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## Urine volume of non-lactating dairy cows in late gestation fed forage based diets in winter

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### Abstract

Quantitative information on urine volume of dairy cows fed winter forages is required to accurately evaluate nitrate leaching risks on these diets. Thirty-two Friesian x Jersey non-lactating, pregnant dairy cows were fed either 10 kg DM of fodder beet with 5.6 kg DM of ryegrass baleage or 16.4 kg DM of kale with 6.4 kg of oat silage. Cows were fitted with a urine meter for 24 to 72 hours to record the frequency and volume of urine events. Daily urine volume ( $28 \pm 3$  L/cow/day), frequency ( $9 \pm 0.89$  events/cow/day), and event volume ( $3.2 \pm 0.3$  L/event) were not significantly different between fodder beet and kale treatments. This was despite respective differences in total apparent water (51 vs. 70 L/cow/day,  $\pm 2$ ,  $P < 0.001$ ) and nitrogen (N) intake (231 vs. 488 g N/cow/day,  $\pm 9.4$ ,  $P < 0.001$ ). In this study, water consumption and N intake did not appear to be linked with urine volume of dairy cows when two different forage diets were compared.

**Keywords:** New Zealand; Brassica oleracea; Beta vulgaris

### Introduction

New Zealand dairy farmers face a major challenge in growing sufficient pasture during winter to meet energy requirements of livestock (Dalley 2011). Farmers aim to regain body condition lost during lactation prior to calving to maximise subsequent productive and reproductive performance (Edwards et al. 2014). Due to the high quality and yield of forages such as kale (*Brassica oleracea*) and fodder beet (*Beta vulgaris*), it is common practice for dairy farmers to graze these forages *in situ* over winter (Brown et al. 2007; Rugoho et al. 2014).

An additional challenge regarding winter feeding is for farmers to reduce nitrogen (N) losses from animals. N leaching from soils growing winter forage grazed by dairy cows accounts for up to 24% of farm annual N leaching losses, despite representing only up to 9% of the farm area (Chrystal et al. 2012). Typically the major driver of N leaching on pastoral dairy farms is urine from grazing animals rather than applications of fertiliser or effluent (de Klein et al. 2010), driven by N concentration of urine, volume of urine deposited, and area covered in any single event (Romera et al. 2012). Consequently, data for total daily N excretion alone may not accurately reflect N leaching risks from individual urine patches. Studies of cattle urination on pasture-based diets reveal highly variable daily urine volumes ranging from 8.7 to 54.7 L (Betteridge et al. 1986; Edwards et al. 2015). Frequency of urination could range from 2 to 73 events per day (Betteridge et al. 1986) with average volumes of 2 to 3 L (Betteridge et al. 2013).

A pilot study by Ravera et al. (2015) testing a urine meter showed that cows fed kale had greater total daily urine volume than those fed fodder beet. The reason was unclear and authors speculated factors such as N and/or water intake may have contributed to the variation. N intake is a strong driver of urine volume (Bannink et al. 1999; Khelil-Arfer et al. 1992) as is water intake, be it through direct or

indirect consumption (Frandsen et al. 2006). To determine the effect of winter management on parameters influencing N leaching, the objective of this research was to compare differences in urine volume, urination frequency, and N concentration of urine from two commercially popular winter diets offered to provide accentuated differences in N and water intake.

### Materials and methods

#### Experimental site and design

The experiment was conducted at the Lincoln University research farm (-43.65, 172.33) for four weeks from 23 June to 18 July 2015 with approval from the Lincoln University Animal Ethics Committee (AEC #620). Diet treatments, representative of industry practice, based on fodder beet and kale winter forages were compared. Allocation of DM was designed to achieve the metabolisable energy requirement of 160 MJ ME/cow/day for body condition score gain for the fodder beet treatment (Edwards et al. 2014). To achieve large differences in N intake between treatments, the DM allocation for the kale treatment was in excess of requirements. Fodder beet allocation was 10 kg DM of fodder beet supplemented with 6 kg DM of ryegrass baleage, while the kale allocation was 16.5 kg DM of kale supplemented with 6.5 kg DM of oat silage, resulting in respective dietary allowances of 275 and 575 g of N/cow/day and 46 and 64 L water/cow/day. Thirty-two Friesian x Jersey adult, non-lactating dairy cows in late gestation, previously adapted to experimental diets, were included in a randomised design. Cows were selected using a restricted randomisation procedure to minimise potential variation in urine volume relating to age and size, ( $6 \pm 0.46$  years;  $513 \pm 8.6$  kg live weight;  $5.0 \pm 0.09$  body condition score;  $120 \pm 8.3$  breeding worth).

#### Animals and management

Four animals per treatment were sampled during

three days each week by removing cows from a larger mob and transferring them to individually fenced pens (area = 48 m<sup>2</sup>) in a paddock with low pasture mass (<1000 kg DM/ha). All cows were independent of each other and received supplement at 0800h followed by forage crop at 1000h. All feed offered (cut and placed next to pens) and refused was weighed using portable, trailer mounted scales (Inconix FX1). Measurements of urine events and volumes commenced between 1300-1500h on the second day by attaching a urine meter to the vulva of each cow using super glue as described by Ravera et al. (2015). The urine meter consisted of a flowmeter and data logger recording time, duration, and volume of each event. Validation of the meter while attached to the cow was carried out by manual stimulation of the vulva, and collection of urine in a measuring jug for comparison with data logger values. Difficulty with manual stimulation of urination whilst meters were attached resulted in a regression based on only 11 measurements with a standard error of 500ml. Urine meters were monitored several times each day ensuring secure attachment for data collection for up to three consecutive days. Following each run (one non-sampling and three sampling days), cows were returned to the mob and a new experimental area was set up. Water troughs, attached to flow meters, were used in each pen.

**Measurements**

Urine samples (n = 14) were collected in the yards opportunistically and by manual stimulation of the vulva at 1400 h. Samples were immediately acidified below a pH of 4.0 using concentrated sulphuric acid preventing volatilization, and stored at -20°C. Thawed samples were assessed for urine N% using an N-analyser Elementar (Vario MAX CN, Analysensysteme, Hanau, Germany). Dung events were counted and sub-sampled for each cow (n = 32). A further two sub-samples (40 g fresh weight) were dried at 100°C for 48h to determine DM content or freeze-dried and ground (1 mm) to determine N content. Faecal N% was measured by an Elementar and OM% through furnace combustion at 550°C for four hours.

Diet samples were collected each week from forage and supplement offered and refused to determine nutrient composition and intake. Forage samples were washed and divided into leaf (including petiole) and kale stem or fodder beet bulb and DM% of these and supplements determined by recording fresh and oven dry weights. Nutritive values of freeze-dried, ground sub-samples of leaf and bulb or stem as well as supplement were determined by near infra-red spectrophotometry (NIRSystems 5000, Foss, Maryland, USA). Mineral composition (Ca, Mg, Na, and K) was determined on freeze-dried, ground material by an Elementar. Apparent DM intake was determined by subtracting dry weight of refusals from dry weight of offered feed. Pasture intake was assumed to be zero. Apparent intake of minerals, ADF, and NDF were determined from content in the offered diet (g/kg DM) less the content in refusals (g/kg DM).

*Statistical analysis*

Apparent intake of nutrient components, water, dry matter, and urination and defecation measurements were compared by ANOVA (Minitab v.17) using diet as the fixed term and cow as the random term. Six animals (four on fodder beet and two on kale) rejected the urine meter and their data was excluded, leaving 26 cows in the statistical analysis.

**Results**

Daily maximum and minimum screen temperatures averaged 11.5°C and 0.8°C respectively during the experimental period with an average weekly rainfall of 9 mm. Chemical and mineral content of diets is presented in Table 1. Compared with fodder beet, leaf constituted a greater proportion of forage DM for the kale. DM% was similar for each of the diet components. Mineral concentrations were higher in leaf compared with stem or bulb and Kale had a greater N content than fodder beet.

The greater DM allowance of the kale diet resulted in greater (P<0.001) apparent intakes of DM, fibre, N, and minerals compared with the fodder-beet diet (Table 2). Based on diet digestibility and DM intake, apparent

**Table 1** Nutrient and mineral composition (g/kg dry matter unless otherwise stated) of feed in winter forage crop diets based on fodder beet and kale. DM = Dry matter.

	Fodder beet			Kale		
	Leaf	Bulb	Ryegrass baleage	Leaf	Stem	Oat silage
% of crop dry weight	17.7	82.3	-	28.6	71.4	-
DM (%)	23.4	18.7	43.2	23.0	14.6	58.0
DM digestibility %	74.4	93.5	69.2	89.7	82.1	60.6
Neutral detergent fibre	355	118	535	150	275	638
Acid detergent fibre	256	128	310	148	230	355
Nitrogen	23.3	11.7	22.5	36.5	23.8	18.8
Calcium	8.37	1.62	5.14	21.6	9.95	3.28
Potassium	31.4	16.5	20.0	11.0	14.5	26.2
Magnesium	2.81	1.39	1.66	1.74	1.85	1.41
Sodium	7.78	3.91	2.85	2.64	3.83	2.45

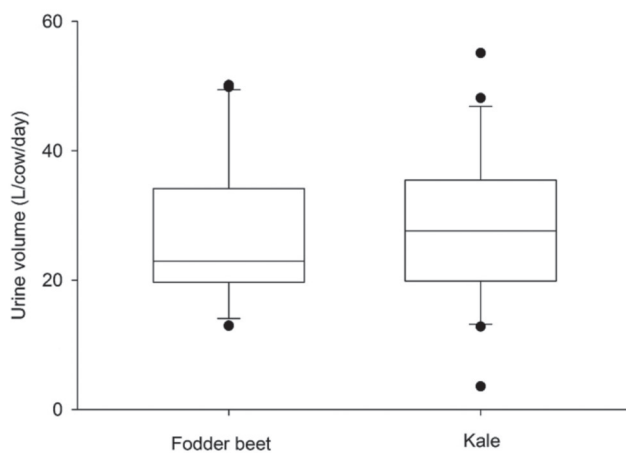
**Table 2** Mean apparent dietary intake and water consumption of non-lactating dairy cows in late gestation fed diets based on fodder beet and kale winter forages (per cow per day). DM = Dry matter.

Intake	Fodder beet	Kale	SEM	P Value
Crop (kg DM)	7.7	11.5	0.44	<0.001
Supplement (kg DM)	5.6	6.0	0.15	0.06
Total intake (kg DM)	13.3	17.5	0.52	<0.001
Acid detergent fibre (kg)	2.89	4.03	0.10	<0.001
Neutral detergent fibre (kg)	4.20	5.75	0.14	<0.001
Nitrogen (g)	231	488	9.4	<0.001
Calcium (g)	26	202	5.9	<0.001
Potassium (g)	263	288	9.7	0.08
Magnesium (g)	21.8	27.9	0.8	<0.001
Sodium (g)	52.8	40.9	1.8	<0.001
Water from trough (L)	11.5	9.68	1.57	0.339
Water from diet (L)	39.2	60.4	1.9	<0.001

**Table 3** Urine and faecal parameters of non-lactating dairy cows in late gestation fed diets based on fodder beet and kale winter forages. Mean values are shown. DM = Dry matter. [Dung on day 1; urine on day 1, 2, and 3].

		Number of Samples	Fodder Beet	Kale	SEM	P value
Dung N% DM		32	2.51	1.93	0.09	0.02
DM%		32	23.6	25.2	1.0	0.33
Organic Matter%		32	54.3	67.4	2.11	0.02
Frequency (#/day)		32	6.75	6.85	0.74	0.93
Urine N (g/L)		14	3.85	5.25	1.32	0.59
Total volume (L/day)		26	27.5	28.7	3.0	0.77
Average volume (L)		26	3.48	2.89	0.30	0.12
Frequency (#/day)		26	8.5	10.3	0.89	0.14

**Figure 1** Box plot of total daily urine volume of non-lactating dairy cows in late gestation fed diets based on fodder beet and kale winter forages.



intake of cows on kale was 210 MJME/kg/day and for cows on fodder beet was 174 MJME/kg/day. Direct water consumption was similar for kale and fodder beet treatments, but the greater dietary water consumption resulted in total apparent water intake being 19 L lower ( $P < 0.001$ ) for the fodder beet treatment (Table 2).

Frequency of defecation events was similar between treatments (Table 3). N content of dung was greater ( $P < 0.05$ ) for cows fed the fodder beet diet than those fed the kale diet. Urine N content, frequency of events, and volumes were similar between diets (Table 3). Average daily volume was 28 litres/cow/day ranging from 12.3 to 50.0 L/cow/day across treatments (Fig.1).

## Discussion

These results confirm large variability between animals for urination behaviour noted in previous studies (Betteridge et al. 2013, Ravera et al. 2015). Daily urine volume (28 L/cow/d), event volume (3.2 L/event) and frequency (9 events/day) were consistent with published values. Under similar conditions, Ravera et al. (2015) observed daily and event volumes of 24 L/day and 2.37 L/event over 10 events/day from cows offered kale and fodder beet diets. The frequency

of urine events was similar to published average values for dairy cattle at 9.4 events/day (Aland et al. 2002; Castle et al. 1950). For pasture-fed dry cows, Betteridge et al. (2013) observed event volumes ranging from 0.30 to 7.83 L and averaging 2.1 L/event with 12 events/day. However, despite large differences in mineral and water intakes, there was a lack of treatment differences in urine volume. The results of Ravera et al. (2015) found urination frequency and total daily urine volume to be greater for cows fed kale than for those fed fodder beet at the same energy allowance and large between cow variation, theorised to be due to greater N intake for cows fed the kale-based diet.

In the current study apparent intakes of N, Ca, Mg, and water were greater for cows fed kale than for cows fed fodder beet. Volume of urine excreted increases according to the need to expel minerals and water from the body (Bannink et al. 1999; Frandson et al. 2006) and cattle fed greater quantities of N would consume and, therefore, excrete more water (Bannink et al. 1999; Khelil-Arfa et al. 2012). Urine volume is also determined by the effect of minerals on urine osmolality (Bannick et al. 1999). Thus, a high intake of K and Na relative to N for cows fed fodder beet may have increased urine excretion in the fodder beet treatment, resulting in a similar urine volume to cows on kale.

Although there were large differences in dietary mineral loading in this experiment, the diet treatments also varied in DM intake and feed type. Given the similarity in faecal event number and DM content, it was unlikely that dung accounted for more than 5 L/cow/day of the variation in water balance. Compared with the fodder beet treatment, DM intake was 32% higher for cows fed kale. At high intakes and cold temperatures, feed digestibility is reduced (Ole Miaron & Christopherson 1992), which may have altered water use in the cow. Given that energy intake has been linked to water loss in mammals, the apparent difference of 50 MJ ME/day may have affected metabolic losses of water (Westerterp et al. 2005). Respiratory and insensible water losses were not measured and the findings from this experiment warrant further investigation into these losses for dry dairy cows in cold climates.

Urinary N concentration from spot sampling is a common variable used to assess potential N leaching risks of grazed diets. Statistically there was no difference in urinary N concentration between diets in this study, which agrees with studies having larger data sets also failing to detect differences using similar diets (Jenkinson et al. 2013; Edwards et al. 2014). Diurnal patterns in urine N concentration have been demonstrated (Betteridge et al. 2013) and information on diurnal changes for cows fed winter forage diets is required.

Predicting animal urinary behaviour *in situ* is not without its challenges, not least because of the variation between animals, but also finding suitable measurement techniques. The urine meter is a novel tool allowing 'in field' data on urination events to be recorded. Although standard errors were greater than desired (0.5 L), similar



variances between treatments suggest bias was unlikely and values fall within the range of published values. In this study parameters which influence N loss from urine events (volume, frequency, and N concentration) did not differ sufficiently to provide an obvious pathway on which to focus changes in animal management practises to reduce N losses.

## Conclusions

Daily urine volumes for cows on diets based on fodder beet and kale forages were similar. Large between-animal variation was confirmed, showing apparent intake of water and N to not be linked to urine volume, when represented in differing diets and allowances. This indicates that predictions of urinary N for cows consuming different levels of water and N would be difficult, given the large between-animal variation. Measurements obtained provide useful information on the urine volume and frequency for dairy cows feeding on commercially popular winter forages.

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