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Forage Based Livestock Systems

FORAGE BASED LIVESTOCK SYSTEMS

Milk production, nitrogen utilization, and methane emissions of dairy cows grazing grass, forb, and legume-based pastures

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Abstract

Achieving high animal productivity without degrading the environment is the primary target in pasture-based dairy farming. This study investigated the effects of changing the forage base in spring from grass-clover pastures to forb or legume-based pastures on milk yield, N utilization, and methane emissions of Jersey cows in Western Oregon. Twenty-seven mid-lactation dairy cows were randomly assigned to one of three pasture treatments: grass-clover-based pasture composed of festulolium, tall fescue, orchardgrass, and white clover (**Grass**); forb-based pasture composed of chicory, plantain, and white clover (**Forb**); and legume-based pasture composed of red clover, bird's-foot trefoil, berseem clover, and balansa clover (**Legume**). Pastures were arranged in a randomized complete block design with three replicates (i.e., blocks) with each replicate grazed by a group of three cows. Production and nutritive quality of the forages, animal performance, milk components, nitrogen partitioning, and methane emissions were measured. Feed quality and dry matter intake (**DMI**) of cows were greater ($P \leq 0.05$) for Legume and Forb vs. Grass, with consequent greater milk and milk solids yields ($P < 0.01$). Cows grazing Forb also had more ($P < 0.01$) lactose and linoleic acid in milk compared with cows grazing the other pastures, and less ($P = 0.04$) somatic cell counts compared with Grass. Cows grazing Forb had substantially less ($P < 0.01$) N in urine, milk, and blood compared with cows grazing the other pastures, with not only a greater ($P < 0.01$) efficiency of N utilization for milk synthesis calculated using milk urea nitrogen but also a larger ($P < 0.01$) fecal N content, indicating a shift of N from urine to feces. Both Forb and Legume had a diuretic effect on cows, as indicated by the lower ($P < 0.01$) creatinine concentration in urine compared with Grass. Methane emissions tended to be less ($P = 0.07$) in cows grazed on Forb vs. the other pastures. The results indicate that Forb pasture can support animal performance, milk quality, and health comparable to Legume pasture; however, Forb pasture provides the additional benefit of reduced environmental impact of pasture-based dairy production.

Key words: methane emission, nitrogen partitioning, sustainability, tannins

Abbreviations

ADF	acid detergent fiber
BCS	body condition score
BW	body weight
CP	crude protein
CT	condensed tannins
DM	dry matter
DMI	dry matter intake
EE	ether extract
FCM 4%	fat-corrected milk yield
GHG	greenhouse gases
HT	hydrolyzable tannins
MUN	milk urea nitrogen
NDF	neutral detergent fiber
NFC	non-fiber carbohydrates
PD	purine derivatives
PM	plate meter
PPO	polyphenol oxidase
RDP	rumen degradable protein
SCC	somatic cell counts
SNF	solid nonfat

Introduction

Greenhouse gas (**GHG**) emissions (CO_2, CH_4, and N_2O) and nitrate (NO_3) leaching from soils by maintaining ruminant livestock on pasture are a growing concern (Lee et al., 2013; Ghahramani et al., 2019). Dairy production in the United States has specifically come under scrutiny despite only contributing 1.3% of the total national GHG emissions (Rotz, 2018). Therefore, maintenance of high-performing pastures while preserving the environment is a primary target of pasture-based dairy farming. The choice of pasture species and their management for grazing have major impacts on pasture productivity and environmental indicators (Ledgard et al., 2009). Grass-clover pastures form the main feed base for dairy cows in temperate climates (Lee et al., 2018). In well-managed dairy farming systems, these pastures can persist under intensive grazing while providing for high animal performance (Dineen et al., 2018). However, pastures often exceed protein requirements of dairy cattle, leading to increased nitrogen (N) waste (Van Vuuren et al., 1992). Urea in urine is quickly transformed to ammonia (NH_3) and volatilizes from the soil surface or, in the soil, is transformed to NO_3 that may leach into nearby groundwater.

Incorporating legumes and herbs in pastures that synthesize natural phytocompounds may help mitigate the deleterious effects of intensive dairy farming on soil, water, and air quality (Villalba et al., 2019; Bryant et al., 2020). In particular, bioactive compounds such as condensed tannins (**CT**) and hydrolyzable tannins (**HT**) lead to reduced CH_4 production and less partitioning of the ingested N to urine through protecting the protein from degradation in the rumen (Aboagye and Beauchemin, 2019; Stewart et al., 2019). Partitioning N away from urine to feces has important environmental implications because fecal N is released via mineralization and is more organically stable, leading to a reduction in N emissions (Bryant et al., 2020).

Legume and forb-based pastures also provide higher production of superior quality forage and complement grass growth (Chapman et al., 2008). These pastures can be grazed as supplementary to grass-clover pastures or as 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). A number of recent studies compared traditional grass-clover pastures with diverse pastures containing non-leguminous forbs or with specialized chicory and plantain pastures for their effects on animal performance and environmental pollution (Totty et al., 2013; Minneé et al., 2017; Bryant et al., 2020). However, there is much less information available on the potential effects of forage legumes with high N content and bioactive compounds on GHG and NO_3 emissions in dairy grazing systems. Thus, this study compared the effects of grass, forb, and legume-based pasture mixtures on milk yield, milk components, N partitioning, and CH_4 yields from individual cows. We hypothesized that specialized forb- and legume-based pastures would maintain their high nutritive value as compared with grass-clover pastures during late spring and summer and, therefore, would support greater milk production with less environmental pollution.

Materials and Methods

All procedures were approved by the Oregon State University Animal Care and Use Committee (ACUP# 5026) prior to the commencement of the experiment. A grazing experiment was carried out at the Oregon State University Dairy Center in Corvallis, OR (44° 34′ N, 123° 18′ W, 78 m a.s.l.) to test the effect of grass-clover vs. specialized legume- or forb-based pastures on milk production, N utilization, and CH_4 emissions from dairy cows. The soil type is a combination of Amity silt loam, Holcomb silt loam, and Bashaw silty clay loam. Soil tests indicated that the site had an organic matter content of 5.8%, 113 kg available P/ha, 1,772 kg Ca/ha, 230 kg K/ha, 298 kg Mg/ha, 0.17 dS/m salinity, and pH 5.9. The site has a long-term mean annual precipitation of 1,086 mm. In 2018 to 2019 growing season (October to September), the total annual precipitation was 885 mm.

Experimental design and grazing management

The pastures were sown on May 20, 2018. A 5.85-ha paddock was divided into three 1.95-ha blocks to serve as replicates. Each block was divided into three 0.65 ha (62 × 105 m) paddocks, which were randomly allocated to a combination of grass, forb, or legume-based pastures, giving a total of nine grazing paddocks. Pasture treatments were: 1) **Grass,** a grass-based pasture that consisted of festulolium cv. Perun (X *Festulolium braunii*), tall fescue cv. Rustler (*Festuca arundinacea*), orchardgrass cv. Sundown (*Dactylis glomerata*), and white clover cv. Domino (*Trifolium repens*); (2) **Forb**, a forb-based pasture that consisted of chicory, cv. Antler (*Cichorium intybus*), plantain cv. Boston (*Plantago lanceolata*), and white clover; and (3) **Legume,** legume-based pastures that contained red clover cv. Raven (*Trifolium pratense*), bird's-foot trefoil cv. Bruce (*Lotus corniculatus*), berseem clover cv. Frosty (*Trifolium alexandrinum*), and balansa clover cv. Fixation (*Trifolium michelianum*). The forages were not grazed but cut for silage twice in the year of establishment.

Twenty-seven Jersey cows in mid-lactation were used in a randomized complete block design with three pasture treatments (nine cows per treatment), two growth periods, and three replicates (blocks). Nine cows were allocated to each treatment with three cows assigned to each replication based on age (mean ± s.d.; 3.2 ± 1.5 yr), live weight (mean ± s.d.; 480 ±

46 kg), milk production (mean ± s.d.; 24.8 ± 6.2 kg/cow per day), and days in milk (mean ± s.d.; 157 ± 64 d). Each plot was grazed by a group of two multiparous and one primiparous cows. Prior to the commencement of the experiment, all cows grazed a diverse pasture mixture together as one herd.

The 39-d grazing experiment was carried out from April 29 to June 6, 2019. Temporary electric fences were used to separate the paddocks and to separate daily pasture allocations. Prior to the grazing trial, Grass pastures were grazed with a group of heifers in mid-March to prevent the accumulation of excess low-quality herbage material. A "put and take" grazing management was applied to match the seasonal forage growth to animal intake (Bransby, 1989) during the grazing experiment. Each treatment had a core group of nine cows (testers) with three spare cows (regulators). Cows strip-grazed and were allocated an estimated 16 kg of dry matter (**DM**)/cow per day (3.3% of body weight [**BW**] with a post-grazing residual of 1,300 kg of DM/ha. Water troughs were moved as needed to ensure ad libitum access to water.

The grazing experiment was split into two periods (regrowth cycles) to consider the effects of changing botanical and chemical compositions of the pastures on measured parameters. The first period was 21 d (April 29 to May 19) and the second period was 18 d (May 19 to June 6). The cows were allowed a 14-d acclimation period from April 29 to May 13 in period 1. Samples from the cows and pastures were collected during the final 7 d of each period.

The cows were milked twice daily (approximately 0500 and 1800 hours) and offered a new pasture allowance after each afternoon milking. Each day all cows received 2 kg DM of rolled grain mix (corn and barley mix 50:50) and 91 g of mineral, with each offered in two equal portions following the morning and afternoon milking throughout the grazing experiment (acclimation and trial periods). No refusals were left after each feeding during the entire experiment. The grain mix contained an average of 9% crude protein (**CP**), 12.4% neutral detergent fiber (**NDF**), and 2.3% ash. Mineral mix contained 170 to 210 g Ca/kg, 70 g P/kg, 80 g Mg/kg, 16.5 g S/kg, 20 to 24 mg Se/kg, and 100 IU/kg vitamin A.

Pasture measurements

Herbage dry matter intake (**DMI**) was estimated from pre- and post-grazing herbage mass in each plot using 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 d). The PM was calibrated by regression against pasture DM by collecting 18 quadrats (each 0.25 m^2; nine pre-grazing and nine post-grazing quadrats) per pasture. Quadrats were cut to 30 mm residual height with electric hand shears. Apparent group DMI of cows was calculated as herbage disappearance between pre- and post-grazing in the allocated area. Calibration was repeated in period 2. Calibration and intake estimation were successfully accomplished except for the forb pastures in period 1 because reproductive stalks of chicory plants prevented the accurate measurements of pasture mass with the 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:

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

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

Period 2:

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

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

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

PRandom pluck samples were collected from pre-grazing allocations of each pasture to determine chemical and botanical compositions of forage on offer. A total of 50 to 75 pluck samples, representative of herbage offered to cows, were collected by hand randomly across pastures (in a "zigzag" pattern) in each plot at 2-d intervals during the 7-d experiment period. Samples were collected within each plot before animals were turned onto fresh pastures. Subsamples 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 screen (Thomas/Wiley, Swedesboro, NJ) for chemical analyses using the Association of Official Analytical Chemists methods (AOAC, 2016). Samples were analyzed for DM (method 930.15), ash (method 942.05), and ether extract (**EE**) (method 920.39). Crude protein (CP = 6.25 × N) concentration of all samples was determined by the Kjeldahl method (AOAC, 2016); LECO FP828, MI, USA). NDF and acid detergent fiber (**ADF**) were analyzed sequentially using an Ankom^{200} Fiber Analyzer (ANKOM Technology Corp., Macedon, NY; Van Soest et al., 1991). The NDF was analyzed with the inclusion of a heat-stable α-amylase and sodium sulfite. Non-fiber carbohydrates (**NFC**) concentration was calculated using a modified (NRC, 2001) equation: NFC = 100 − [(%NDF − 2) + CP + Ash + EE)], which assumes a concentration of neutral detergent insoluble CP concentration of 2.0% (Hall, 2001). Total apparent N intake was calculated using the individual N contents (%) of the pasture on offer and concentrate × the average daily apparent DM intake (kg DM) of each component.

Forage samples were also analyzed for total CT according to previously described method by Grabber et al. (2013). The CT contents of Legume and Grass pastures were assayed using standards isolated from bird's-foot trefoil, whereas standard isolated from chicory was used for Forb pastures. HT in Forb pastures were analyzed as previously described (Hartzfeld et al., 2002) using a methyl gallate standard. Bird's-foot trefoil and chicory foliage and white clover flowers are known to contain CT (Foo et al., 2000), while the absorbance spectrum of plantain is consistent with other forbs known to contain HT (Mueller-Harvey I., The University of Reading, UK, personal communication). Since pasture botanical composition was known and only Forb pastures contained plantain, only Forb samples were assayed for HT.

Milk and body condition score measurements

Daily individual milk yield was automatically recorded by the AfiMilk system (Kibbutz Afikim, Israel). Milk samples were collected from each cow after morning (0500 hours) and evening (1800 hours) milking on days 0 (baseline), 15, 18, and 21 for period 1 and days 12, 15, and 18 for period 2 to determine the milk composition. Samples were analyzed commercially (Willamette DHIA Laboratory in Salem, OR) for fat, protein, lactose, solid nonfat (**SNF**), somatic cell counts (**SCC**), and milk urea nitrogen (**MUN**) by near-infrared spectrophotometry. Fat-corrected milk yield (**FCM, 4%**) was calculated using the following formula: (0.4 × kg milk) + (15 × kg fat).

Total daily milk N output was calculated by dividing the milk protein concentration (%) by 6.38 to obtain the N concentration (%), which was then multiplied by the milk yield (kg/d) to obtain milk N output (kg/d). Cows were scored weekly by two trained, independent evaluators using a 5-point body condition score (BCS) scale (1 = thin; 5 = fat).

Milk fatty acid profile

Milk fatty acid profiles were determined as previously described (Folch et al., 1957; Morrison and Smith, 1964). Approximately, 1 mL of milk sample was combined with 5 mL of chloroform: methanol solution (2:1, v/v) to extract lipids. Each sample was vortexed with 8 mL of 0.74% potassium chloride. Samples were incubated at room temperature for 2 h to achieve phase separation. The upper phase was discarded, whereas the lower phase was retained and evaporated to dryness under N gas at 70 °C using a Meyer N-Evap Analytical Evaporator (Organomation Associates Inc., Berlin, MA, USA); 1 mL of 0.5 N potassium hydroxide in methanol was added, followed by heating in a water bath at 70 °C for 10 min. Then, 1 mL of 14% boron trifluoride in methanol was added, followed by heating in a water bath at 70 °C for 30 min. The sample was then cooled to room temperature, followed by addition of 2 mL of High Performance Liquid Chromatograph (**HPLC**) grade hexane and 2 mL of saturated sodium chloride. The upper phase was removed and mixed with 800 mg of sodium sulfate to remove moisture. The remaining sample was placed in a water bath at 70 °C and evaporated under N. A Varian 420 gas chromatograph (Varian, Palo Alto, CA, USA) was used to analyze fatty acid methyl esters. A fused silica capillary column (SPTM-2560; 100 m × 0.25 mm × 0.2 µm film thickness; Supelco, Bellefonte, PA, USA) was used. Conditions were as follows: injector temperature, 240 °C; flame ionization detector, 260 °C; helium carrier gas, 37 psi; and oven temperature, 2.5 °C/min to a maximum 240 °C and held for 16 min. Individual fatty acids were normalized to total fatty acids. External analytical standards (Supelco, Bellefonte, PA, USA) were used to identify fatty acids.

Blood, urine, and fecal measurements

Immediately after the morning and afternoon milking on days 0 (baseline), 15, 18, and 21 in period 1 and days 12, 15, and 18 in period 2, the cows were taken into the Oregon State University 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 N volatilization, and then stored at –20 °C until analysis. Feces were collected via manual stimulation or collected as cows defecated and frozen at –20 °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 no anticoagulant for plasma and serum isolation, respectively. After blood collection, tubes with lithium heparin were placed on ice and tubes without additive were kept at room temperature until centrifugation (~1 h). 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. Plasma urea was measured using an ILab 600/650 kit (Instrumentation Laboratory, Bedford, MA, USA) by the Department of Animal Sciences Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore (Piacenza, Italy), following the kit instruction (Calamari et al., 2016).

Fecal samples were thawed, weighed, and dried in an oven at 55 °C for 72 h to determine DM content. Dry fecal samples were ground to 1 mm and analyzed for DM, ash, and N contents. The N contents of feces, plasma, and urine samples were determined by using an N analyzer (LECO FP828, MI, USA).

Samples of urine collected after the morning and afternoon milking from each cow on days 0 and 21 in period 1 and day 18 in period 2 were analyzed for concentration of purine derivatives (**PD**) and urea by HPLC (Agilent 1260 Infinity, Agilent Technologies, Waldbronn, Germany) fitted with a Luna 5 µm C18(2) 100 Å, LC Column 250 × 4.6 mm (00G-4252-E0, Phenomenex Inc., Torrance, CA), and SecurityGuard cartridges for C18 HPLC columns (AJ0-4287, Phenomenex Inc.). Urine samples were diluted 10-fold with double-distilled water and filtered using syringe filters and 1 mL disposable Luer Lock syringes (57022-N04-C and 58901-S, MicroSolv Technology Corporation, Leland, NC). Filtered diluted samples were inserted into 1 mL 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 a previously published method (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 35 °C). 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, decreasing the derivatization of urea. To address that issue, we ran the second point of the standard curve every 10 runs and we used three samples that were added into the sequence every 10 samples and used the data to adjust for the final urea concentration. Urea was quantified using a 5-point standard curve (4-fold dilution) of purified urea (BDH4602-500G, VWR) prepared in pH 2.4 double-distilled water to match the acidified urine. Creatinine, uric acid, and allantoin concentrations were analyzed following a previously developed method (George et al., 2006). A standard curve constituted of 480 µg/mL of allantoin (Spectrum, New Brunswick, NJ, USA), 120 µg/mL of creatinine (TCI, Portland, OR, USA), and 108 µg/mL of uric acid (Alfa Aesar) diluted in five steps to a 4-fold dilution was used for final quantification.

Urinary N excretion (g/d) was estimated as urinary g of N/d = 21.9 (mg/kg) × BW (kg) × [1/ urinary creatinine (mg/kg)] × urine N (g/kg), as previously described (Pacheco et al., 2009). Due to high diurnal variations in urinary N and creatinine concentrations, urinary N excretion (g/d) estimation was also performed using the relationship with MUN and urinary N output as described by Nousiainen et al. (2004). The following formula was used to estimate urinary N output: 14.1 × MUN + 26. Milk N efficiency was calculated as N in milk divided by N intake. Milk N efficiency was also estimated using a formula based on MUN according to Nousiainen et al. (2004) (N efficiency,

% = –0.73 × MUN + 38). The latter also accounts for the protein balance in the rumen and urinary-N excretion.

Methane emission

Methane 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 administered to the rumen or reticulum of each cow at the beginning of the study using a bolus gun. The release rate from the permeation tubes was about 1,200 ng/min or 2 mg/d. Each permeation tube was loaded with 600 mg of SF_6 and the release rates were measured gravimetrically for 6 wk before using them 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 (< 0.2 psi) had a negative pressure, which drew air continuously for a 24-h period through the filter. After the samples were collected, the canister was removed and pressurized with high purity N gas (N_2).

The collected gas was sampled and assayed using a gas chromatograph to determine the concentrations of CH_4 and SF_6 (Johnson et al., 2007). The emission rate of the permeation tube and the ratio of SF_6 to CH_4 in the collection canister, corrected for background concentrations, 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 the two multiparous cows in each grazing plot) for six consecutive days (on day 16 to 21) during period 1. The measurement of CH_4 emission from one cow in the Forb group failed. For the same 6 d, two ambient air samples (background concentrations) were collected in canisters located in different paddocks.

Statistical analysis

All variables were subjected to 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 CH_4 emissions data that were analyzed by pasture type as this variable was only collected during period 1. The analyses of CH_4 emissions were performed by unbalanced one way-ANOVA as the measurement of one cow in the Forb group failed. Treatment means for urine, feces, milk, and blood parameters were determined using data collected from individual cows during the experimental periods (morning and evening of days 15, 18, and 21 in period 1 and days 12, 15, and 18 in period 2). Individual cow means within each replicate were combined to give group as the experimental unit (pasture plots) within each period. Herbage and total DMI intakes were estimated as means for the treatment group as cows grazed pastures as small herds (three cows). 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., Rothamsted, UK) by ANOVA (Payne, 2009). Significant differences among treatment means were compared by Fisher's protected least significant difference at $P < 0.05$.

Results

DMI and pasture quality

Pregrazing Forb herbage mass was greater than that of Grass ($P = 0.05$; Table 1). In general, pre-grazing herbage mass ($P = 0.01$) was less in period 2 than period 1, and the difference was larger for Forb and Legume as compared with Grass (interaction, $P = 0.01$). Herbage DMI differed among treatments ($P = 0.05$) with the cows in Legume having the greatest herbage DMI. Overall, cows had similar herbage DMI in both periods ($P = 0.29$). Herbage DMI as % of BW of cows in Grass was 2.78 and this was lower than those in Legume and Forb ($P = 0.01$).

The chemical composition of offered pastures was significantly different among pastures for all measured parameters except EE and CT concentrations. Pastures had greater CP and ash but lower NFC values in the second than in the first period as well (Table 2). There was a tendency for an interaction between pastures and grazing periods for CP concentration ($P = 0.06$). The CP concentration increased in Legume and Grass pastures from the first to the second period, while CP concentration in Forb pasture remained generally stable. The ADF concentration of Legume was less than for Grass and Forb pastures ($P = 0.01$). Grass pasture had greater NDF concentration than Forb and Legume pastures ($P = 0.01$). The NFC and ash concentrations differed among pastures with the greatest concentrations in Forb followed by Legume and Grass pastures, which had similar NFC and ash contents ($P = 0.01$). CT concentrations did not differ among the pasture types ($P = 0.34$), while Forb pastures had 17.1 mg/g DM of HT content.

Botanical composition of pastures

White clover proportion in Grass ranged from 7% in period 1 to 20% in period 2 (Table 3). Chicory and plantain were the main components of Forb, comprising over 56% of the DM. Legume was predominantly composed of red clover, bird's-foot trefoil,

Table 1. Effect of pasture type on feed intake of grazing dairy cows in period 1 (April 29 to May 19) and period 2 (May 19 to June 6)

							P-values*		
	Grass	Forb	Legume	Period 1	Period 2	SEM	Pas	Per	P × P
Pre-grazing herbage mass, kg DM/ha	3,071[b]	3,399[a]	3,140[ab]	4,089	2,317	122.7	0.05	0.01	0.01
Post-grazing herbage mass, kg DM/ha	1,601[a]	1,651[a]	1,335[b]	1,598	1,460	109.1	0.05	0.15	0.06
Herbage DMI, kg	13.6[b]	14.5[ab]	15.0[a]	14.5	14.2	0.42	0.05	0.29	0.63
Total DMI, kg	15.6[b]	16.5[ab]	17.0[a]	16.5	16.2	0.42	0.05	0.29	0.63
Herbage DMI, % BW	2.78[b]	3.03[a]	3.19[a]	3.03	2.98	0.091	0.01	0.51	0.76
Total DMI, % BW	3.19[b]	3.61[a]	3.45[a]	3.44	3.40	0.094	0.01	0.57	0.79
BCS	2.9	3.1	3.0	3.0	2.9	0.15	0.49	0.17	0.93

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
*Pas, pasture; Per, period; P × P, pasture and period interaction; SEM, standard error for interaction.

Table 2. Chemical composition and secondary metabolites of grass-clover, forb-, and legume-based pastures in period 1 (April 29 to May 19) and period 2 (May 19 to June 6)

							P-values*		
	Grass	Forb	Legume	Period 1	Period 2	SEM	Pas	Per	P × P
Ash, % DM	9.2[b]	11.3[a]	9.8[b]	9.2[b]	10.9[a]	0.32	0.01	0.01	0.71
CP, % DM	17.9[b]	18.7[b]	23.0[a]	18.2[b]	21.5[a]	0.79	0.01	0.01	0.06
ADF, % DM	25.4[a]	24.6[a]	22.8[b]	24.9	23.6	0.76	0.01	0.06	0.35
NDF, % DM	45.5[a]	35.5[b]	36.5[b]	40.0	38.4	1.27	0.01	0.14	0.43
EE, % DM	1.8	1.9	2.1	2.0	1.8	0.14	0.16	0.07	0.26
NFC[1], % DM	28.2[b]	35.5[a]	30.6[b]	33.6[a]	29.3[b]	1.33	0.01	0.01	0.69
CT, mg/g DM	2.96	3.11	3.25	2.95	3.26	0.196	0.34	0.06	0.07
HT, mg/g DM	—	17.1 (±2.10)	—	15.9 (±1.98)	18.4 (±1.33)	—	—	—	—

[1]NRC (2001).
[a–b]Means within a row and category with different superscripts differ ($P < 0.05$).
*Pas, pasture; Per, period; P × P, pasture and period interaction; SEM, standard error for interaction.

Table 3. Botanical composition (% of total DM) of the grass, forb, and legume-based pastures in period 1 (April 29 to May 19) and period 2 (May 19 to June 6)

	Grass		Forb		Legume	
Component	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
Sown grasses	81	69	—	—	—	—
Annual ryegrass (volunteer)	12	11	22	26	15	27
White clover	7	20	9	15	—	—
Chicory	—	—	44	33	—	—
Plantain	—	—	23	23	—	—
Red clover	—	—	—	—	56	40
Bird's-foot trefoil	—	—	—	—	14	13
Berseem clover	—	—	—	—	4	10
Balansa clover	—	—	—	—	8	9
Dead material	0	0	0	3	0	1
Weeds	0	0	2	0	3	0

with lesser amounts of berseem and balansa clovers. Overall, the average legume content decreased to 72% as volunteer annual ryegrass increased by 12% from period 1 to period 2. Weeds comprised 2% to 3% of the total DM in period 1 but were absent in period 2.

Milk production and composition

Milk yield (kg/d) of the cows differed among pasture treatments ($P = 0.01$) but it remained similar in both periods ($P = 0.43$; Table 4). The cows that grazed Legume and Forb had greater milk yield, FCM, and yield of milk components than the cows that grazed Grass ($P < 0.01$). We detected a tendency ($P = 0.06$) for a decrease in 4% FCM between the first and second periods. The average milk fat yield decreased in all treatments from period 1 to period 2 ($P = 0.01$). Milk fat concentration tended to be greater for cows that grazed Legume and Forb compared with Grass ($P = 0.07$). Overall, milk fat concentration of all treatments decreased from 4.8% in the first period to 4.5% in the second period ($P = 0.05$). Milk protein concentration did not differ among pasture treatments but increased from the first to the second period ($P = 0.05$). Concentrations of SNF and lactose were greater in the milk of cows that grazed Forb than in milk from cows that grazed Legume or Grass ($P \leq 0.05$). Cows that grazed Forb had similar SCC in milk to Legume but less compared with those grazing Grass ($P = 0.03$) and values decreased from period 1 to period 2 ($P = 0.03$).

The fatty acid profiles of milk revealed an effect of pasture type on linoleic acid (C18:2n6c) with cows grazing Forb pastures having the highest concentration followed by cows grazing Legume with the lowest concentration for cows grazing Grass pastures ($P = 0.01$; Table 5). Few of the other measured fatty acids in milk were affected by pasture treatments. Those include lauric acid (C12:0), which was greater for cows grazing Forb vs. cows grazing Grass pastures ($P = 0.05$), and palmitoleic (C16:1) and stearic (C18:0) acids that were greater for cows grazing Grass compared with cows grazing other pastures ($P = 0.01$). Grass cows also had a tendency ($P = 0.09$) for greater monounsaturated fatty acids in milk compared with the other groups. The activity of the Δ9 desaturase was also greater for cows grazing Grass compared with the cows grazing the other pastures ($P = 0.01$).

Nitrogen in urine, feces, milk, and plasma

Cows that grazed Legume had greater N intake than cows grazing the other pastures, while cows that grazed Grass consumed less N than cows grazing Forb ($P = 0.01$; Table 6). The N intake of cows was greater in the second than the first grazing period ($P = 0.01$). Percentage of N in urine was less for cows grazing Forb than Grass or Legume, while urine N concentration of cows grazing Legume was greater than for those grazing Grass ($P = 0.01$). Urine N content was 26% greater in the second than the first period ($P = 0.01$). Similarly, cows that grazed Forb had lower ($P \leq 0.05$) urine NH_3 and creatinine concentrations and a tendency

Table 4. Milk yield and components of dairy cows grazing grass, forb, and legume-based pastures in period 1 (April 29 to May 19) and period 2 (May 19 to June 6)

							P-values*		
	Grass	Forb	Legume	Period 1	Period 2	SEM	Pas	Per	P × P
Milk, kg/d	20.5[b]	22.0[a]	22.9[a]	22.0	21.6	0.63	0.01	0.43	0.47
4% FCM, kg/d	21.7[b]	24.2[a]	25.3[a]	24.6	22.9	0.97	0.01	0.06	0.66
Dairy efficiency[1]	1.40	1.47	1.49	1.49	1.42	0.08	0.46	0.34	0.91
Milk solids, kg[2]/d	1.8[b]	2.0[a]	2.1[a]	2.0	2.0	0.06	0.01	0.60	0.50
Milk fat, g/d	902[b]	1,023[a]	1,073[a]	1,051[a]	952[b]	50.0	0.05	0.01	0.74
Milk protein, g/d	705[b]	761[a]	776[a]	745	751	20.7	0.01	0.77	0.53
Components									
Fat, %	4.43	4.70	4.82	4.84[a]	4.46[b]	0.151	0.07	0.05	0.73
Protein, %	3.47	3.50	3.43	3.43[b]	3.51[a]	0.041	0.31	0.05	0.54
SNF, %	9.05[b]	9.24[a]	9.11[b]	9.10	9.16	0.064	0.05	0.27	0.93
Lactose, %	4.59[b]	4.76[a]	4.66[b]	4.68	4.66	0.045	0.01	0.58	0.68
SCC, log_2[3]	15.41[a]	14.62[b]	14.83[ab]	15.23[a]	14.67[b]	0.215	0.03	0.03	0.27

[1]Dairy efficiency was calculated by dividing FCM with total DMI.
[2]Nonfat milk solids: SNF and SCC.
[3]Log_2 of (10^3 cells/mL).
[a,b]Means within a row and category with different superscripts differ ($P < 0.05$).
*Pas, pasture; Per, period; P × P, pasture and period interaction; SEM, standard error for interaction.

Table 5. Fatty acid profile of milk of dairy cows grazing grass, forb, and legume-based pastures in period 1 (April 29 to May 19) and period 2 (May 19 to June 6)

							P-values*		
Milligrams/100 mg	Grass	Forb	Legume	Period 1	Period 2	SEM	Pas	Per	P × P
C10:0	0.71	0.95	0.94	0.49[b]	1.24[a]	0.11	0.21	0.01	0.49
C12:0	2.30[b]	2.91[a]	2.57[ab]	2.30[b]	2.90[a]	0.17	0.05	0.01	0.72
C14:0	11.5	11.6	11.5	11.8	11.4	0.25	0.88	0.18	0.45
C14:1	2.31	2.28	2.25	2.32	2.24	0.06	0.76	0.21	0.72
C16:0	34.8	34.0	35.2	35.9[a]	33.5[b]	0.64	0.45	0.01	0.17
C16:1	2.33[a]	1.93[b]	2.09[b]	2.18	2.05	0.08	0.01	0.17	0.09
C18:0	15.0[a]	14.1[b]	14.1[b]	14.4	14.4	0.21	0.01	0.97	0.20
C18:1n9t	2.69	2.20	2.40	2.19[b]	2.67[a]	0.20	0.21	0.05	0.20
C18:1n9c	21.8	20.8	20.9	21.7	20.7	0.48	0.29	0.10	0.33
C18:2n6c	1.17[c]	2.41[a]	1.77[b]	1.91[a]	1.66[b]	0.09	0.01	0.02	0.11
C20:0	0.68	0.86	0.78	0.85[a]	0.70[b]	0.06	0.11	0.03	0.01
Other FA	4.74[b]	5.88[a]	5.49[a]	4.09	6.65	0.24	0.01	0.01	0.22
SFA[1]	65.0	64.5	65.1	65.6	64.0	0.76	0.85	0.08	0.11
MUFA[2]	29.1	27.2	27.7	28.3	27.7	0.63	0.09	0.34	0.22
De novo[3]	37.1	38.7	38.6	37.9	38.4	0.87	0.31	0.66	0.37
Preformed[3]	61.6	61.3	61.4	62.1[a]	60.7[b]	0.44	0.87	0.02	0.22
Δ9 14:0[4]	0.17	0.16	0.16	0.16	0.16	0.004	0.78	0.82	0.23
Δ9 16:0[4]	0.062[a]	0.054[b]	0.056[b]	0.057	0.058	0.002	0.01	0.43	0.03
Δ9 18:0[4]	0.59	0.60	0.59	0.60	0.59	0.01	0.78	0.41	0.37
Δ9[4]	0.30	0.29	0.29	0.30	0.30	0.01	0.57	0.94	0.14

[1]Sum of saturated fatty acids.
[2]Sum of monounsaturated fatty acids.
[3]De novo synthetized fatty acids = sum of fatty acids with chain-length <16 + 50% of C16:0 and 50% of unknown fatty acids; preformed is all fatty acids – de novo fatty acids.
[4]Delta 9 desaturase indexes calculated as the sum of delta-9 unsaturated fatty acid/[delta-9 unsaturated fatty acid + saturated fatty acid] for C14:0, C16:0, and C18:0 separated and as a sum of the three (Δ9).
[a,b]Means within a row and category with different superscripts differ ($P < 0.05$).
*Pas, pasture; Per, period; P × P, pasture and period interaction; SEM, standard error for interaction.

($P = 0.08$) for lower urea concentration than those grazing Grass. Cows grazing Legume had almost 4-fold greater urea in urine compared with cows grazing Forb. Creatinine concentration of urine from cows that grazed Forb and Legume was less than cows that grazed Grass ($P = 0.01$). There was a significant interaction between treatment and period for creatinine in urine ($P = 0.02$). The level of creatinine in urine was greater for cows grazing Grass vs. the other pastures only during period 1. The urea normalized by creatinine concentration was greater in the urine of cows grazing Legume vs. the other pastures ($P < 0.01$; Table 6).

Table 6. Nitrogen partitioning of dairy cows grazing grass, forb, and legume-based pastures in period 1 (April 29 to May 19) and period 2 (May 19 to June 6)

							P-values*		
	Grass	Forb	Legume	Period 1	Period 2	SEM	Pas	Per	P × P
N intake, g/d	420[c]	467[b]	578[a]	460[b]	517[a]	20.9	0.01	0.01	0.21
Urine									
N, %	0.33[b]	0.23[c]	0.39[a]	0.27[b]	0.36[a]	0.028	0.01	0.01	0.80
NH_3, mM	2.34[a]	3.21[b]	3.10[ab]	2.47[b]	3.30[a]	0.18	0.01	0.01	0.23
Urea, mM	56.2[bc]	30.9[c]	116.3[a]	57.4	78.2	13.8	0.01	0.09	0.60
Creatinine, mM	2.43[a]	1.81[b]	1.80[b]	2.01	2.02	0.09	0.01	0.89	0.02
Urea:creatinine	24.5[b]	18.0[b]	67.0[a]	31.1	41.9	6.59	0.01	0.06	0.65
Allantoin, mM	14.7[a]	11.2[b]	11.6[b]	12.2	12.8	0.87	0.03	0.56	0.36
Uric acid, mM	1.39[a]	1.07[b]	1.22[b]	1.14[b]	1.31[a]	0.08	0.01	0.02	0.02
PD[1], mM	15.9[a]	12.2[b]	12.9[b]	13.4	14.0	0.88	0.02	0.52	0.24
Allantoin:creatinine	6.17	6.62	6.66	6.27	6.70	0.24	0.31	0.14	0.40
PD:creatinine	6.69	7.25	7.36	6.83	7.37	0.25	0.17	0.09	0.52
N output[2], g/d	154.3[b]	134.2[b]	228.8[a]	141.8[b]	203.0[a]	21.77	0.01	0.01	0.82
N output[3], g/d	187.6[b]	131.5[c]	271.8[a]	178.5[b]	215.4[a]	11.90	0.01	0.01	0.28
Feces									
N, %	2.1[c]	2.8[a]	2.6[b]	2.6[a]	2.5[b]	0.05	0.01	0.01	0.39
Ash, %	19.8[a]	20.6[a]	18.0[b]	19.9	19.0	0.66	0.01	0.14	0.49
DM, %	10.6[b]	10.8[b]	11.6[a]	11.0	11.0	0.24	0.01	0.88	0.01
Milk									
MUN, mg/dL	11.5[b]	7.50[c]	17.4[a]	10.8[b]	13.4[a]	0.56	0.01	0.01	0.28
N output, g/d	111.2[b]	120.8[a]	123.4[a]	118.2	118.6	2.94	0.01	0.84	0.49
Blood plasma									
Urea-N, mM	4.5[b]	2.8[c]	6.9[a]	4.4[b]	5.0[a]	0.32	0.01	0.05	0.12
N efficiency[3], %	29.6[b]	32.5[a]	25.3[c]	30.1[a]	28.2[b]	0.62	0.01	0.01	0.28
N efficiency[4], %	27.3[a]	25.9[a]	21.6[b]	26.3[a]	23.5[b]	1.43	0.01	0.05	0.17

[2]Estimated using urine creatinine concentration according to Pacheco et al. (2007).
[3]Estimated according to Nousiainen et al. (2004).
[4]Milk N efficiency was calculated as milk N ÷ N intake × 100.
[a–c]Means within a row and category with different superscripts differ ($P < 0.05$).
*Pas, pasture type; Per, period; P × P, pasture type and period interaction; SEM: standard error for interaction.

The concentration of the PD allantoin and uric acid in urine was less in cows grazing Forb and Legume vs. cows grazing Grass ($P \leq 0.03$; Table 6). The allantoin:creatinine and PD:creatinine ratios in urine were not affected ($P = 0.31$ and $P = 0.14$, respectively) by pasture type (Table 6). The concentration of PD also decreased from period 1 to period 2. Total N outputs of cows that grazed Forb and Grass were similar; however, Legume led to substantially greater urine N output than the other pastures ($P = 0.01$). When estimated based on the relationship with MUN, the total N output of cows that grazed Forb was also lower than those grazed Grass pastures ($P = 0.01$).

The N concentration of feces was greater for cows grazing Forbs compared with cows on the other pastures, with a lower value detected for cows on Grass compared with cows on Legume ($P = 0.01$; Table 6). The feces of cows grazing Forb and Grass had a greater concentration of ash compared with those grazing Legume ($P = 0.01$). The DM content of feces was greater for cows on Legume pastures compared with cows on Forb and Grass, due to a greater value only in the first period (pasture × period interaction, $P = 0.01$).

Cows on Legume pastures had the highest MUN concentration, while cows that grazed Forb had the lowest MUN, with intermediate MUN concentration for cows that grazed Grass ($P = 0.01$; Table 6). Milk N output was greater for cows that grazed Forb and Legume than for those that grazed Grass ($P = 0.01$). Blood plasma urea concentration of cows that grazed Legume was greater than those grazing Grass or Forb, while cows that grazed Forb had the least concentration ($P = 0.01$). The plasma urea concentration was greater in the second compared with the first period ($P = 0.05$).

Overall, cows that grazed Forb had higher efficiency of utilization of N for milk production than those grazed Legume and Grass when calculated using MUN but a similar efficiency compared with Grass when calculated as N output in milk in proportion to N intake. In addition, cows grazed on Legume had lower efficiency of using N for milk production compared with cows grazed on Grass.

Methane emission

Daily CH_4 production (g/d) of cows on Forb was 14% and 20% less than those grazing Grass or Legume, respectively ($P = 0.07$; Table 7). Methane emissions calculated based on productivity parameters (DMI, milk yield, FCM, milk protein yield, and milk fat yield) did not differ among pasture treatments ($P \geq 0.13$).

Discussion

Legume- and forb-based pastures improve performance of lactating dairy cows

The greater milk yield of the cows that grazed alternative pastures (i.e., Legume and Forb) compared with grass-clover pasture aligned with our hypothesis. Although grass-clover pastures had been grazed by heifers in early spring to prevent the accumulation of low-quality forage, both legume and forb

Table 7. The effect of pasture type on methane emissions and their relationship to animal productivity during period 1 (April 29 to May 19)

	Grass	Forb	Legume	SED[1]	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 4% 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

[1]SED, standard error of the differences of means.

pastures provided higher-quality forages than grass-clover pasture regrowth, as evidenced by lower NDF and higher CP concentrations that translated into greater DMI, at least for the cows grazing Legume. The positive effect of legumes on DMI and animal production has been well-established with different classes of animals and in various grazing systems (Harris et al., 1997; Waghorn and Clark, 2004; Steinshamn, 2010). The increased DMI of cows that grazed legume-based pastures is associated with their higher rate of ruminal fermentation, physical breakdown, and passage rates through the rumen (Waghorn and Clark, 2004).

Although the DMI was not statistically different than cows grazed on Grass, cows grazed on Forb had greater milk and milk solids yields. Our results are very similar to a prior study that reported a tendency for increased milk production when forbs (chicory or plantain) were incorporated from 20% to 60% in dairy cow diets that mainly consisted of perennial ryegrass/white clover (Minneé et al., 2017). In more recent studies (Mangwe et al., 2019, 2020; Bryant et al., 2020), a positive effect on the yield of milk solids and/or DMI was detected in dairy cows that grazed pastures containing forbs compared with ryegrass–white clover pastures. In contrast, another study (Muir et al., 2014) reported no effect on herbage DMI and milk yield by adding chicory to perennial ryegrass (50:50, DM basis). The discrepancy among the studies can be partly explained by the quality of the pasture on offer, being substantially lower in the latter study compared with our study or the other cited studies.

A consistent finding in our and all the above studies was the increase in the yield of milk solids with forb-based pastures. The effect of forbs and legumes on milk solids may also be partially due to the presence of secondary metabolites and high NFC concentrations. For example, increasing bird's-foot trefoil containing CT in cows' diets positively affects milk yield (Woodward et al., 2000). In the present study, we detected measurable concentrations of CT compounds in all pastures but the reason for the improved performance in the cows grazed on Forb and Legume is likely due to pasture chemical composition, digestibility, and factors other than a change in rumen degradability of protein given that CT concentration was small and not different among pastures. It is probable that the CT in grass-clover pastures came from the white clover flowers (Burggraaf et al., 2006).

The increase in milk lactose concentration for cows grazing Forb in our study agrees with the data from Minneé et al. (2017); however, the increase in lactose concentration is peculiar. Due to its strong osmolarity, lactose synthesis should drive milk volume (Osorio et al., 2016) such that lactose concentration should remain relatively stable in milk. Thus, the increase in the concentration of lactose in cows in the Forb group requires an explanation. Besides lactose, other osmotic compounds in milk are minerals and proteins; however, the increase in SNF content was driven exclusively by lactose content in our study. An increased concentration of lactose in milk without substantially changing the concentrations of protein and fat has been previously observed in transgenic animals overexpressing lactalbumin (Osorio et al., 2016). Thus, it is possible that some unknown compounds in chicory and/or plantain might affect the expression of genes coding for proteins involved in lactose synthesis (Osorio et al., 2016). However, the reduced proportional increase in milk volume in Forb remains to be explained.

The lower milk SCC content of cows grazing Legume and Forb compared with cows grazing Grass pastures is a desirable outcome, considering the well-established negative association of SCC with milk quality and mammary health. We are not aware of any prior work where SCC was measured in the milk of cows that grazed forb pastures compared with grass-clover pastures. In a prior study, the SCC was not affected in prior studies where legumes were fed to dairy cows (Eriksson et al., 2012). It is unclear why SCC decreased in Forb and Legume pastures in our study; however, red clover, chicory, and plantain have a number of beneficial secondary metabolite compounds, including anthelmintic, antimicrobial, and digestive aid compounds (Koner et al., 2011; Flythe et al., 2013; Das et al., 2016; Sahan et al., 2017). The high content of vitamins, such as β-carotene and vitamin E in chicory, plantain, and legumes (Elgersma et al., 2013), might also explain the observed lower SCC content of milk. These vitamins have been associated with better immune system function and healthier mammary glands (O'Rourke, 2009). However, further studies are needed to draw more concrete conclusions on the effect of forb or legume pasture on the immune system (Eriksson et al., 2012; Flythe et al., 2013).

The higher proportion of linoleic acid (the only Polyunsaturated fatty acids [**PUFA**] annotated in our study) and a lower proportion of stearic acid in Forb vs. Grass pastures confirmed the findings of Mangwe et al. (2020). Our findings confirmed that Forb has the potential to increase the level of PUFA in the milk of dairy cows, which is a desirable outcome for consumers (Nguyen et al., 2019).

Forb pasture shifts N output from urine to feces and milk

The observed shift in N output from urine to feces and milk, improving the efficiency of N utilization for milk synthesis when calculated using MUN for the cows that grazed Forb compared with Grass pastures, indicated improved environmental impact. The calculation of N utilization efficiency using N-output in milk and N-intake does not account for the efficiency of the utilization of intestinally absorbed N and the protein balance in the rumen. In our study, compared with Grass, Forb not only increased the amount of N into feces, which can be considered a

loss, but also decreased the N output in the urine increasing the N output in the milk, indicating a higher efficiency of using the absorbed N for milk yield. The apparent higher efficiency was captured by the formula using MUN (Nousiainen et al., 2004).

The larger N output in feces in cows grazed on Forb pastures compared with the other pastures, specifically Legume, can be attributed to the presence of HT, which decreases the degradability of protein in the rumen (Koenig and Beauchemin, 2018; Bryant et al., 2020). Increased N output in feces due to CT and HT in feed has been observed in dairy cows consuming bird's-foot trefoil hay (Ghelichkhan et al., 2018) and beef cattle fed bird's-foot trefoil, sainfoin (*Onobrychis viciifolia* Scop.) and small burnet (*Sanguisorba minor* Scop.) (Stewart et al., 2019). Others have reported dramatically decreased urinary N output of dairy cows when forbs such as chicory were incorporated into pasture mixtures (Totty et al., 2013; Vibart et al., 2016). As observed in our study, in the above studies, the shift of N excretion toward feces by Forb pastures was concurrent with a reduction of the amount of N in urine, which can be readily converted to NH_3. Nitrogen in feces is in a less readily volatilized form than N in urine, thus Forb pastures have important environmental implications for N pollution (Marini and Van Amburgh, 2005).

The reduction in the concentration of urea and other N compounds in the urine of cows that grazed Forb pastures, as well as the lower plasma N and MUN concentrations, was likely due to the diuretic effect of plantain (Bryant et al., 2020). This was demonstrated in a recent study of dairy cows grazing chicory or plantain, where the amount of urine produced daily was between 1.5- and 2-fold greater than for cows grazing ryegrass–white clover pasture (Mangwe et al., 2019). The mechanism of the diuretic effect of plantain is still unclear; however, a recent study with rats demonstrated an inhibitory effect on the angiotensin-converting enzyme by phenylethanoid glycosides present in a human-consumed plantain species *Plantago asiatica* (Tong et al., 2019). Total N excretion and N output in urine in our study were estimated based on previously reported algorithms (Pacheco et al., 2009; Totty et al., 2013). That calculation of urinary N is based on the excretion of creatinine, which is a function of animal BW. Thus, our calculated total N output in urine should account for any diuretic effect. However, caution is needed in interpreting the estimated excretion of N in urine from spot urine samples as diurnal variation in urinary N concentration and excretion of N and creatinine in urine is known to occur (Bryant et al., 2018; Lee et al., 2019); (Whittet et al., 2019). In order to partially address that issue, we calculated the urea N excretion also based on its relationship with MUN (Nousiainen et al., 2004), which should be less affected by diurnal variation because milk accumulates urea during the 12-h milking intervals. Both calculations provided a similar outcome, clearly indicating a lower N urinary output in Forb compared with the other pastures (between 13% and 50% reduction). Thus, reducing urinary N concentration through grazing non-leguminous forbs can be a powerful tool to decrease nitrate leaching from urine patches even in the cases where the total N output of forb-grazing cows is not lower than those grazing grass-clover pastures (Bryant et al., 2020).

Reduced N concentration of urine and plasma in cows grazing forb-based pasture, especially urea and NH_3 concentrations, could also be due to a combination of reduced rumen degradable protein (**RDP**) and greater NFC content (Dijkstra et al., 2013). Pastures are known to contain excessive amounts of RDP (Bargo et al., 2003), often in the order of 70% to 80% of total CP (NRC, 2001). Reduction of RDP due to CT (Broderick and Albrecht, 1997) and a concomitant increase of rumen undegradable protein is generally associated with greater milk yield (Santos et al., 1998), especially in grazing cows (McCormick et al., 1999) due to the elevated RDP concentrations in herbage. Excessive RDP also increases deamination and, thus, the production of NH_3 in the rumen. When readily available carbohydrates are not coupled with NH_3 availability in the rumen, NH_3 escapes the rumen and is cleared by the liver via the ureagenesis pathway. In our study, Forb pasture had a substantially larger NFC concentration compared with the other pastures, similar to a prior study (Mangwe et al., 2020). Energy-protein coupling (or nutritional synchrony) in the rumen is key to increasing the efficiency of rumen fermentation (Niwińska, 2012). Therefore, lower urea and NH_3 contents of plasma from cows on Forb pastures could have resulted from better nutritional synchrony. The greater utilization of urea in the rumen despite a significantly larger amount of CP intake in cows grazing Forb compared with Grass pastures is supported by the similar urea to creatinine ratio, which can be considered a proxy for urea utilization in the rumen (assuming the same ureagenesis). Furthermore, Legume pastures provided 20% more N intake but the urea to creatinine ratio was >3-fold higher compared with Forb pastures. The apparent better nutritional synchrony by Forb pastures appeared not to have promoted greater microbial protein production, as suggested by the lack of effect on PD:creatinine (Orellana et al., 2004).

The greater moisture and mineral contents of the pasture may also have contributed to the decreased plasma urea content of cows that grazed Forb pastures. Specifically, greater moisture concentration may have increased urine volume and diluted plasma urea concentration, while mineral intake, specifically NaCl intake, has a diuretic effect and thus decreases urea in plasma and in milk, as previously reviewed (Dijkstra et al., 2013). It is of note that the Forb pastures in our study had the highest ash content of all pastures.

The Legume pasture in our study had the highest nutritive value compared with the other pastures. Legumes are known to be of high nutritive quality and highly digestible by ruminants compared with grass-based pastures (Dewhurst et al., 2009). Thus, it is not surprising that in our study cows grazing Legume had greater milk production and yield of milk components compared with Grass. However, excess N intake of cows consuming legumes with high CP concentrations and insufficient NFC can lead to greater N excretion and N leaching. In the present study, the cows grazing Legume had higher urine N concentrations and greater N output through urine as compared with those grazing Grass and Forbs. The presence of bird's-foot trefoil in the mix of the Legume pasture should have helped shift the N from urine to feces due to the content of CT (Ghelichkhan et al., 2018; Koenig and Beauchemin, 2018). It is of note that CT content of pastures in our study was low (2.96 to 3.25 mg/g DM) and not discernibly different among pastures, probably due to the relatively low proportion of bird's-foot trefoil in Legume pasture. It is possible that higher content of bird's-foot trefoil than in the legume pastures in the current study would have reduced the N excretion in urine. It is of note that red clover and berseem clover (>50% in our legume pastures) are known to contain polyphenol oxidase (**PPO**) enzyme that provides protection against protein degradation in the rumen that leads to greater N utilization efficiency (Lee et al., 2004). It is likely that PPO activity contributed to the N presence in feces and the reduction of RDP in Legume pastures since this compound can complex with plant proteins rendering them unavailable for rumen utilization.

The study also investigated the effect of grazing period to account for changing botanical composition and chemical composition of the pastures. The superiority of Forb and Legume

pastures over Grass pastures was consistent across grazing periods even with increased legume content and nutritive quality of the Grass pastures in period 2. It is of note that the N intake of cows and urine N concentrations increased substantially in response to increased CP content of pastures. Although Legume pastures enhanced milk yield, the environmental burden of higher N output of cows grazing legumes should be carefully considered, in particular, in pastures established on light soils due to higher risk of N leaching. Within a system context, the N use efficiency of different pasture types should also be considered to understand the potential effect of each pasture type on animal performance and environmental pollution (Welten et al., 2019).

Forb-based pastures tend to decrease methane emission

Changing the diet of grazing ruminant livestock to include plants containing secondary metabolites such as CT and HT can affect both enteric CH_4 emissions and N utilization by dairy cows and beef cattle (Grainger et al., 2009; Williams et al., 2011; Ghelichkhan et al., 2018). Depending upon the source and concentration of CT and HT in the diet, enteric CH_4 emissions can be decreased by up to 50% (Bodas et al., 2012; Naumann et al., 2017). There is no consensus on the mechanism for CT inhibition of CH_4; however, CT are assumed to directly inhibit the CH_4-producing archaea in the rumen and bind with compounds in the rumen to reduce fermentation, thereby lessening the availability of substrates for use by the methanogens (Aboagye and Beauchemin, 2019).

In our study, the cows grazing Forb pastures tended to produce 15% less CH_4 daily compared with cows grazed on Grass, likely due to the content of HT. A similar CH_4 reduction was observed in beef cattle fed small burnet hay containing 4.5% HT (Stewart et al., 2019). A 9% reduction in CH_4 yield (g/kg DMI) in beef cattle was detected by Aboagye and Beauchemin (2019) when using levels of dietary HT (15 mg/g DM) similar to that of the Forb pastures in the current study (15.9 to 18.4 mg/g DM).

The lack of a stronger effect of Forb pastures when CH_4 emissions were expressed relative to DMI or milk is somewhat in line with the divergent results observed in prior work. Reported effects of legume or forb on CH_4 emissions of animals resulted in decreased emissions in sheep fed chicory and bigleaf trefoil (*Lotus pedunculatus*) compared with sheep fed ryegrass (Waghorn et al., 2002), no differences in sheep grazed on ryegrass compared with chicory pastures (Sun et al., 2011), or even greater CH_4 emissions in dairy cows fed a chicory-rich diet (26.1 g CH_4/kg DMI) compared with cows fed concentrate (21.0 g CH_4/kg DMI) or a forage *Brassica*-based diet (20.5 g CH_4/kg DMI; Williams et al., 2016). It is noteworthy that the chicory in Williams et al. (2016) study was reported to be at the reproductive stage, indicating that the overall feeding value of forages is the primary factor affecting the enteric CH_4 emissions. Similarly, in the current study, chicory plants had developed visible reproductive stems in period 1. It is probable that the CH_4 emissions from more vegetative Forb pastures could have been lower. However, this is also true for other pastures in particular for the Grass that had also reproductive stems.

Conclusions

Our study indicated that incorporating legume- and forb-based pastures offers a viable option to manage pastures as evidenced by greater milk yield, more efficient rumen fermentation, and, for forb-based pasture, less environmental pollution potential compared with the classical ryegrass–white clover pasture. The results indicated that forb and legume pastures have the potential for maintaining high milk yields, especially when the nutritive quality of grasses is poor due to the accumulation of low-quality forage. Overall, the tendency for less CH_4 emissions together with improved N use efficiency for forb-based pastures indicates improved environmental efficiency over legume-based pastures. Thus, including forb-based pastures that contain chicory and plantain in the feedbase of dairy cows may be more effective for reducing the environmental impact of pasture-based dairy farming than legume pastures. Our data clearly support the hypothesis that alternative pastures, containing both chicory–plantain and legume, outperform traditional grass-clover pasture.

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Conflict of interest statement

None declared.

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