 60% into the dairy cows' diet that mainly consisted of low quality perennial ryegrass (9.6 MJ/kg of DM). However, the superiority of forbs on DMI and milk yield was not consistent when the perennial ryegrass had moderate quality (10.5 MJ/kg DM), indicating the positive effect of herbs may be highly related to the nutritive quality of the grass-clover pastures. In a more recent study, Mangwe *et al* (2019) reported substantially greater DMI (17.7 kg) and milk-solid yield (1.93 kg of milk solid) from plantain and chicory pastures as compared to ryegrass-white clover pastures (15.6 kg DM and 1.65 kg milk solid). In contrast, Muir *et al*. (2015) reported similar herbage DMI from mix chicory-perennial ryegrass (50:50) pastures (14.0 kg) as compared to perennial ryegrass pastures (14.0 kg). The discrepancy in the DMI and milk yield responses of dairy cows to forb vs. grass-based pastures appears to be related to the pasture management, plant stage of growth and agro-ecological conditions.

Milk components, in particular milk fat and lactose contents varied across pastures and forage quality. In a review paper, Elgersma *et al*., (2006) noted that milk fat content does not change with forage type, while milk fat composition can be heavily regulated by forage type and forage DMI. Similarly, Muir *et al*. (2014) reported comparable milk protein and fat concentrations from different herbage types. It is probable that the higher milk fat content obtained from the legume pastures in the present study was rather a function of it superior feeding value. It can be speculated that in similar nutritive qualities, the pastures tested in the current study may have provided milk with comparable butterfat contents.

A feature of the results was that cows that grazed legume, and in particular forb pastures, had consistently low milk somatic cell counts. This may be because chicory has a number of beneficial secondary metabolite compounds that provide the antihelmitic, antimicrobial, and digestive aid properties (Das *et al*., 2016; Sahan *et al*., 2017), but also have antibacterial properties (Yoon *et al*., 2013; Nohynek *et al*., 2006). In a study analyzing the antimicrobial compounds in chicory, it was found that the compounds deterred bacterial reproduction and growth (Koner *et al*., 2011). However, further studies are needed to draw more concrete conclusion on the effect of chicory and plantain for their effect in the immune system.

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4.4.2 Nitrogen Partitioning

Pastures generally exceed protein requirements for dairy cattle leading to increased nitrogen waste in the form of urea in the urine (NRC, 2001; Byrant *et al*., 2019). Urea in urine quickly transforms through nitrification or is leached into nearby groundwater, contributing to about 80% of N_2O emissions from pastures with the remain 20% coming from N fertilizer application (de Klein and Ledgard, 2005). Therefore, partitioning of N away from urine to feces has become of focus for much research due to fecal N being much more organically stable, allowing for a reduction in N emissions (de Klein and Ledgard, 2005).

In the current study, in the dairy cows that grazed forb-based pastures there was distinct shift in nitrogen (N) output from the urine, milk, and plasma to the feces. This was indicated by the decrease in of multiple nitrogen indicators in all sources except for feces, which increased in cows that grazed forb-based pastures. Milk urea nitrogen (MUN) was the lowest in cows grazing forb-based pastures and highest in legume pastures. This is intuitive since the N intake was the highest in legumes but does not explain the low MUNs for forb pastures since CP for those pastures was similar to that of legume pastures and much higher than grass-clover pastures which had intermediary MUN concentrations. Previous research has shown incorporating forbs such as chicory into the pasture mixtures can dramatically decrease urinary N output through reducing the source of degradable dietary protein (Totty *et al*., 2012; Vibart *et al*., 2016). Combined with the decrease in MUN, decreased urinary N output suggests N partitioning in dairy cows has been affected by the diet (Edwards *et al*. 2007). High phenolic compounds and condensed tannins that chicory and plantain contain are assumed to be the primary instigators of this shift in N partitioning through reducing the degradability of protein in the rumen (Byrant *et al*., 2019; Koenig and Beauchemin, 2018). Another possible source of the shift in N partitioning in cows grazing forb-based pastures is the ratio of water-soluble carbohydrates (WSC) to crude protein. Forage cultivars with high WSC such as tetraploid perennial ryegrass improve efficiency of dietary N and decrease N waste through the urine in dairy cattle and beef steers (Edwards *et al*. 2007). Though WSC was not measured in this experiment, chicory is known to have much higher values than grasses and legumes (Minnee *et al*. 2017). Furthermore, the decreased MUN of cows few forb pasture could be explained by their mineral content. Other research has shown that increasing mineral intake, specifically NaCl, can decrease MUN concentrations (Dijskra *et al*., 2013). Forb pastures had the highest ash content of all pastures, indicating high mineral contents. Plantain is known for high mineral concentrations, specifically Ca, Mg, Na, P, Zn, Cu, and Co (Stewart, 1996).

Cows that grazed legume pastures had greater N intake and urine and milk N outputs. The main components of the legume pastures were red clover and birdsfoot trefoil (64%) in the current study. Both legume species were classified as containing lower water-soluble carbohydrates as compared to alfalfa and white clover, indicating lower N output potential than more commonly grown pasture legumes (Krawutschke *et al*., 2013). However, the bioactive compounds found in these legumes were probably could not offset the high N concentration of legume pastures as compared to forb-based pastures. An indoor feeding experiment with Holsteins by Christenson *et al*. (2015) compared birdsfoot trefoil hay and alfalfa hay based total mixed ration (TMR) and showed no significant difference urinary N output. Hymes-Fecht *et al*. (2013) compared urinary N output of lactating Holsteins fed red clover, alfalfa, and birdfoot trefoil silages. Though there was not a significant difference between treatments, birdfoot trefoil silages made from cultivars of normal and high condensed tannin concentrations had a tendency to decrease urinary N output.

4.4.3 Methane Emissions

Current research has shown that changing the diet of grazing ruminant livestock to include plants containing plant secondary metabolites such as condensed tannins can affect both enteric methane emission and nitrogen utilization by dairy cows (Grainger *et al*., 2009; Williams *et al*., 2011; Ghelichkhan, 2018). Depending upon the source and the inclusion concentration of condensed tannin in the diet, the plant secondary metabolite can decrease enteric methane emissions up to 50% (Bodas *et al*., 2012; Naumann *et al*., 2017). Furthermore, pastures that contain high digestible forages have potential to decrease CH_4 emissions from grazing animals through increased production efficiency (Hegarty 1999; Hristov *et al*, 2013). Though there is no consensus on the mechanism for condensed tannins inhibition of methane production, condensed tannins are assumed either to inhibit the methane-producing bacteria in the rumen or bind the proteins the methanogens break down in order to produce methane. In relation to the effects of condensed tannins on methane yields, the results from the current study are not conclusive, although methane emissions from cows that grazed forb pastures tended to be the lowest as compared to the cows that grazed legume and grass-based pastures. Legume and forb pasture had a similar forage nutritive value but legume pastures had almost five times higher CT content as compared to forb pastures in period 1. It was of note that the methane emission from cows that grazed legume pastures appeared to be higher, although the methane cost of production (methane/kg milk) were similar with forb and grass based pastures associated with higher DMI of high quality feed that legume pastures offered. It is possible to attribute this effect to the presence of CT in legume pastures.

The results from various previous studies are also quite divergent. Waghorn *et al*. (2002) reported lower CH_4 emissions from sheep that fed chicory and bigleaf trefoil (*Lotus pedunculatus*) (12 to 17 g CH_4/kg DMI) as compared to the sheep fed ryegrass (21 g CH_4/kg DMI). Sun *et al*. (2011) did not report any differences in CH_4 emission from sheep that grazed ryegrass or chicory pastures. Although not directly comparable to the results of the current study, Williams *et al*. (2016) reported higher methane yield (26.1 g CH_4/kg DMI) of dairy cows that were fed chicory rich diet as compared to the cows that consumed concentrate (21.0 g CH_4/kg DMI) or forage brassica based diets FBR (20.5 g CH_4/kg DMI) diets. However, the methane yield of Jersey cows that grazed forb-based pastures (17.4 g CH_4/kg DMI) in the current study compare favourable to the methane yield of Holstein–Friesian cows that were fed chicory rich diet reported by Williams *et al*. (2016). Overall, The tendency for a lower level of methane emission together with the possible inhibition of protein breakdown in the rumen may have increased the energy-protein coupling (or nutritional synchrony, Niwińska, 2012) in the rumen increasing the efficiency of the fermentation, as indicated by the higher microbial N, higher dairy efficiency, higher milk yield, and the tendency for the lower methane emission compared to grass.

4.5 Conclusions

The current study indicated that incorporating legume and forb based pasture offers a viable option to manage wet soils as evidenced with higher milk yield and less environmental pollution potential. The findings of these studies indicated that the potential of forbs and legume pastures for maintaining high milk yields were particularly apparent when the nutritive quality of grasses was lower. It is also noteworthy that despite the fact that the nutritive value of forb pastures was comparable to legume pastures both DMI and milk yields were not at par with the cows that grazed legume pastures. However, including forb-based pastures that contain chicory and plantain in feedbase of dairy cows may be more effective for reducing the environmental impact of pasture based dairy farming than legume pastures.

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