Does silage containing Plantain affect the urination behaviour and urinary nitrogen of dry cows grazing diverse or conventional pasture?

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Abstract

The objective of this study was to investigate the effect of diverse silage containing plantain on urination behaviour and urinary and faecal nitrogen (N) concentration of dairy cows when grazing standard ryegrass-white clover (RGWC) or diverse pasture containing plantain. Four groups of six non-lactating dairy cows were balanced and randomly assigned to one of four dietary treatments in a 2 × 2 factorial design: RGWC pasture plus RGWC silage; RGWC pasture plus diverse silage containing plantain; diverse pasture plus RGWC silage; diverse pasture plus diverse silage. Urination behaviour (frequency and volume) was continuously measured for a minimum of 24 hours on four cows per treatment using the Lincoln University PEETER V1.0 sensor. There was no silage or silage × pasture type interaction effects on urination behaviour, though diverse pasture regardless of silage increased daily urination frequency (p=0.046) and daily urine volume (p=0.003) of cows by 27% and 31% respectively, compared to RGWC. Spot urine N% concentration was similar among treatments, but faecal N% tended (p=0.07) to be higher in cows grazing diverse pasture compared to those grazing RGWC with no silage type effect. Unlike fresh plantain, silage containing plantain does not seem to alter urination behaviour or N excretion of dairy cattle.

Keywords: *Plantago lanceolata* L; dairy cattle; winter study; urination frequency

Introduction

The total number of dairy cattle in New Zealand has doubled in the last two decades (2.4 million in 1990/91 to 4.9 million in 2020/21; StatsNZ 2020), while the area of land in dairy has increased by only 81% from 2002 (1,230,484 ha) to 2019 (2,221,459 ha) (StatsNZ 2020). Along with this intensification, dairy farming has imposed nutrient surpluses such as nitrogen (N) to the environment. The dairy cow urine patch is the major source of N loss from pastoral dairy systems (Di & Cameron 2002, Di & Cameron 2007). The high N load per urine patch exceeds the capability of plant N uptake, resulting in excess N being lost through N leaching or nitrous oxide emission (Selbie et al. 2015). The N lost to waterways has been associated with the risks of environmental degradation and human health issues (Ansari & Gill 2014, Johnson 2019). Consequently, regulations have been implemented by regional councils throughout New Zealand to reduce the impact of intensive farming, such as dairy systems, on the environment (Ministry for the Environment 2020). Hence, mitigation strategies should be sought for farmers to meet the N leaching related environmental regulations, while maintaining the profitability and practicality of pastoral dairy systems.

Many researchers have investigated the impact of diverse pastures containing plantain (*Plantago lanceolata* L.) on urinary N excretion from dairy cattle (Box et al. 2017, Bryant et al. 2018, Minnee et al. 2020, Al-Marashdeh et al. 2021, Navarrete et al. 2022). Those studies showed that in comparison to ryegrass/white clover mix, plantain reduces urinary N concentration and increases urination frequency of dairy cows and, therefore, is recommended as a viable strategy to reduce nitrate leaching losses from dairy systems. Farmer adoption of plantain is supported through recognition of the effect of plantain on urinary N losses in regulatory modelling tools such as Overseer (Shepherd 2020). However, integration of this herb into farming systems requires information about managing feed supply. For example, plantain growth is rapid during summer compared with conventional ryegrass pastures (Moorhead & Piggot 2009) but slower during winter (Nobilly et al. 2013). During periods of surplus supply, plantain could be conserved as silage and fed out during periods of pasture deficits (Bariroh et al 2018). Whether plantain silage holds similar environmental benefits in reducing N load per urine patch of cows as the fresh plantain forage remains unclear. Previous research has demonstrated that plantain can be ensiled (Bariroh et al 2018) but questions arise whether the benefits of plantain, in terms of altering urinary N load, are maintained when plantain is fed as a silage. Therefore, the objective of this research was to investigate the effect of diverse silage containing plantain on urination behaviour and nitrogen excretion of non-lactating pregnant dairy cows grazing either standard ryegrass/white-clover pasture or diverse pasture containing plantain.

Materials and methods

The experiment was conducted in June 2021 over two weeks at Lincoln University's Research Dairy Farm, Lincoln, New Zealand, with the approval of the Lincoln University Animal Ethics Committee (AEC application # 2021-08).

Experimental design and management

The experimental design was a 2 × 2 factorial with two pasture types; diverse vs ryegrass/white clover (RGWC) and two silage types; diverse vs RGWC. The four treatments were: 1) cows grazing RGWC pasture and supplemented with RGWC silage (RP+RS); 2) cows grazing RGWC pasture and supplemented with diverse silage (RP+DS); 3) cows grazing diverse pasture and supplemented with RGWC silage (DP+RS); and 4) cows grazing diverse pasture and supplemented with diverse silage (DP+DS). All pasture types and silages were derived from the same area which had been established in autumn 2018. The pastures were sown with Italian ryegrass (*Lolium multiflorum* Lam. Cv Asset AR37), plantain (*Plantago lanceolata* L. cv. Tonic*),* red clover (*Trifolium pratense* cv Relish) and white clover (*Trifolium repens* cv. Tribute).

Twenty-four multiparous Holstein Friesian x Jersey late-gestation (224 ± 13 days pregnant) dry cows were blocked by age (5.3 years ± 2.0), liveweight (517.6 kg ± 39.7), body condition score (4.7 ± 0.3) and expected calving date (11/08/2021 ± 2) and randomly assigned into the four dietary treatments (n=6). A total area of 4.0 ha was used in this study. Two paddocks (0.5 ha each) containing diverse pasture and two paddocks (0.5 ha each) containing RGWC pasture were allocated to each treatment group. Groups from the same pasture treatments grazed adjacent breaks on the same 0.5 ha paddock and were kept separate using temporary electric fencing. Based on the botanical composition of the pre-cut pasture used for ensiling, bales of diverse silage, previously harvested in summer, were selected to contain a high proportion (>30%) of plantain in the mixed sward. The experiment was conducted over 14 days, including 7 days of adaptation and 7 days of measurement.

Prior to the experiment, all cows were rotationally grazed together on RGWC pasture. During the period of this experiment, cows were allocated daily to 7 kg dry matter (DM)/cow of fresh pasture above the target post-grazing residual of 1400-1500 kg DM/ha and 3 kg DM/cow of silage. Pasture allowance (7 kg dry matter (DM)/cow per day) and estimated pasture mass per unit area were used to determine the area to be allocated per cow to graze daily. The pasture mass was assessed using an electronic rising plate metre (RPM; Jenquip F150 Electronic Pasture Meter, Fielding, New Zealand). The compressed pasture height of the paddock was recorded from 20-30 random raising plate meter (RPM) readings daily, and pasture mass was estimated using the winter equation (Kg DM /ha = 140 × RPM + 500; Lile et al. 2001). Daily pasture allocations were back-fenced to prevent grazing of residual regrowth. Silage was offered in a 1.5m x 1m plastic trough every morning, and the required quantity was calculated based on the pre-measured DM content for each silage type. Cows had *ad libitum* access to water.

Pasture and silage measurements

The RPM measurements were calibrated against 0.2 m^2 quadrat cut to the ground level on day 0, and three times during the experimental period (total number of cuts; diverse pasture = 34 and RGWC pasture =32). Two RPM measurements were recorded in each quadrat prior to pasture harvest. The cut pasture was washed and then oven-dried at 65°C for 48 hours to determine DM in each quadrat. Then, pasture mass (kg DM/ha) was calculated as dry weight x 50. The regression analysis was performed between dry weight of quadrant cut and measured RPM to generate the RPM calibration equations.

Representative silage sample and pasture plucks were collected on day 0 and three times over the experimental period and were subsampled for DM and chemical composition analysis. A subsample of pasture and silage were ground, sieved through a 1 mm and analysed for chemical composition by infra-red spectrophotometer (NIRS, NIRS DS 2500 F, Foss, Maryland, USA) at Lincoln University Analytical Laboratory, New Zealand. Metabolisable energy (ME) contents of pasture and silage (MJ ME/kg DM) were calculated as 0.16 x digestible organic matter content (Freer et al. 2007). A second pasture subsample was sorted into each herbage species to determine the botanical composition. Each of those herbage species was individually bagged, dried at 65°C for 24 hours and dry weights were recorded. Then the proportion of each species was determined as a percentage of total pasture DM.

Animal measurement

Group pasture intake was estimated from the difference between pre-and post-grazing herbage mass. Silage refusals were measured daily and silage intake was measured as silage offered minus refusals.

On days 0, 7, 10 and 13, faecal, urine and blood spot samples were collected from all cows at approximately 0900 hours. Faecal samples were collected via rectal grab and subsampled into two sets. One faecal subsample was oven-dried at 65°C for 48 hours and used for DM content determination. The second subsample was freeze-dried, grounded, sieved through a 1 mm and analysed by NIRS for N and organic matter content.

Urine samples were collected by vulval stimulation and one millilitre of concentrated sulfuric acid was added to minimise ammonia volatilisation. Urine samples were subsampled and frozen until analysed for creatinine, urea and nitrogen content at the Lincoln University Analytical Laboratory, New Zealand as per commercial kits instructions (Randox Rx Daytona Plus, Randox Laboratories Ltd). Blood samples were collected to 10 ml EDTA-containing vacutainers via the coccygeal vein and immediately placed in ice before being centrifuged at 3000 x g for 15 min at 4°C. The plasma was separated and analysed for urea content by the Lincoln University Analytical Laboratory as per commercial kits instructions (Randox Rx Daytona Plus, Randox Laboratories Ltd).

Urination behaviour was measured via the Lincoln University PEETER V1.0 sensors attached to four cows per treatment for 48 hours as described in Marshall et al. (2021). Eight urination sensors attached to a 3D printed harness were glued to the vulva of two random cows from each treatment on day eight and then switched over to another two random cows from each treatment on day ten. The sensors work by urine pressure travelling through a flow metre which records the urination time, pressure, volume and temperature to the built-in SD memory card. Data on SD card was downloaded. To maintain consistency, the first 24 hours of sensor's data was recorded from each cow, except for one cow in the RP+RS treatment where the sensor failed before the completion of the first 24 hours after attachment. Where possible, a second 24-hour of urination behaviour data obtained from sensors had no issues or breakage during the 48 hours of attachment and included in the data analysis. Fifteen cows had complete sensor data set for the first 24 hrs while only eight cows (n=2 for each treatment) had the second 24 hrs data set.

Statistical analysis

Urine, faecal, blood and urination volume per unit liveweight data were analysed using two-way ANOVA where pasture type, silage type and their interaction were included as a fixed effect and cows as replicate. Urination behaviour data were analysed using a linear mixed models (REML) approach. The model included day, time of day, pasture type, silage type and their interactions as fixed effects and cow as a random effect. All statistical analyses were performed using Genstat v19 statistical software (VSN International Ltd., Hemel Hempstead, UK). Results were declared significant at $P < 0.05$, and tendency was declared at $P < 0.01$. Figures on diurnal variations in urine volume and urination frequency were generated using the "ggplot2" in "tidyvers" package in RStudio (RStudio team, 2020, PBC, *Massachusetts*, USA).

Results

Chemical and botanical composition, intake and ME content of pasture allocated to cows are presented in Table 1. The average DM content of the DP (14.3%) was similar to the RP (14.4%). The average content of water-soluble carbohydrates (WSC) was higher in DP (12.8%) compared with RP (8.8%). Neutral detergent fibre (NDF; 33.7 vs 48.8%) and acid detergent fibre (ADF; 21.2 vs 26.2%) were lower in DP compared with RP, respectively. The crude protein (CP) and ME content were similar between the two pasture types and among treatments.

Ryegrass was the dominant pasture species in all the treatments (Table 1). The average plantain content (% DM) in the DP treatments was 28.7% and was higher in the DP+RS (33%) than DP+DS (22%) treatment. The average content of white clover (% DM) was higher in the DP treatment compared to the RP (4.0 vs 1.7%) but lower in the RP+DS (0.51%) than RP+RS treatment (2.9%). The content of dead material was lower in the DP compared to the RP (on average, 7.7 vs 24.5 %, respectively).

Botanical composition of pre-cut pasture for the ensiling and chemical composition of silage offered are presented in Table 2. The proportion of plantain in DS was 30.4 % and RS was free of plantain. The proportions of

Table 1 Pasture chemical and botanical composition (% DM), metabolisable energy (MJ/kg DM), pre/post grazing pasture mass (Kg DM/ha), and pasture and silage intake (Kg DM/cow/day) of non-lactating dairy cows grazed either diverse (DP) or ryegrass/white clover pasture (RP) and supplemented with either diverse (DS) or ryegrass/white clover silage (RS). Values are Mean ± SD.

	Treatment			
	DP+RS	DP+DS	RP+RS	RP+DS
Pasture Nutritive Content				
Dry matter	12.7 ± 0.04	15.8 ± 3.00	14.0 ± 2.83	14.8 ± 0.12
Crude protein	23.1 ± 2.10	22.2 ± 2.03	21.2 ± 1.86	21.4 ± 3.01
Water-soluble carbohydrates	11.9 ± 2.89	13.7 ± 1.82	9.1 ± 3.23	8.5 ± 2.88
Neutral- detergent fibre	34.7 ± 3.35	32.7 ± 3.11	47.6 ± 7.32	49.9 ± 4.88
Acid detergent fibre	21.6 ± 2.01	20.8 ± 1.10	25.7 ± 3.32	26.6 ± 2.25
Dry matter digestibility	78.6 ± 2.81	79.8 ± 0.94	76.2 ± 2.16	75.6 ± 2.31
Organic matter	88.0 ± 0.92	88.3 ± 2.20	89.8 ± 0.95	90.4 ± 0.88
Digestible organic matter	74.6 ± 3.92	76.4 ± 2.56	72.8 ± 2.50	72.4 ± 2.86
Organic matter digestibility	83.4 ± 3.43	84.8 ± 1.06	80.7 ± 2.70	80.1 ± 2.87
Metabolisable energy	11.5 ± 0.50	11.5 ± 0.41	11.4 ± 0.27	11.4 ± 0.28
Pasture Botanical Composition				
Ryegrass	60.8 ±10.10	55.7 ± 9.82	76.5 ± 7.73	70.3 ± 10.08
Plantain	22.4 ± 8.17	33.1 ± 10.77	-	-
White clover	4.0 ± 2.15	3.9 ± 2.71	2.9 ± 4.71	0.51 ± 0.54
Red clover	0.43 ± 0.83	0.73 ± 0.65	-	-
Ryegrass reproductive material	-	-	0.05 ± 0.25	-
Weed	2.0 ± 2.10	1.4 ± 1.82	0.70 ± 1.42	-
Dead material	10.3 ± 9.01	5.2 ± 2.84	26.1 ± 11.15	22.9 ± 7.99
Herbage mass				
Pre-grazing	2752 ± 275.1	3052 ± 252.4	3217 ± 184.7	3500 ± 143.8
Post-grazing	1641 ± 172.9	1638 ± 161.8	1676 ± 84.0	1606 ± 90.7
Intake				
Pasture	6.9 ± 1.37	7.3 ± 0.78	7.2 ± 0.45	7.6 ± 0.81
Silage	3.0 ± 0.11	3.0 ± 1.19	3.0 ± 0.11	3.0 ± 1.14

ryegrass were 28.5 and 56.5 % DM respectively for DS and RS. The average DM content (36 vs 52 %) and NDF (40 vs 48 %) content were lower in DS compared to RS, respectively. The CP content of silage was high but similar between DS (21.8) and RS (22.0). Ammonia N was similar between DS and RS, but ammonia N % in total N content was higher in DS (7.1%) compared to the RS (4.5%). Content of WSC was lower in DS compared to RS (4.3 vs 6.2%, respectively).

Concentration of N, urea and creatinine in urine, content of DM, organic matter and N in faeces, and plasma urea and urea N concentration from non-lactating pregnant dairy cows are presented in Table 3. There were no significant effect of pasture type, silage or pasture × silage interaction on concentration of creatinine, urea or N in urine of cows (P>0.05). Faecal content of DM was higher (P=0.01) and N tended to be higher (P=0.07) in cows grazed DP compared to those grazed RP, with no silage or pasture × silage effect. Faecal organic matter content and plasma urea and urea-N concentration were similar among treatments.

Table 2 Botanical composition of pre-cut pasture and chemical composition of diverse (DS) and ryegrass/white clover (RS) silage supplemented to non-lactating pregnant dairy cows during winter (Mean ± SD, % DM).

Item	DS	RS
Botanical Composition		
Ryegrass	28.5	56.5
Plantain	30.4	-
Clover	13.2	29.6
Chemical Composition		
DM	36.0 ± 0.03	52.0 ± 0.04
Crude protein	21.8 ± 2.7	22.0 ± 2.3
Acid-detergent fibre	26.5 ± 2.4	26.8 ± 3.5
DM digestibility	69.9 ± 11.3	72.5 ± 9.7
Digestible organic matter	70.3 ± 11.9	70.1 ± 12.9
Neutral-detergent fibre	40.4 ± 2.4	47.8 ± 4.9
Organic matter	90.3 ± 1.5	92.8 ± 2.4
Organic matter digestibility	82.9 ± 18.3	80.9 ± 16.6
Water-soluble carbohydrates	4.3 ± 1.2	6.2 ± 3.6
Ammonia N DM	0.21 ± 0.0	0.16 ± 0.0
Ammonia N in N %	7.1 ± 4.3	4.5 ± 2.5

The effect of pasture and silage type on the urination behaviour of non-lactating pregnant dairy cows is presented in Table 3. Daily urination frequency (P=0.046), urine volume (P=0.003), urine volume per kilogram of liveweight (P<0.001) were significantly affected by pasture type. Cows grazed DP urinated more frequently (16.2 vs 12.7 urination event/day) and excreted larger urine volume (47.4 vs 36.0 L/day) compared to cows grazed RP, respectively. There was no silage effect on urination frequency or urine volume.

Because there was no pasture × silage interaction effect on urination behaviour of cows, the diurnal variation of urine volume and urination events per hour were presented separately in Figure 1, as affected by pasture or silage type. Urine volume per urination event was not affected by pasture or supplement type (Table 3 and Figure 1a and c). Urination frequency per hour was greater during 1600 -1800 hours in cows grazed DP compared to those grazed RP (Figure 1b). Diurnal variation in urination frequency was not affected by silage type (Figure 1d).

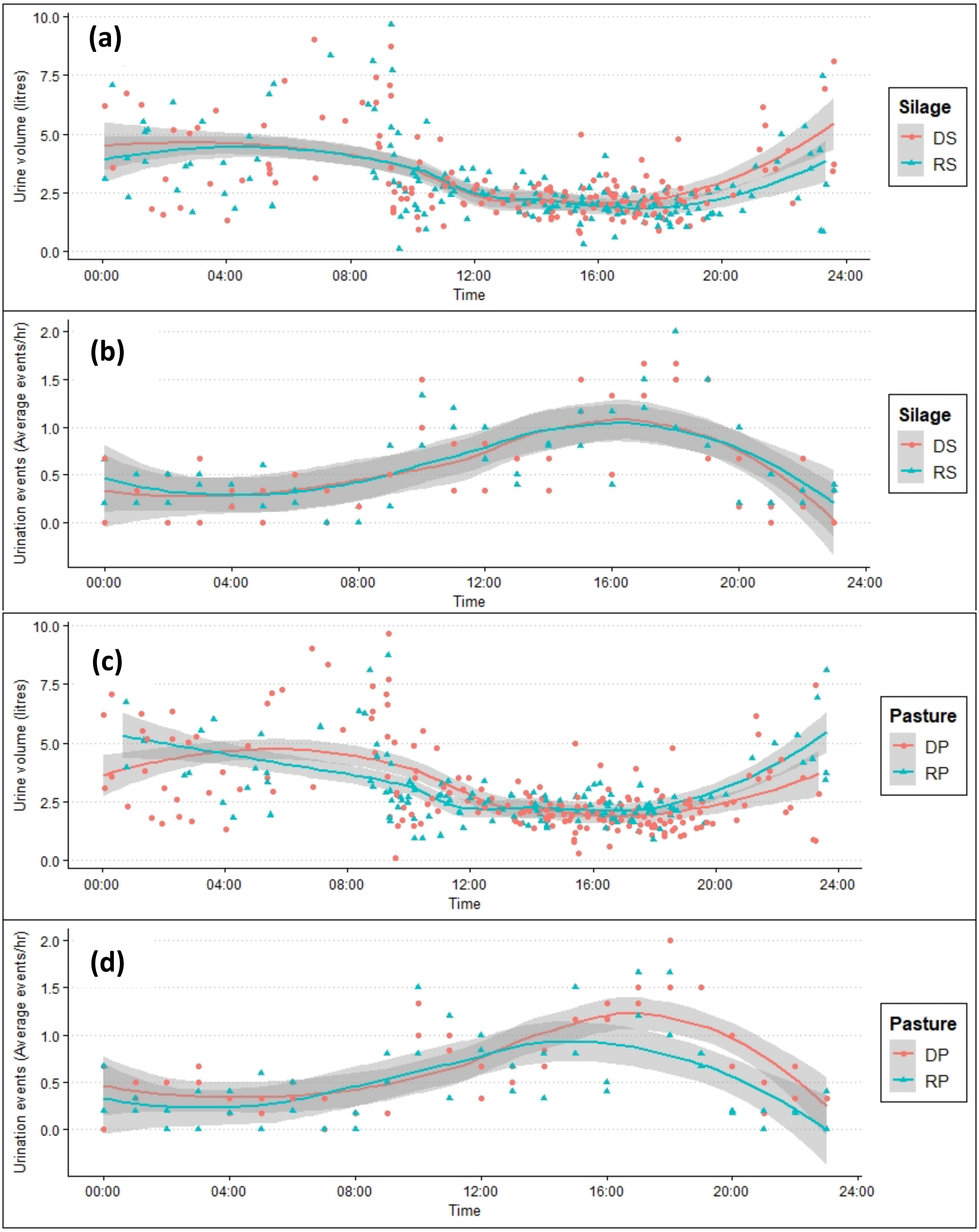
Discussion

Daily urination frequency of cows grazed DP was increased by 27% and total urine volume by 31% compared to the RP grazed cows. Comparably, Minnee et al. (2020) showed an increase of 15% in urination frequency and 14% in urine volume of dairy cows fed RGWC -based diet containing 45% DM plantain compared to those fed

Table 3 Urinary nitrogen, urea and creatinine concentrations, faecal DM, nitrogen and organic matter contents, plasma metabolites and urination behaviour of non-lactating pregnant dairy cows grazed either diverse (DP) or ryegrass/white clover (RP) pasture and supplemented with either diverse (DS) or ryegrass/white clover (RS) silage.

	Treatment					P-Value		
Item	DP+DS	DP+RS	RP+DS	RP+RS	SED	Pasture	Silage	Pasture × Silage
Urine								
Nitrogen %	0.17	0.19	0.18	0.17	0.002	0.86	0.73	0.577
Urea (mmol/L)	49.6	52.7	49.8	48.3	7.00	0.67	0.88	0.650
Creatinine (mmol/L)	1.10	1.11	1.06	1.16	0.015	0.96	0.61	0.642
Faeces, (% DM)								
DM	15.7	14.8	13.2	12.3	0.849	0.01	0.15	0.981
Nitrogen	3.35	3.21	3.11	3.02	0.154	0.07	0.29	0.848
Organic matter	74.3	74.4	73.7	72.4	2.16	0.45	0.68	0.635
Blood, (mmol/L)								
Urea	5.17	5.17	4.86	5.10	0.207	0.203	0.454	0.407
Urea-N	10.34	10.33	9.72	10.19	0.413	0.203	0.454	0.407
Urination Behaviour								
Urination frequency (events/day)	15.1	17.3	12.5	12.9	2.53	0.046	0.473	0.717
Urine volume (L/day)	46.9	47.9	36.3	36.0	4.89	0.003	0.986	0.978
Urine volume L/ kg liveweight	0.01	0.01	0.07	0.07	0.007	<0.001	0.714	0.925
Urine volume (L/event)	3.4	2.8	2.9	2.8	0.52	0.506	0.345	0.588

Figure 1 Effect of pasture (diverse; DP vs ryegrass/white clover; RP) and silage (diverse; DS vs ryegrass/white clover; RS) type on diurnal variation of urine volume (a and c, respectively) and average urination events per hour (b and d, respectively) of non-lactating pregnant dairy cows during winter. The shaded area represents the 95% confidence level.



RGWC only diet. In previous research the higher urine volume of cows fed plantain was attributed to water induce diuresis caused by intake of plantain-containing pasture which had a greater content of water (Box et al. 2017, Minnee et al. 2020, Mangwe et al. 2019, Beck et al 2020). However, in the present study there was little difference in the water content between DP and RP (14.3 vs 14.4% DM, respectively). In addition, pasture intake was similar for cows grazed DP or RP (7.1 and 7.4 kg DM/cow/day). Thus, the calculated water consumption from pasture ingestion was approximately similar for cows grazed in either DP or RP (43 vs 44 L/d/cow, respectively). Therefore, it is unlikely the greater urine volume and urination frequency of DP grazed cows resulted due to more water intake from DP compared to counterparts grazed in RP. The reasons for increased urine excretion from diverse pasture type is unclear, and there has been speculation that the mineral content and plant secondary compounds alter urination behaviour in plantain fed animals (O' Connell et al 2016; Box et al 2019), however, the present study did not measure the secondary components in the plantain-containing pasture. Beck et al (2020) investigated the role of minerals and secondary compounds in plantain 'diuresis' in deer and showed little relationship between urine volume and these plant characteristics. A more likely explanation for the results in this study is the change in the partitioning of water to dung and urine as cows grazing DP had increased fecal DM% compared with cows grazing RP. This is supported by the findings of Minnée et al. (2020) who reported less water excretion via faeces of cows fed with ryegrass-based diet containing 30 or 45 % plantain (on a DM bases) compared to those fed ryegrass only.

Compared to RP, DP including plantain tended to increase faecal N content of cows. A similar effect for plantain on N partitioning was reported by Minnée et al. (2020) who showed a higher proportion of dietary N partitioned to the faeces of lactating dairy cows fed increasing levels of plantain in their diet compared to those fed ryegrass white clover. This supports the benefit of plantain in reducing N leaching from the urine patch by shifting N from urine into the more stable form in faeces.

The urine volume per event was higher in DP than in RP grazed cows, hence, cows in DP treatment might have excreted diluted urine, depositing less N per urine patch. This could reduce the N accumulation in soil and consequently, reduce the risk of N leaching. However, urinary N% in spot sample was similar among treatments, suggesting that DP cows may have excreted more urinary N compared to RP cows due to the larger urine volume. This is contrary to the results of Minnée et al. (2020) and Box et al. (2017) who showed less N concentration in urine and total urinary N excretion for cows fed plantain compared to ryegrass-based pasture. It is worth noting that in this study, the N percentage of urine was measured from one spot sample per day collected at 0900 hours. Urinary N concentration of dairy cows fluctuates significantly during the day and there were variations even if measured at the same time of the day (a peak N concentration reported by Minnée et al. (2020) vs the nadir reported by Shepherd et al. (2017) at 0900 hours). Hence, one spot sample per day potentially was not enough to capture the expected dilution effect of the DP on the urinary N concentration of cows in this study, thus, a more frequent urine sampling per day should be considered in future research.

Unlike the DP, DS containing plantain did not alter the urination behaviour (urine volume and urination frequency) of cows in this study. The DS was composed of high water content compared to RS (64 vs 48 %) and there was no difference in silage intake of cows fed either DS or RS. Thus, DS cows had greater water consumption via silage intake. Nevertheless, the greater water content in silage did not change the urine volume and urination frequency of cows. One possible reason could be the degradation of secondary plant components in plantain during the ensiling process. Bariroh (2020) reported a reduction in the plant secondary compounds (full degradation for Aucubin and Catapol, and reduction from 18.16 to 2.27 mg/g for Acteoside) when plantain forage was ensiled. Although minimal research has been conducted on the effect of each of these secondary compounds on the urination behaviour of cows, we speculate that the degradation of secondary compounds in plantain silage diminished the effect on urination volume or frequency. This means that, unlike fresh plantain, silage containing plantain could not be used as a mitigation strategy to N loss of dairy systems. The identification of the changes in plant secondary components during ensiling and storage and their primary mode of action in altering the urination behaviour of cows, if there is any, warrant future research.

Diverse silage containing plantain also had no significant effect on urinary or faecal N content, or plasma metabolites concentrations. Beatson (2017) reported a tendency of urinary N concentration reduction in cows fed plantain silage (100 % plantain) compared to those fed ryegrass silage supplement. Also, Judson & Edwards (2016) reported a reduction in urinary N concentration in heifers grazing kale supplemented with plantain baleage (100% plantain) compared to those fed ryegrass baleage. The reason for this discrepancy in the results of urinary N concentration is unclear, but one possible reason that the pure plantain silage used in the study of Beatson (2017) and Judson & Edwards (2016) resulted in greater water intake via feed compared with the diverse silage used this study. This in turn may have resulted in greater urine volume and hence, dilution effect. Additionally, there may be differences in dietary N partition into the live weight based on level of DM intake and physiological state (growing heifers in the study of Judson & Edwards (2016) vs mature cows in this study) of animal.

Conclusion

The result from this study found that cows allocated diverse pastures containing fresh plantain (average 30% of pasture DM) increased urination frequency and daily urine volume, compared to cows grazed ryegrass/white clover. Diverse silage containing plantain does not affect urine N concentration, urination frequency and urine volume of cows. Unlike fresh plantain, ensiled plantain containing pasture is less likely to be used as a mitigation option to reduce nitrate leaching by dairy farmers.

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References

Al-Marashdeh O, Cameron K, Hodge S, Gregorini P, Edwards G 2021. Integrating Plantain (*Plantago lanceolata L.*) and Italian Ryegrass (*Lolium multiflorum Lam.*) into New Zealand Grazing Dairy System: The Effect on Farm Productivity, Profitability, and Nitrogen Losses. Animals 11: 376.

Ansari AA, Gill SS 2014. Eutrophication: causes, consequences and control. Volume 2. Springer Dordrecht Heidelberg, London, New York. http://www.dawsonera.com/depp/reader /protected / external/AbstractView/S9789400778146 [Accessed 2nd August 2022].

Bariroh N, Bryant R, Black A 2018. Plantain silage quality under variable management practices. Journal of New Zealand Grasslands 80: 119-124.

Bariroh NR2020. Fermentation properties and nutritive value of plantain (Plantago lanceolate) silage. PhD thesis, Lincoln University, Lincoln New Zealand.

Beatson C 2017. Urine N excretion and urine N loading of lactating dairy cows fed ryegrass and plantain as forage and silage. A dissertation, Lincoln University, New Zealand.

Beck MR, Konagh Garrett, Bryan RT, David RS, Barrell GK, Gregorini P 2020. Plantain (*Plantago lanceolata*) reduces the environmental impact of farmed red deer (*Cervus elaphus*), *Translational Animal Science* 4: 1-11.

Box LA, Edwards GR, Bryant RH 2017. Milk production and urinary nitrogen excretion of dairy cows grazing plantain in early and late lactation. New Zealand Journal of Agricultural Research 60: 470-482.

Box LA, Edwards GR, Bryant RH 2019. Seasonal and diurnal changes in aucubin, catalpol and acteoside concentration of plantain herbage grown at high and low N fertiliser inputs. New Zealand Journal of Agricultural Research 62: 343-353.

Bryant RH, Welten B, Costall D, Shorten P, Edwards G 2018. Milk yield and urinary-nitrogen excretion of dairy cows grazing forb pasture mixtures designed to reduce nitrogen leaching. Livestock Science 209: 46-53.

Di HJ, Cameron KC 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64: 237-256.

Di HJ, Cameron KC 2007. Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor – a lysimeter study. Nutrient Cycling in Agroecosystems 79: 281-290.

Freer M, Dove H., Nolan JV 2007. Nutrient requirements of domesticated ruminants, CSIRO publishing. Australia.

Johnson SF 2019. Methemoglobinemia: Infants at risk. Curr Probl Pediatr Adolesc Health Care 49: 57-67.

Judson HG, Edwards GR 2016. Urinary nitrogen concentration from dairy heifers grazing kale supplemented with either plantain or perennial ryegrass baleage in winter. Journal of New Zealand Grasslands 78: 99-102.

Lile J, Blackwell M, Thomson N, Penno J, Macdonald K, Nicholas P, Lancaster J, Coulter M 2001. Practical use of the rising plate meter (RPM) on New Zealand dairy farms. Proceedings of the New Zealand Grassland Association 159-164.

Mangwe MC, Bryant RH, Beck MR, Beale N, Bunt C, Gregorini P 2019. Forage herbs as an alternative to ryegrass-white clover to alter urination patterns in grazing dairy systems. Animal Feed Science and Technology 252: 11-22.

Marshall C, Beck M, Garrett K, Beale N, Gregorini P 2021. Evaluation of PEETER V1. 0 urine sensors for measuring individual urination behavior of dairy cows. JDS Communications 2: 27-30.

Ministry for the Environment 2020. National Policy Statement for Freshwater Management. In: Environment, Mft (ed.). Wellington, New Zealand.

Minnee EMK, Leach C and Dalley DE 2020. Substituting a pasture-based diet with plantain (*Plantago lanceolata*) reduces nitrogen excreted in urine from dairy cows in late lactation. Livestock Science 239: 104093.

Moorhead, A. J. E. & Piggot, G. J. 2009. The performance of pasture mixes containing 'Ceres Tonic' plantain (Plantago lanceolata) in Northland. Proceedings of the New Zealand Grassland Association, 71**:** 195-199.

Navarrete S, Rodriguez M, Horne D, Hanly J, Hedley M, Kemp P 2022. Nitrogen Excretion by Dairy Cows Grazing Plantain (Plantago lanceolata) Based Pastures during the Lactating Season. Animals 12: 469.

Nobilly F, Bryant R.H, McKenzie BA, Edwards GR 2013. Productivity of rotationally grazed simple and diverse pasture mixtures under irrigation in Canterbury. In Proceedings of the New Zealand Grassland Association 165-172.

O'Connell C, Judson H, Barrella G. 2016. Sustained diuretic effect of plantain when ingested by sheep. Proceedings of the New Zealand Society of Animal Production 76: 14-17.

Selbie DR, Buckthought LE, Shepherd MA 2015. Chapter Four – the challenge of the urine patch for managing nitrogen in grazed pasture systems. Advances in Agronomy 129: 229-292.

Shepherd M 2020. Recommendations for the implementation of plantain in Overseer. Report for Forages for Nitrate Leaching (FRNL) and Overseer Ltd, AgResearch Report RE450/2019/069.https://www.dairynz.co.nz/media/5793403/frnl-overseer-2-recommendations-for-implementation-of-plantain-based-pasture-in-overseer-after-frnl-feedback-final-20-feb-2020.pdf.

Shepherd M, Shorten P, Costall D, Macdonald KA 2017. Evaluation of urine excretion from dairy cows under two farm systems using urine sensors. Agriculture, Ecosystems and Environment 236: 285-294.

StatNZ, 2020. Livestock numbers. https://www.stats.govt.nz/indicators/livestock-numbers/ [Accessed 2nd August 2022].