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

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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.

## 1. 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

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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., 2017a). 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., 2017b). 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.

## 2. 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 × 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, (Cich*orium 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).

### 2.1. 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 × 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 (0700 h) and afternoon (1500 h) 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™, 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 faeces were sub-sampled following consecutive afternoon and morning milkings on days 11 and 15 of the experiment (0730–0800 h and 1530–1600 h). 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 fecal 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).

### 2.2. 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