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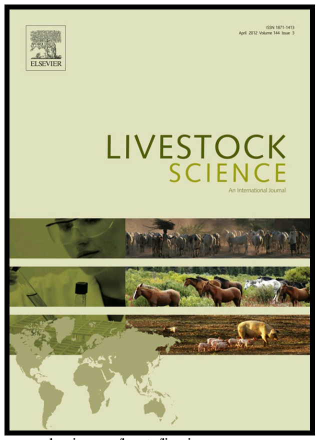

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R.H. Bryant, B. Welten, D. Costall, P.R. Shorten, G.R. Edwards

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Milk yield and urinary-nitrogen excretion of dairy cows grazing forb pasture mixtures designed to reduce nitrogen leaching

Bryant RH[1], Welten B[2], Costall D[3], Shorten PR[2] and Edwards GR[1]

[1]Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 7647, Canterbury New Zealand

[2]Farm Systems and Environment Group, AgResearch, Ruakura Research Centre, Private Bag 3115, Hamilton 3240, New Zealand

[3]AgResearch Grasslands, Private Bag11008, Palmerston North 4442, New Zealand

Corresponding Author: Racheal Bryant, racheal.bryant@lincoln.ac.nz

Abstract

The effect of including a mixture of forbs in a standard perennial ryegrass-white clover pasture, with or without Italian ryegrass was investigated in a two-period grazing study comparing urinary nitrogen (N) excretion and milk yield of dairy cows in late lactation. Forty-eight mixed age, Holstein Friesian x Jersey cows were assigned to replicated, balanced, groups of six and offered one of four pasture treatments. Pasture types were either a perennial ryegrass-based pasture (PRG) with white clover or a forb-containing mixed pasture (MIX) including perennial ryegrass, white clover, chicory, plantain and lucerne. A second factor was the inclusion of Italian ryegrass (PRG+I and MIX+I). During Period I, ten cows in each of the MIX and PRG treatments were fitted with urine sensors to measure urine N parameters (urination frequency, volume and N concentration). During Period II, milk yield was measured from cows on all four pasture types. In both the ryegrass and forb pasture types, legume content exceeded 30% of the DM and in forb pastures chicory and plantain accounted for over 30% of the DM. Italian ryegrass in the MIX+I and PRG+I was respectively 5 and 15

% of the DM. Digestibility and crude protein was similar for all pasture types, but fibre content was lower for forb pastures. During Period I, there was no effect of pasture type on the amount of N per urination event (averaged 13 g N/event) and daily urine-N excreted (190 g N/cow/day). However, pasture type affected the diurnal pattern in urine-N excretion with MIX pastures having a lower urine N loading per event during the day and higher urine N loading at night compared with PRG pastures. During Period II, milk yield was greater for forb compared with ryegrass pasture types (1.66 vs 1.50 kg MS/cow/d). There was no effect of Italian ryegrass on milk yield due to low botanical content. This study demonstrated that forb containing pastures altered the diurnal pattern in urine-N excretion and offers opportunities to mitigate N leaching from grazed pastures whilst supporting high milk production in late lactation.

Keywords: *Cichorium intybus, Plantago lanceolata, Medicago sativa, Lolium multiflorum,* diverse pasture, multi-species

Introduction

Agricultural food production is under increasing pressure to meet the demands of a growing population. Compounding this issue is the reduction in land available for farming as urban areas expand further into agricultural areas. For temperate farming systems, which graze livestock year round, there has been an increased focus on intensification of existing pastoral farm systems to increase food production. However, concerns exists on the adverse environmental effects related to intensification of pastoral grazing systems, most notably is nitrogen (N) leaching loss to local water bodies (surface and groundwater systems. Erisman et al., 2015). Assessment of the contribution of individual farms to N pollution is likely to be carried out through modelling of nitrate leaching to help identify farm activities which pose a threat to water quality. Therefore, not only is it desirable to control aquatic pollution from N leached from grazed pastoral systems, but there is also a strong need to ensure confidence in modelling tools which are used to predict N leaching.

While N from grazing ruminants is excreted in both dung and urine, the primary source of N leached to nearby agricultural waterways is attributed to urine, which is greater than ten-fold that leached from dung (Wachendorf et al., 2005), and is deposited to soil at high rates of up to 1000 kg N/ha (Cameron et al., 2013; Whitehead, 1970). The majority of this urine-N is comprised of urea and undergoes rapid transformation in soil to nitrate, which is highly mobile and readily leached during the autumn-winter drainage period (Scholefield et al., 1993). Consequently, N mitigation solutions have been sought that include the use of alternative forages and grazing practises to mitigate leaching loss of urinary-N. For example, winter active plants such as Italian ryegrass have attributes which reduce N leaching by capturing more of the soil derived urinary-N, through rapid establishment (Bartholomew et al., 1981), high winter growth activity and high annual yields (Malcolm et al., 2014, 2015; Woods et al., 2016). In addition to these attributes, Italian ryegrass possesses high feed quality characteristics which support animal production relative to traditional perennial ryegrass-white clover pastures (Cosgrove et al., 2007).

Alternative forages also offer an opportunity to reduce N losses by improving the efficiency of dietary N utilisation and thereby reducing loading of urine N onto soil (Beukes et al., 2014; Romera et al., 2017). Early animal studies examined the use of condensed tannins (CT) and included CT-containing plants, such as lotus, in grazing studies which reduced protein degradability in the rumen of sheep (Barry and Manley, 1984). More recently, grazing studies were undertaken to assess the effects of CT in dairy cows with lotus sown in a mixed pasture with ryegrass and forbs. However, the lotus failed to establish after being outcompeted by other species (Totty et al., 2013). While the attributes of lotus were not realised in that study, the forbs in the same mix appeared to reduce urinary-N loss evidenced through reduced N concentration in urine compared to the control ryegrass-clover pasture. The incorporation of forbs in grass clover mixtures were investigated in subsequent studies which, when fed to grazing dairy cows, again showed lower urinary N concentration (Bryant et al., 2017). However, those studies measured urine N concentration from mid-stream spot samples collected after milking in the morning and afternoon and therefore may not accurately reflect differences in total N loading from those pasture types. In order to conclude whether or not mixed pastures reduced total urine N load from animal to soil, or alter urination patterns in such a way as to reduce soil N loading, requires more complete measurement of the urination behaviour of dairy cows.

The need to determine voluntary urinary N excretion from outdoor grazing cows is well recognised, but so has the difficulty in achieving these measurements (Betteridge et al. 2010). The AgResearch urine sensor has been developed to measure individual urination events (frequency, volume, N concentration) from grazing cattle (Betteridge et al., 2013, Shepherd et al., 2017). Recent studies have demonstrated large variation in urine N excretion throughout the day, which was, in part, affected by feeding regime (Misselbrook et al., 2016; Shepherd et al., 2017). The purpose of this study was to use this urine sensor to compare urination behaviour of dairy cows grazing pastures with or without forbs. Additionally, the question was asked whether inclusion of plant species which improve soil N uptake, such as Italian ryegrass, could be integrated into an existing grazing system to support late lactation milk production.

Materials and methods

The experiment was carried out at the Lincoln University Research Dairy Farm (43°38′S, 172°28′E; 17 m above sea level) on a free-draining Templeton fine sandy loam soil (Hewitt 2010). The experimental design consisted of a 2 x 2 factorial with two replicates. The first factor was pasture type which consisted of a control ryegrass-based pasture (PRG) and mixed pasture with forbs (MIX). The second factor was Italian ryegrass where half of the area of PRG and MIX was sown with Italian ryegrass (PRG+I, MIX+I).

The experimental area (12 ha total) was cultivated and divided into 4 blocks of 3 ha which were separated into paddocks in October 2013. The ryegrass pasture types (6 ha) were sown with perennial ryegrass, (*Lolium perenne* cv. Arrow AR1, 20 kg/ha) and white clover, (*Trifolium repens* cv. Weka, 3 kg/ha). Forb pasture types (6 ha) were also sown with perennial ryegrass (12 kg/ha) and white clover (3 kg/ha), but the mix also included: chicory, (Cichorium intybus cv. Choice, 1.5 kg/ha), plantain, (*Plantago lanceolata* cv. Tonic, 1.5 kg/ha), and lucerne, (*Medicago sativa* cv. Torlesse, 8 kg/ha). In February 2015 half of each paddock was mown to a standing height of 3.5 cm, grazed by cows, and direct drilled with 20 kg/ha of Italian ryegrass seed (*Lolium multiflorum* cv Asset). An additional 2 kg/ha of chicory and plantain was direct drilled into the mixed pasture to ensure sufficient forb population the following year.

The experiment took place over 30 days between February and March 2016 which included a covariate period (15 days), an adaptation period (5 days), urine measurement period (5 days, Period I) and a milk yield measurement period (5 days, Period II). The urine measurement period compared two of the four pasture treatments (PRG and MIX) which represented pasture mixtures similar to those in previous studies. The milk yield measurement period included the Italian ryegrass treatment comparing the four pasture types (PRG, MIX, PRG+I and MIX+I).

Animals and management

All animal measurements were carried out with the approval of the Lincoln University Animal Ethics Committee (#2016-06). Forty-eight mixed-age, lactating, pregnant, Friesian x Jersey cows were blocked according to milk yield (1.43 ± 0.01 kg MS), live weight (493 ± 6.1 kg LW), age (5.4 ± 0.3 years), and days in milk (193 ± 2.2) following a 15-day covariate period where all experimental cows grazed together on the same pasture diets. On 29 February 2016, cows were allocated to 8 groups of 6 cows and adapted to a 50% change in diet over a 5 day period. Botanical measurements prior to the experiment indicated that the plant species in the covariate period accounted for half of the botanical composition in the treatment diets. Each day cows were offered a new allocation of pasture of 35 kg DM/cow/day above ground level. For allocation of pasture, herbage mass was estimated from compressed height of a calibrated rising plate meter (Jenquip F150 Electronic Pasture Meter, Fielding, New Zealand) using the manufacturers equation of mass (kg DM/ha) = 140 x height reading +500. Additionally, daily observations of post grazing residuals and utilisation of pasture were used to assess suitability of allocation for each treatment. If visual estimates of post grazing residuals fell outside the range of 1480-1900 kg DM/ha, equivalent to a compressed height between 3.5 and 5.0 compressed cm, the allocation for that treatment was reviewed.

Cows were milked twice daily, in the morning (0700h) and afternoon (1500h) where liveweight was also recorded on walk over scales after milking. Milk yield was measured at each milking (Delaval Alpro Herd Management system) and sub-samples were collected on days 11, 13, 14, and 15 at pm and am milkings and determined for fat, protein and lactose concentration by near-infrared (Milkoscan^{TM}, Foss Electric, Denmark). Milk urea N was determined on skimmed milk after cooling and centrifuging to remove fat and analysed by an automated Modular P analyser (Roche/Hitachi) (Talke and Schubert, 1965).

Urine and feces were sub-sampled following consecutive afternoon and morning milkings on days 11 and 15 of the experiment (0730-0800h and 1530-1600h). Urine sampling was carried out by manual stimulation under the vulva, whilst fecal collection occurred by rectal stimulation. Urine was acidified with sulphuric acid to reduce pH and prevent volatilisation of N. Faeces were freeze dried and the N concentration of acidified urine and ground faecal samples was determined by combustion (Vario MAX CN, Analysensysteme GmbH. Hanau, Germany) and creatinine concentration in urine by Jaffe method (Daytona RX Clinical Analyser, Randox, Nishinomiya, Japan).

Urine N excretion

To measure urine-N excretion from cows grazing PRG and MIX pastures (excluding Italian ryegrass treatments), urine sensors were attached to five cows in each treatment replicate (10 cows per treatment; 20 cows total). The urine sensors were attached by using a custom-made collection device glued around the vulva and retained on the animal using harnesses attached to a ventilated cow cover. The urine sensor automatically records the frequency, volume, nitrogen concentration and time of each urination event (refer to Betteridge et al. 2013, Shepherd et al. 2017a, b, for further details). Cows selected to wear the sensor were balanced across the treatments by age and LW. The sensors were attached to the cows from 7 to 11 March 2016, with a total of 4 days data collection.

Herbage measurements

Herbage was sampled for botanical and nutrient composition by cutting to ground level at multiple (n=10-12) random locations, within each paddock treatment, before and after each grazing event. Herbage samples were collected in the morning immediately prior to cows receiving a new paddock. After collection, each sample was thoroughly mixed and sub-sampled for botanical composition (50 g FW) and nutrient composition (30 g FW). Botanical composition was determined by manually sorting into sown species and dead material and oven drying for 24 hours before weighing. The fresh weight of the combined sample was recorded prior to sorting and drying to determine DM%. Samples for nutritive analysis were placed into the freezer (-18 C) followed by freeze drying. Post-graze herbage samples were washed prior to freezing to remove any urine or fecal debris. The freeze dried herbage was ground to pass through a 1mm sieve and analysed for organic matter (OM), soluble sugars and starch (SSS), neutral and acid detergent fibre (NDF, ADF), crude protein and digestible organic matter in the dry matter (DOMD) by near infrared spectrophotometry (NIRS, Model: FOSS NIRSystems 5000, Maryland, USA).

Average apparent DM intake for cows in each group was determined as the difference in herbage mass before and after each grazing event ie apparent intake (kg DM/cow/day) = (((pre kg DM ha^{-1} – post kg DM ha^{-1}) ÷ No. cows) x area). Apparent N intake was estimated using the same equation but multiplying herbage mass by the herbage N concentration in the mass before and after grazing. Herbage mass was measured using quadrat cuts before and after grazing on days 8, 11 and 15 for each group. All herbage within 2 x 0.2m^2 quadrat per paddock was harvested to soil level using an electric hand-piece. Each sample was washed prior to oven drying at 60°C to a constant weight. Additionally, four multiparous cows in each mob were randomly selected and fitted with a grazing activity recorder (SensOor eartag, Agis Ltd, Netherlands). The activity recorder monitored grazing time and ruminating behaviour. Technical details and validation are described by Bikker et al. (2014).

Statistical analysis

Herbage and milk variables, and urine and dung variables from spot samples were analysed using linear models in Genstat where pasture type and Italian ryegrass and their interaction were included as fixed terms in the model and sampling date and replicate were random terms. Means were compared using Fishers protected LSD and reported at the 5% significance.

The urine sensor data was processed and analysed using the method described by Shepherd et al. (2017a). The effects of cow, treatment (PRG, MIX) and live weight on the urine variables were analysed using the mixed effects statistical model

$$y_{ijk} = trt_i + cow_j + weight_k ,$$

where cow was treated as a random effect. The diurnal variation in model parameters and measured variables were examined using a 3 hour smoothing average. Time series in the measured variables were also assessed for autocorrelation. Calculations were performed in R and Matlab (The Mathworks).

Results

Climate

Between 1 and 15 March 2016 the average minimum temperature was 10.5 °C (range 1 – 16 °C) and the average maximum temperature was 21.3°C (range 14 - 31°C). During the experiment, conditions were dry but on the final day of the study (15/16 March) there was 25 mm of rain. During the pasture growth period prior to the study accumulated thermal time was 450 - 500 °C days (assuming a base temperature of zero). During the 15 day study a further 230 °C days accumulated. Average day length was approximately 12 hrs 50 minutes with sunrise occurring between 0607 - 0625 h and sunset occurring between 1916 - 1852 hr.

Diet

Herbage mass was similar across treatments, with ryegrass pasture types consisting of greater ryegrass, dead material and white clover compared with forb pasture types (Table 1). Undersowing Italian ryegrass resulted in disappearance of lucerne in forb pastures compared with treatments without Italian ryegrass. Dry matter content, fibre and soluble sugars and starch were lower in forb compared with ryegrass pastures. Protein content was similar across all treatments and there was no effect of Italian ryegrass on nutrient composition. There was no difference in herbage mass after grazing (Table 2). Selection for legumes, plantain and chicory across treatments was evident as composition before grazing was respectively 21%, 18%, and 15% compared with 12%, 9%, and 2% after grazing. A high proportion of dead material in post grazing residual coincided with elevated fibre content and low DOMD. There was no effect of Italian ryegrass on trough water intake but cows on PRG consumed 8.5 litres more water from the trough than cows on MIX pastures (37.6±4.5 versus 29.5±2.6 litres/cow/day, $P<0.05$).

Urine N excretion

There was no effect of pasture type on total daily urine volume and N excreted (Table 3). Urine volume per event fluctuated from low volume events during the day to high volume events during the evening and early morning (Figure 1). Although the pattern was similar for PRG and MIX, urination volumes were higher for MIX during the night and early morning, and higher for PRG in the afternoon. Average urine N concentration was similar for both treatments but, as with volume, there were diurnal effects of diet on urine N concentration (Figure 2). During the day, between 7am and 6pm, cows grazing MIX had lower urine N concentration compared with cows grazing PRG pastures ($P<0.05$). Cows grazing PRG pastures had a lower urine N concentration than MIX between 3pm and 10pm ($P<0.05$). Diurnal differences between the two pasture types for urine N load are depicted in Figure 3 and reflect trends in urine N concentration. The effect of pasture type on the diurnal variation in the time between urination events are depicted in Figure 4 and exhibit a similar pattern between treatments.

The apparent N intake was similar for both PRG and MIX pasture types (Table 5). Similarly, total daily urine N excretion was unaffected by pasture type and averaged 191± 12 g N/cow/day. There was no difference between pasture type in the amount of N voided in milk during the urine measurement period (101 ± 2.8 g/cow/d). There was no significant effect of animal live-weight on all urination parameters.

Urine N concentration from spot samples collected during Period II showed no time of day effect on urine N concentration, (0.42 ± 0.016 and 0.48 ± 0.034 % N for morning and afternoon respectively). Urine N concentration was greater for PRG than MIX, though an interaction between pasture type and Italian ryegrass showed inclusion of Italian ryegrass in a ryegrass mix (PRG+I) resulted in lower urine N concentration but did not lower N concentrations when included in a forb mix (Table 4). There was no effect of treatment on urine ammonia or urine creatinine concentration, though cows fed forb pastures had higher fecal N% compared with those fed ryegrass pastures.

Milk yield

Greater herbage mass estimated in the forb pasture types resulted in a smaller grazing area compared with ryegrass pasture types (111 and 130 ± 2.7 m^2/cow/day $P<0.05$). On the first day of the milk yield period (experimental day 11) animals on ryegrass pasture types (PRG and PRG+I) received only half of their required area (74 m^2/cow) due to a calculation error. Visual observation of the post grazing residual identified the problem and the allocation was corrected the following day, but the negative effect of the restriction is evident on day 11 and 12 (Figure 5).

Milk yield throughout the study is presented in Figure 5. During adaptation, cows grazing forb pastures had increased milk production, but differences in production disappeared during urine measurements in Period I (days 6 to 10) and by the end of Period II (days 11-15) milk yield was again greater for forb pastures. The results for milk production and composition during Period II are presented in Table 5. Milk yield and milksolid production were greater from cows grazing forb compared with ryegrass pastures (Table 5). There was no effect of pasture type or Italian ryegrass treatment on milk composition. Variation in milk solids yield was due to both increased milk fat ($P<0.05$) and protein ($P<0.10$) content.

Cow live-weight and grazing behaviour

There was no effect of treatment on cow live-weight at the start and end of the study (490 ± 4.9 and 502 ± 6.1 kg LW respectively) though cows fed ryegrass pastures gained 16.7 compared with 8.9 kg LW gained by cows fed forb pastures ($P<0.05$). There was no effect of treatment on body condition score on day 0 (4.06 ± 0.04 BCS) and day 15 (4.11 ± 0.05 BCS).

Apparent cow feed intake ranged between 14 and 18 kg DM/cow/day between treatments which did not differ statistically (Table 4). However, time spent grazing was affected by treatment (Figure 6). During urine measurements in Period I, all cows were held in the yards to check urine sensors before returning to their paddocks. Consequently cows spent less time grazing during Period I compared with Period II of the study (526 vs 593 ± 12 minutes/ day, $P<0.05$). However, the diurnal pattern of grazing was similar for both periods whereby cows had three grazing bouts, two major bouts after each milking and a smaller bout around midnight. There was no treatment interaction between Period and grazing or ruminating time. Across both periods, cows offered forb pasture treatments spent an extra 52 minutes/day grazing than those on ryegrass pasture treatments (585 vs 533 ± 11.8 minutes/cow/day, respectively. $P = 0.01$), whereas cows on ryegrass pastures tended to spend more time ruminating than cows offered forb pastures (436 vs 411±8.2 minutes/cow/day, $P = 0.06$). There was no effect of Italian ryegrass treatment on grazing time, but cows offered pastures with Italian ryegrass spent an extra 40 minutes per day ruminating compared with cows on treatments without Italian ryegrass (443 vs 404±8.2 minutes/cow/day P = 0.01).

Discussion

Effect of pasture type on urinary-N excretion

Under the conditions of this study, the results of this experiment did not support the hypothesis that forb-containing pastures, including chicory and plantain, reduce urinary N excretion. However, the results did identify a distinction between diurnal patterns in urinary N excretion caused by the different pasture types which may provide opportunities to manage 'high risk' large N loading periods.

The first aim of the current study was to ascertain whether the previously reported low urine N concentration, from cows fed forb-containing pasture, reflected a lower total urine N excretion (Totty et al., 2013; Bryant et al., 2017). We hypothesized this to be the case based on previous indoor feeding studies which showed reduced total urine N excretion from cows fed forb pastures compared with ryegrass pastures (Woodward et al., 2013). However, those authors also reported lower herbage crude protein content and lower N intake on forb pastures and attributed differences in urine N excretion to differences in total N intake. As N intake is the major driver for urine N loss (Kebreab et al., 2001) and apparent N intake in the current study was similar for the two treatments, a lack of treatment effect on daily urinary N excretion is expected.

Prior to this study, we speculated that the inclusion of forbs in a pasture mix may alter the quantity or pattern of urinary N excretion of dairy cows as a result of either morphological or chemical characteristics of the different plant species. For example, changes in urine N concentration from spot samples could be due to differences in urine volume. Evidence of a diuresis effect of plantain had previously been demonstrated in sheep (O'Connell et al., 2016), dairy heifers (Cheng et al., 2017) and dairy cows (Box et al., 2016, 2017). Although no specific plant mechanism has been attributed to the observed increases in urine volume from ruminants fed plantain (O'Connell et al., 2016; Box et al., 2017), it is accepted that, generally, urine volume is influenced by water and/or mineral intake such as sodium (Spek et al., 2012). In spite of differences in daily drinking water intake measured in the present study, we estimated that cows had similar total daily water intake for both ryegrass (115 litres/cow/day) and forb (110 litres/cow/day) pasture due to the lower DM content of the forb pastures. This was validated from both urine sensor data and creatinine concentration of the urine indicating no diuresis effect of forb pastures. Thus, if plantain does increase urine volume, then our findings suggest that the proportion in the diet required to elicit a response should exceed 20% of the offered herbage. This conclusion is in line with previous studies which observed no difference in urine volume from cows fed a forb pasture with less than 20% plantain (Woodward et al., 2012; Edwards et al., 2015).

While it is desirable to reduce total N losses in pasture based systems, a key outcome for farm management is to reduce the risk of N leaching. This includes solutions which reduce N loads from urine patches (Cameron et al., 2013). Even at similar urinary N excretion, it is feasible that different forages can alter N leaching risk through changes in the type or availability of protein in the forage (Beukes et al., 2014). In the current study, the results of the urine excretion investigation demonstrate this possibility. Although typical circadian urine behavior was evident, whereby cows fed either pasture type had greater amounts of N per urination event at night than during the day, the results depicted in Figures 3 and 6 indicated that the peaks in urine N excretion corresponded with the onset of a grazing bout. These results support previous research which demonstrate close links between timing of feeding, and rumen ammonia and plasma urea concentrations (Rodriquez et al., 1997; Piccione et al., 2007).

There were also significant temporal effects between pasture types. For instance, for a large proportion of the day (between 7am and 5pm), the amount of N per urination event was lower for forb compared with ryegrass pastures. The contrast is likely to arise from the quantity and location of legumes in the different pasture types, ie vertical distribution of herbage N in the sward profile. It was evident that white clover was abundant in the ryegrass pasture treatments and that selection for clover occurred in these treatments. Thus, it is anticipated that a large proportion of the total daily N intake on ryegrass pastures would have occurred in the morning driving the elevated urinary N excretion during the day. Interestingly, cows were observed to avoid plantain until after the afternoon milking and we would speculate that any nutritional influence of consumption of plantain was likely to occur overnight. Consequently we suggest that the combination of spatial and temporal diet selection for plant parts – which vary in N concentration – could be important drivers in diurnal urine N loading.

Our findings showed that urinary N excretion in late lactation accounted for roughly 32% of the N apparently consumed, while milk N excretion accounted for 17% of N eaten. The proportion of dietary N in milk and urine are in line with the range of 15-23% N in milk and 29-43% N in urine for housed cows in late lactation (Woodward et al. 2013). We were unable to determine N loss in dung during our grazing study but we estimated 23% of consumed N would be excreted in dung based on our values for apparent DM intake, digestibility and fecal N concentration. Although there was weight gain in the present study it's expected that only a small amount of N is retained for liveweight change and fetal growth (<15 g/day) which would leave roughly 25% of N unaccounted for. Balancing N in feeding studies is notoriously difficult even during total collection studies (Spek et al., 2013; Woodward et al., 2012). Spek et al. (2013) noted that at increasing N intake the proportion of unaccounted N increased. At N intakes of 480 g/day, 14% could not be accounted for in milk, tissue, dung or urine. Over- and under- estimates of N can occur with each variable and balancing for N in grazing studies is an area which requires further investigation.

Finally, the question remains as to what effect the inclusion of Italian ryegrass is expected to have on urinary N excretion? An interaction for urine N concentration between pasture type and Italian ryegrass, showed inclusion of <15% DM as Italian ryegrass to pastures reduced urine N concentration in PRG but not MIX pastures. The reason for a reduction in urine N concentration from PRG diets we believe to be the result of dilution of clover from more Italian ryegrass being ingested in PRG pastures. We also expect that most of the ingestion took place during the day due to taller tillers presenting more leaf bulk at the top of the sward which would be grazed earlier in the grazing bout. The establishment of Italian ryegrass did not seem to affect the proportion of white clover in either MIX or PRG pastures, but it did reduce (remove) lucerne from the MIX+I pasture resulting in a greater legume content in the MIX versus the MIX+I treatments. However, due to the low Italian ryegrass content in the MIX+I and the more uniform vertical distribution of leaf on lucerne compared with clover in the MIX, we suggest this avoided any potential diurnal variation in N intake between the two treatments. Thus, if the change in species composition of a pasture mixture alters the rate of ingestion of dietary protein (as likely occurred for PRG and PRG+I), then it is likely to achieve diurnal variation in urinary N excretion. Further, if the addition of another plant species doesn't alter crude protein content of the herbage or apparent N intake (which, in this case, it did not) then it is unlikely to alter total urinary N excretion. The advantage of using pasture mixtures to shift diurnal urination patterns presents an opportunity for farmers, which have stand-off facilities, to remove cows from pasture during periods of high risk urine N loading. The N in effluent can then be captured from those stand-off areas and applied to pastures at a rate which plants can utilize for growth (ie <100 kg N/ha), reducing the proportion of N leached compared with that of N deposited as urine on pasture (>600 kg N/ha).

Milk yield

Offering forb-containing pastures to dairy cows in late lactation resulted in greater milk yield than offering a ryegrass and clover mix. Previous studies have reported similar or greater milk yield from cows offered pastures containing one or both forbs (Minnee et al., 2017; Bryant et al., 2017; Pembleton et al., 2016; Muir et al., 2013). In those studies differences in milk yield were observed in early or mid lactation and were attributed to improved digestibility in warm environments (Minnee et al., 2017, Pembleton et al. 2016; Muir et al., 2013) or to the legume content in a protein-limiting diet (Bryant et al., 2017). In the current study digestibility was similar across pasture types and dietary protein was not limiting, indeed legumes were more abundant in the grass clover mix which had the lower milk yield. In pastoral systems, allocation of herbage is a major influence on herbage intake. With the exception of experimental day 11, the observed herbage residuals did not indicate that allocation was constraining intake. Moreover, the positive effect of the forb pasture treatment on milk yield had started to become apparent during the adaption period (Figure 4).

There are two possible explanations for the milk yield differences, with the first being due to a difference in apparent intake. Under grazing conditions it is often very difficult to detect differences in intake due to large variation in prediction parameters such as herbage mass and between-animal variation on a day to day basis. Numerically, apparent intake of forb pastures in the current study was 1kg DM greater than ryegrass pastures. This might explain the lower milk yield on ryegrass treatments, as milk yield from cows fed ryegrass pasture did not alter appreciably from milk yield during the covariate period (1.49 versus 1.43 kg MS). On the other hand, milk yield increased from cows grazing forb pastures. When offered forb pastures, cows spent more time grazing which may reflect a stronger motivation to graze because choice is increased (Gregorini et al., 2017).

Our second explanation for the observed difference in milk yield may be due to the influence of herbage fibre content on the end products of fermentation and partitioning of assimilates to milk or body tissue. Cows on the ryegrass pasture treatments consumed more fibre than those on the forb pasture treatments. This is evident from the difference in pre and post grazing NDF and in the longer rumination times of cows on ryegrass pastures. The difference in weight gain during the 15 day study was greater for ryegrass compared with forb pastures and this may also indicate an increase partitioning of fermentation end products to body in favour of milk. High fibre diets result in increased assimilation of acetate which is used in fatty acid synthesis (McDonald et al., 1995). Conversely, a higher proportion of carbohydrates in the forb pasture would have been non-structural, and readily fermented which could increase propionate production in the rumen. The increased lactose yield from cows on forb pastures suggests that these animals had either increased supply of the glucogenic proprionate through increased total intake or a lower acetate:propionate ratio, or both.

The addition of Italian ryegrass appears to have had little impact on milk yield under the conditions of this study. Italian ryegrass presents an opportunity to reduce nitrate leaching through it's cool-season activity and increased soil N uptake during winter (Malcolm et al., 2014, 2015; Woods et al., 2016). Animal production studies have previously also demonstrated milk yield and urine N benefits of Italian ryegrass (Cosgrove et al., 2007). The lack of milk response to Italian ryegrass is likely due to the low content of this species in the diet. Italian ryegrass is an annual plant and is expected to have poor persistence - particularly in mixed swards. Given that Italian ryegrass in the present study had been sown 13 months prior to this experiment, the decline in tiller populations after flowering in spring would have contributed to the low botanical content.

Conclusion

Given that farm nutrient management is being increasingly regulated and audited through modelling, assumptions used in those models should adequately reflect the impacts of the variables used to mitigate N loss. Forb pastures containing a combined chicory and plantain content of 30-35% of the dry matter was insufficient to reduce the total daily amount of N excreted in urine. However, the content of forbs was sufficient to alter the diurnal pattern of urinary N excretion, and explained the differences in urine N concentration observed in spot samples from earlier research. Alternative forage species offer opportunities to manage nutrient loss risk without compromise in milk production in late lactation.

Conflict of interest statement
We wish to confirm that there are no known conflicts of interest associated with this manuscript and there has been no significant financial support for this work that could have influenced its outcome.

Racheal Bryant

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Table 1. Pre grazing herbage mass, botanical composition and nutritive content of ryegrass (PRG) and forb (MIX) based pasture types when sown with (+I) or without Italian ryegrass

	Ryegrass		*Forb*			*P value*		
	PRG	PRG+ I	MIX	MIX+I	SEM	Type	Italian	T x I
Pre graze mass (kg DM/ha)	3096	2923	3221	3320	171	0.14	0.83	0.44
Pre height (cm)	8.3	7.6	9.8	10.0	0.49	<.001	0.57	0.43
Perennial ryegrass (% of DM)	55	41	27	28	4.0	<.001	0.11	0.08
White clover (% of DM)	33	33	19	23	3.3	0.01	0.57	0.45
Italian ryegrass (% of DM)	-	15	-	4	5.5	0.12	-	-
Plantain (% of DM)	-	-	16	21	3.1	-	0.22	-
Chicory (% of DM)	-	-	14	15	1.6	-	0.62	-
Lucerne (% of DM)	-	-	17	0	5.5	-	0.05	-
Dead and stalk (% of DM)	11	11	6	7	1.7	0.02	0.81	0.70
Dry matter (% of DM)	17.7^{ab}	18.4^{a}	16.3^{b}	14.5^{c}	0.6	<.001	0.42	0.04
Organic matter (% of DM)	91.6	91.6	91.4	91.0	0.2	0.11	0.33	0.47
Soluble sugars	20.5	19.2	16.2	18.7	1.0	0.03	0.57	0.07

and starch (% of DM)								
Crude protein (% of DM)	18.5	18.3	19.7	18.6	0.7	0.28	0.36	0.52
NDF (% of DM)	41.9	42.5	35.7	35.4	1.0	<.001	0.85	0.68
ADF (% of DM)	24.8	24.7	24.2	23.9	0.3	0.05	0.66	0.77
DOMD (% of DM)	75.4	75.1	74.8	75.5	0.6	0.83	0.80	0.41

NDF is neutral detergent fibre; ADF is acid detergent fibre; DOMD is digestibility of the organic matter in the dry matter; SEM is standard error of the mean

Table 2. Post grazing herbage mass, botanical composition and nutritive content (% of DM) of ryegrass (PRG) and forb (MIX) based pasture types when sown with (+I) or without Italian ryegrass

	Ryegrass		*Forb*			*P value*		
	PRG	PRG+ I	MIX	MIX+I	SEM	Type	Italian	T x I
Post graze mass (kg DM/ha)	1489	1635	1783	1754	211	0.34	0.79	0.68
Post height (cm)	4.5	4.4	4.5	4.5	0.24	0.77	0.90	0.77
Perennial ryegrass (% of DM)	58	56	52	57	4.9	0.57	0.85	0.45
White clover (% of DM)	10	10	12	12	2.4	0.47	0.98	0.98
Italian ryegrass (% of DM)	-	7	-	5	2.5	0.47	-	-
Plantain (% of DM)	-	-	11	7	2.3	-	0.23	-
Chicory (% of DM)	-	-	3	2	1.0	-	0.32	-
Lucerne (% of DM)	-	-	3	0	1.8	-	0.23	-
Dead and stalk (% of DM)	30	26	18	18	4.8	0.05	0.68	0.75
Dry matter (% of DM)	17.7	18.8	16.7	17.6	1.3	0.42	0.48	0.94
Organic matter (% of DM)	92.4	92.4	91.2	90.8	0.05	0.01	0.71	0.62

Soluble sugars and starch (% of DM)	22.9	23.4	19.0	20.8	1.4	0.03	0.42	0.68
Crude protein (% of DM)	13.1	14.1	14.2	13.8	1.0	0.68	0.79	0.50
NDF (% of DM)	50.8	49.2	46.1	46.6	1.6	0.03	0.73	0.51
ADF (% of DM)	30.1	28.3	29.1	28.3	1.0	0.60	0.19	0.62
DOMD (% of DM)	69.4	72.2	70.3	71.4	1.6	0.98	0.25	0.61

NDF is neutral detergent fibre; ADF is acid detergent fibre; DOMD is digestibility of the organic matter in the dry matter; SEM is standard error of the mean

Table 3 Urine volume, frequency and N concentration of cows fed a ryegrass (PRG) or forb (MIX) pasture type

	PRG	*MIX*	*Treatment difference*	*P value*
Daily urine volume (L/cow/day)	29.0	28.0	1.0 ± 3.5	0.8
Mean event size (L)	2.39	2.30	0.09 ± 0.19	0.6
Mean Urine N %	0.63	0.60	0.03 ± 0.04	0.5
N loading per event (g)	15.3	13.9	1.5 ± 1.9	0.4
N load per day (g/day)	195	187	-8 ± 26	0.8
Time between urination events (day)	0.077	0.076	0.0008 ± 0.01	0.9

Table 4 Urine composition and fecal N concentration of spot samples collected from cows fed ryegrass (PRG) or forb (MIX) based pasture types when sown with (+I) or without Italian ryegrass

	Ryegrass		*Forb*			*P value*		
	PRG	PRG+ I	MIX	MIX+I	SEM	Type	Italian	T x I
Fecal N%	3.35	3.30	3.51	3.52	0.05	0.002	0.62	0.59
Urine N %	0.519[a]	0.425[b]	0.402[b]	0.457[ab]	0.022	0.10	0.50	0.009
Urea N (mmol/L)	381[a]	315[b]	299[b]	342[ab]	19.3	0.19	0.57	0.02
Ammonia (mmol/L)	1.66	2.25	1.54	2.76	0.74	0.80	0.24	0.68
Creatinine (mmol/L)	1.64	1.48	1.43	1.59	0.18	0.80	0.98	0.39

Table 5 Apparent intake (kg DM/cow/day), milk yield and milk composition from cows fed ryegrass (PRG) or forb (MIX) based pasture types when sown with (+I) or without Italian ryegrass

	Ryegrass		*Forb*			*P value*		
	PRG	PRG+I	MIX	MIX+I	SEM	Type	Italian	T x I
Apparent intake	16.5	14.3	15.2	17.5	2.6	0.72	0.99	0.39
Apparent N intake	604	542	637	661	89.8	0.45	0.84	0.66
Milk yield (kg/c/day)	16.6	15.9	17.5	18.5	0.85	0.05	0.88	0.35
Milk solids (kg MS/c/d)	1.54	1.45	1.64	1.68	0.072	0.03	0.72	0.38
Fat %	5.18	5.05	5.15	5.11	0.18	0.93	0.64	0.79
Protein %	4.18	4.12	4.30	4.10	0.07	0.51	0.06	0.32
Lactose %	4.89	4.79	4.89	4.87	0.08	0.59	0.49	0.61
Fat yield (g/cow/d)	845	795	891	925	38.5	0.03	0.84	0.29
Protein yield (g/cow/d)	691	651	750	753	37.9	0.04	0.63	0.56
Lactose yield (g/cow/d)	762	821	907	892	71.2	0.05	0.66	0.48
MUN (mmol/L)	6.55[a]	6.12[ab]	5.54[b]	6.83[a]	0.27	0.60	0.14	0.008

MUN is milk urea nitrogen

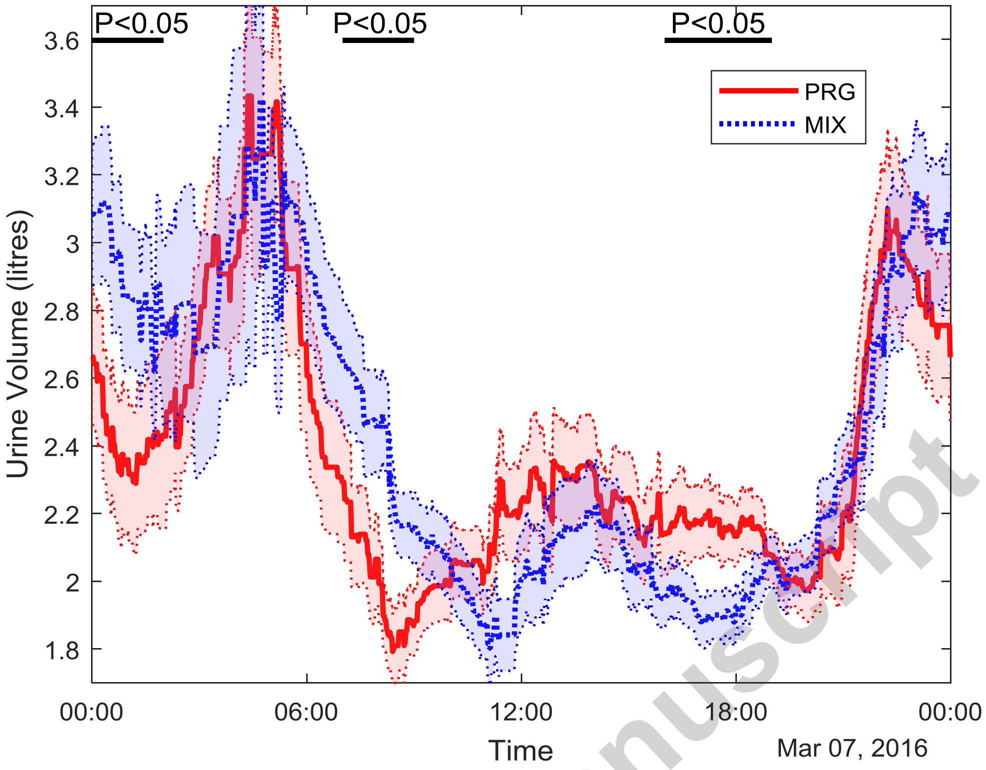

Figure 1: The dynamic temporal changes in urine volume over the trial. Thick lines denote a 3 hour smoothing average and thin lines denote SEM.

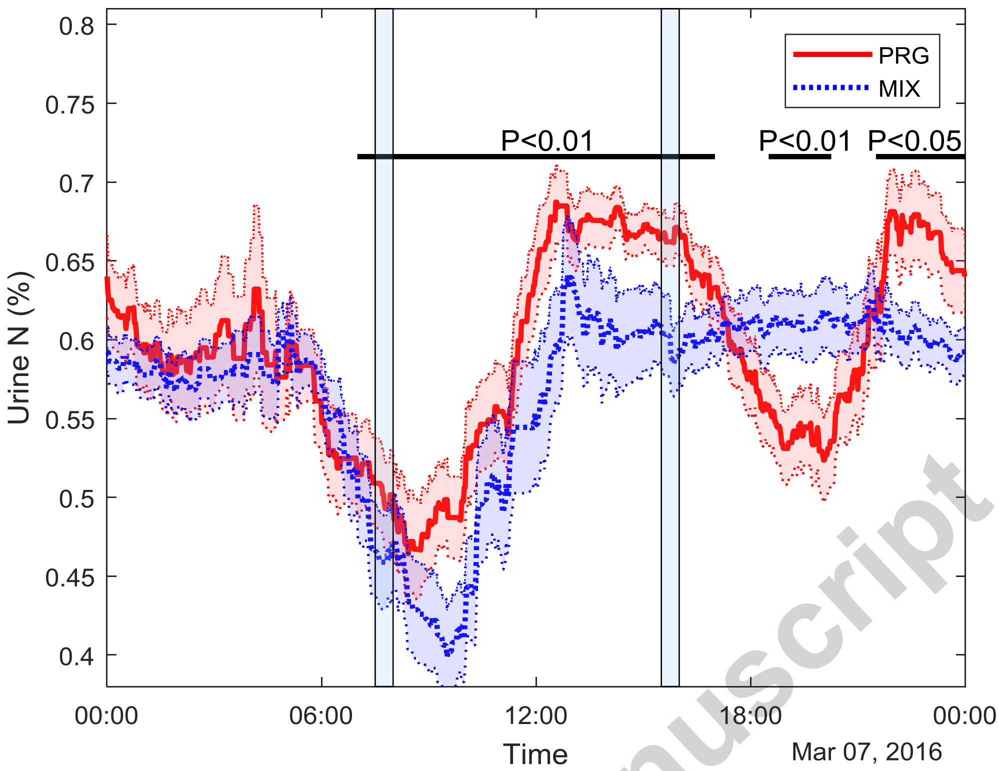

Figure 2. The dynamic temporal changes in urine N concentration per urination event over the trial. Thick lines denote a 3 hour smoothing average and thin lines denote SEM. Shaded bars indicate sampling time of spot urine samples after the removal of the urine sensors

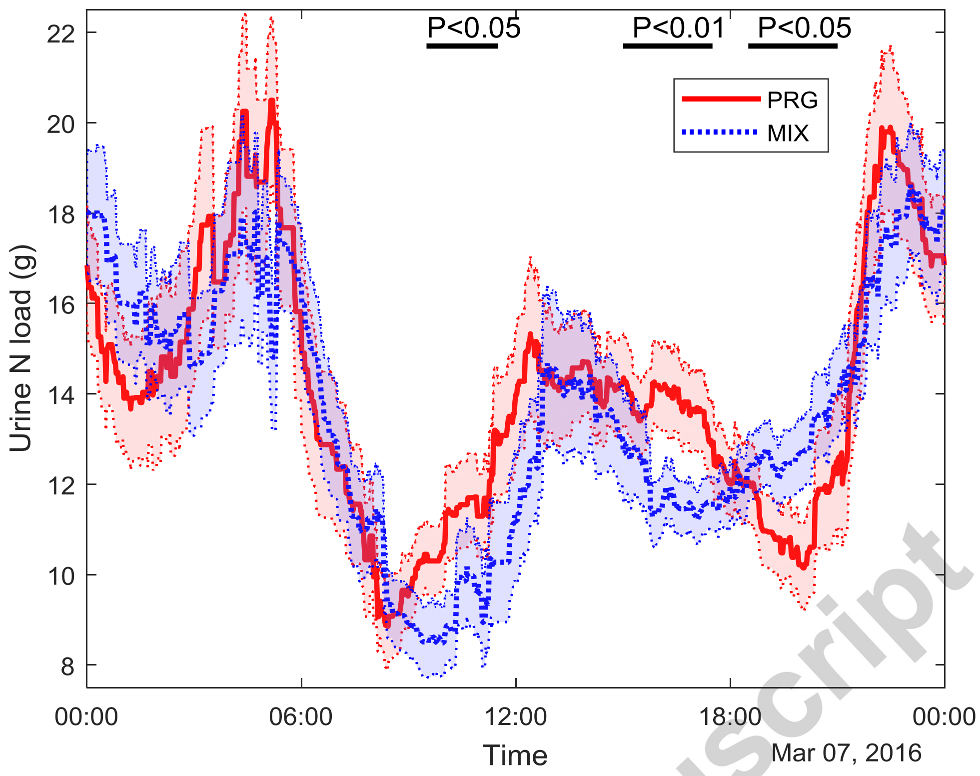

Figure 3: The dynamic temporal changes in urine N load per urination event over the trial. Thick lines denote a 3 hour smoothing average and thin lines denote SEM.

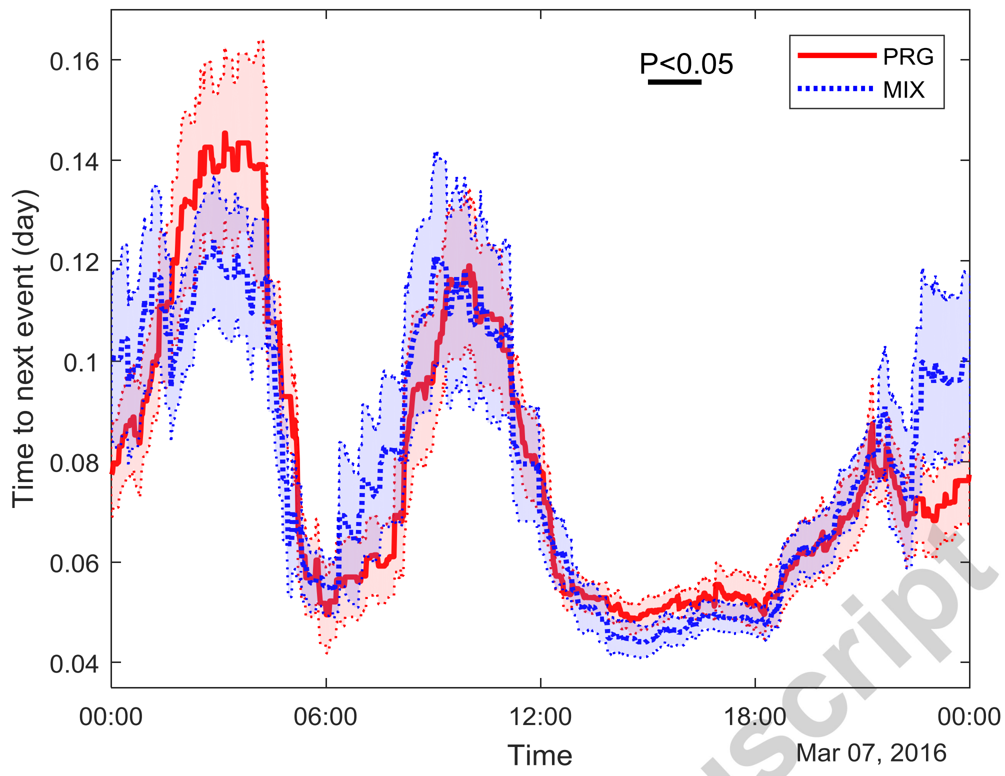

Figure 4: The dynamic temporal changes in the time to the next urine event over the trial. Thick lines denote a 3 hour smoothing average and thin lines denote SEM.

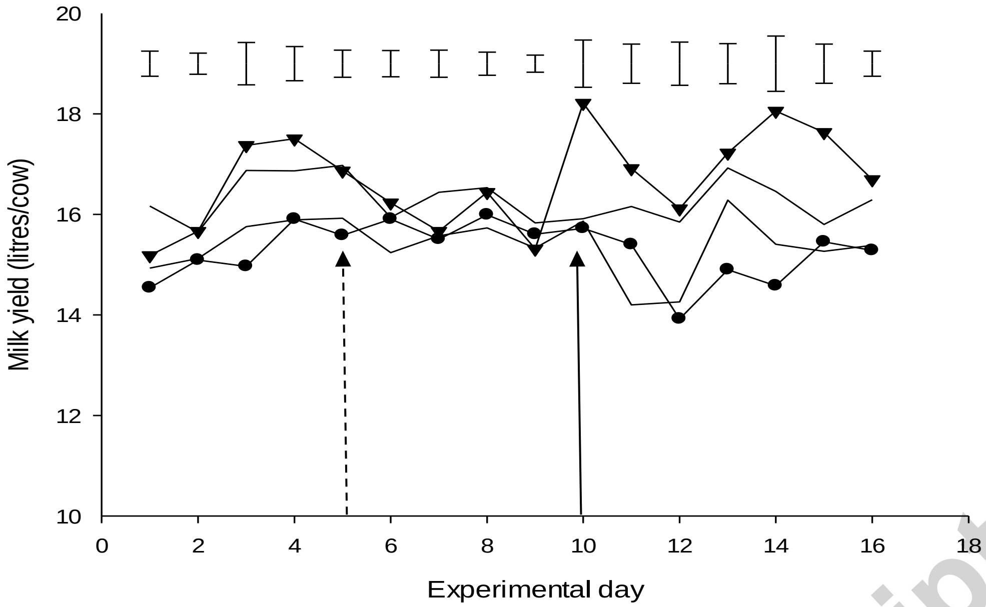

Figure 5. Daily milk yield (litres/cow/day) from cows grazing ryegrass and white clover pastures with (open circles) or without (closed circles) Italian ryegrass or forb-containing pastures with (open triangles) or without (closed triangles) Italian ryegrass. The arrows indicate the start of the urine (dashed line) and milk yield (solid line) measurement periods. Error bars represent the standard error of the mean.

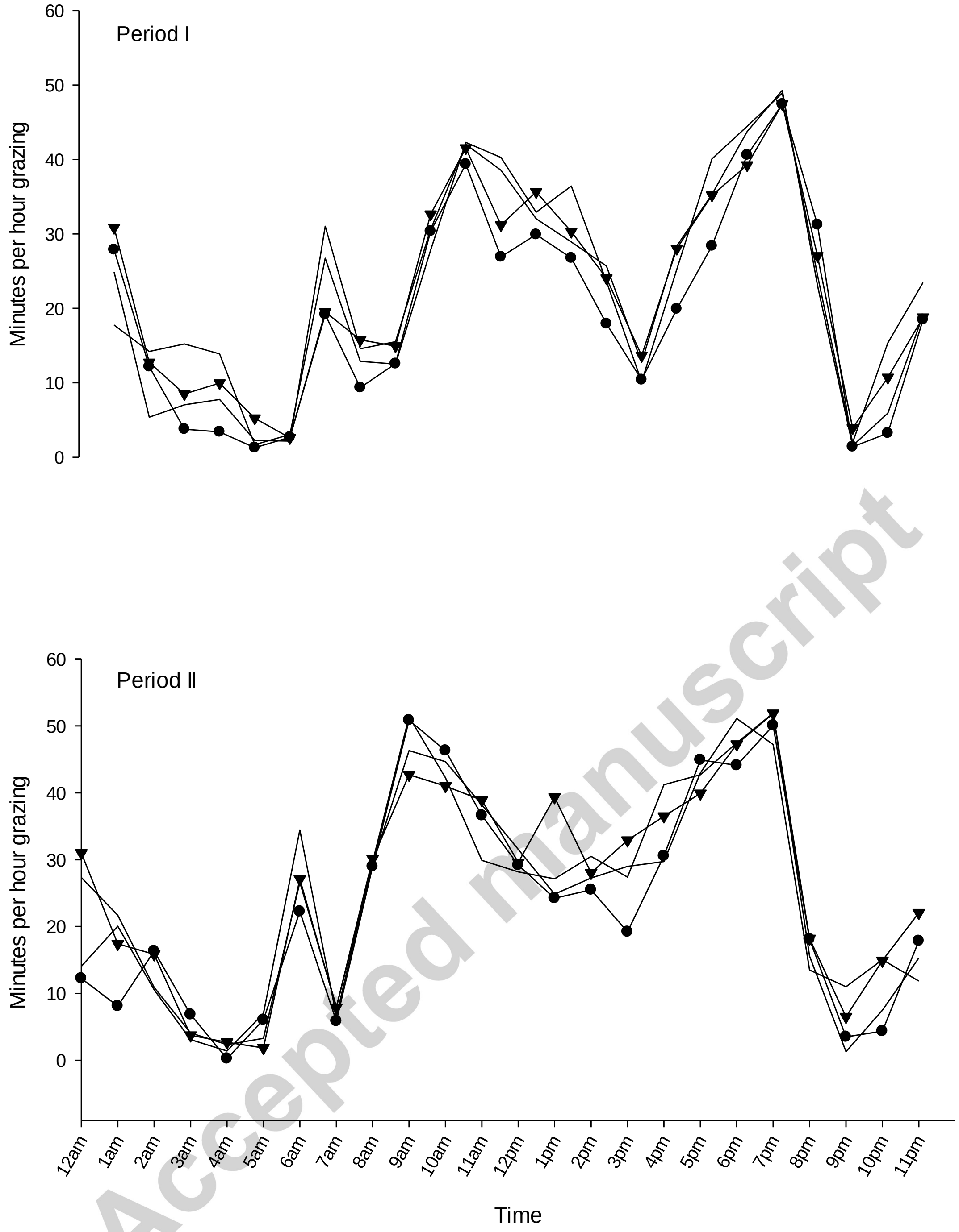

Figure 6 Time spent grazing simple ryegrass and white clover pastures with (open circles) or without (closed circles) Italian ryegrass or forb-containing pastures with (open triangles) or without (closed triangles) Italian ryegrass. Where Period I represents the five urine measurement days and Period II represents the five milk yield measurement days.

Highlights

- In late lactation, feeding forb pastures can improve milk yield compared with perennial ryegrass white clover pastures
- Manipulating pasture species in a sward mixture can alter urination pattern of grazing dairy cows
- Including herbs in pastures offers an opportunity to reduce soil nitrate leaching through removal of cows from pasture during peak urine N loading periods