# Urinary nitrogen excretion, grazing and urination behaviour of dairy heifers grazing pasture, chicory and plantain in autumn

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## Abstract

The objective of this study was to investigate urinary nitrogen (N) excretion, grazing and urination behaviour of dairy heifers grazing pasture, chicory and plantain. A 35-day trial was conducted with 56 Friesian x Jersey heifers aged 9-10 months. Heifers were blocked into five treatments balanced for their live weight and breeding worth: 100% ryegrass/white-clover pasture (PA; n = 12); 100% chicory (CH; n = 10); 100% plantain (PL; n = 12); 50% pasture + 50% chicory (PA+CH; n = 10); and 50% pasture + 50% plantain (PA+PL; n = 12). Feed was offered every three days with allowance calculated according to feed requirement for maintenance plus live weight gain of 0.8 kg/day. Measured urinary-N concentration, estimated daily urinary-N excretion (UN) and urinary output were similar among treatments. During the first six hours after feed allocation, heifers spent more time idling and less time ruminating on CH and PL than on PA. The CH and PA+CH groups urinated more frequently than other groups. Data from this study indicate that heifers grazed chicory urinated more frequently without increasing daily UN and may potentially reduce N loading from soil. Future study is needed to take account of urinary N excretion diurnal variation.

**Keywords:** metabolisable energy; prediction equation; herbs; mixed swards; sustainability

## Introduction

New Zealand pastoral farming systems are primarily based on perennial ryegrass (*Loliun perenne*) and white-clover (*Trifolium repens*) pasture. However, in recent years, there has been an increased interest in adding herbs such as chicory (*Cichorus intybus*) and plantain (*Plantago lanceolata*) to the diet of livestock to improve animal performance (Schreurs et al. 2002). Previous studies demonstrated that the use of pasture containing chicory and plantain is associated with reduced internal parasite load (Scales et al. 1995) and increased sheep and deer live weight (LW) gain (Schreurs et al. 2002; Golding et al. 2008), compared to perennial ryegrass/white-clover pasture. Further, recent work (Totty et al. 2013) showed lactating dairy cows grazing mixed-species pasture containing chicory and plantain had lower urinary-nitrogen (N) concentration and urinary-N excretion (UN) than those grazed perennial ryegrass/white-clover pasture. This demonstrates the potential of herbs to reduce N losses in urine, and potentially, losses as nitrate to the environment. However, there is little information on which pasture species or how much each of the species is required to achieve an environmental benefit without losing productivity. Therefore, the objective of this study was to investigate UN, grazing and urination behaviour of heifers grazing pasture, chicory and plantain in autumn.

## Materials and methods

The study was undertaken at Ashley Dene Pastoral Systems Research Farm, Lincoln University, New Zealand with the approval of Lincoln University Animal Ethics Committee (No. 557). A 35-d trial was conducted from 13 May 2014 to 16 June 2014. This consisted of a seven-day feed-adaptation period and a 28-day measurement period. Perennial ryegrass/white-clover pasture and pure swards of chicory and plantain were sown as adjacent strips on 15 January 2014. A total of 56 heifers were blocked into five dietary treatments balanced for their LW (mean = 210 kg; SD = 17.5 kg) and breeding worth (mean = NZ$155; SD = NZ$ 33.7): 100% pasture (PA; n = 12); 100% chicory (CH; n = 10); 100% plantain (PL; n = 12); 50% pasture + 50% chicory based on area of feed allocated (PA+CH; n = 10); and 50% pasture + 50% plantain based on area of feed allocated (PA+PL; n = 12). The number of heifers differed between treatments reflecting lower feed available in CH and PA+CH than other treatments.

Herbage was offered every three days at 0900 h. Allowance was calculated according to feed requirements for maintenance plus a LW gain of 0.8 kg/day (Nicol and Brookes, 2007). For each species (PA, CH and PL), 25 pre- and post-grazing herbage mass measurements were estimated by a rising plate meter (Jenquip, Feilding, New Zealand) and calibrated with 0.1 $m^2$ quadrat cuts, prior to the study. The calibration curves were:

CH (kg DM/ha) = 89.8 × rising plate meter (clicks) + 364.7; $r^2 = 0.82$; $P < 0.001$

PL (kg DM/ha) = 59.9 × rising plate meter (clicks) + 696.7; $r^2 = 0.73$; $P < 0.001$

PA (kg DM/ha) = 97.6 × rising plate meter (clicks) + 103.5; $r^2 = 0.85$; $P < 0.001$

Herbage samples were harvested from pre- and post-grazing breaks from ground level every three days and oven dried at 60˚C. Samples were ground for quality analysis using Near-Infra Red Spectrometry to predict contents of neutral detergent fibre (NDF), N and metabolisable energy (ME) according to the methods of Totty et al. (2013). Ingested nutrient composition was calculated as:

Ingested nutrient composition = [pre-grazing herbage mass (kg DM/ha) × pre-grazing herbage nutrient (MJ ME/kg DM or g N/kg DM or g NDF/kg DM) – post-grazing herbage mass (kg DM/ha) × post-grazing herbage nutrient (MJ ME/kg DM or g N/kg DM or g NDF/kg DM)] ÷ [pre-grazing herbage mass (kg DM/ha) – post-grazing herbage mass (kg DM/ha)]

On day 16 of the study, grazing and urination behaviour were recorded during the first six hours of feed allocation. Grazing, ruminating and idling of each heifer were recorded at 15-minute intervals by trained observers between 0900 and 1500 h. Each occurrence of urination per heifer was also recorded. Bite rate was measured for one minute from four randomly selected heifers three times (1045, 1445 and 1645 h) during the observation period, by recording of head movements and sound associated with selected pasture prehension.

One spot urine sample was obtained at 1300 h on each of days 17 and 23, and subsequently analysed for N and creatinine concentration according to the methods of Totty et al. (2013). Urinary output (UO) was estimated from a published equation (Chizzotti et al. 2008):

UO (kg/day) = [0.28 ± 0.01 – 0.000097 ± 0.000015 × LW (kg)] × LW (kg) ÷ urinary creatinine concentration (mmol/l)

In addition, a 10 ml blood sample was harvested on each of days 17 and 23, and analysed for blood urea-N (BUN) according to Totty et al. (2003). The UN was estimated from a published equation using BUN and LW (Khon et al. 2005):

UN (g/day) = 1.3 ± 0.24 × BUN (g/l) × LW (kg)

Data were analysed by ANOVA using Genstat (version 15.1; Payne et al. 2015), with forage type as treatment and individual animal as replicate. A multiple comparison test (Student-Newman-Keuls Test) was performed to differentiate the mean values among treatments when $P < 0.05$.

## Results

The DM content of CH and PL were lower than PA. Estimates of ingested NDF and ME by heifers differed among treatments (Table 1). The CH (13.9 MJ/kg DM) ingested by heifers contained higher ME than from other treatments (mean = 11.7 MJ/kg DM; SEM = 0.16 MJ/kg DM) and PA ingested by heifers had 55% higher NDF compared with CH and PL. Urinary-N concentration, estimated UN and UO per heifer were not different among treatments (Table 2). Each heifer spent an average of 243 minutes grazing during the first six hours of feed allocation, but this did not differ among treatments (Table 2). Heifers offered PA spent the least amount of time idling (Table 2). Heifers offered CH (12 minutes) and PA (71 minutes) spent the least and most amount of time ruminating ($P < 0.001$), respectively. Bite rate tended ($P = 0.076$) to be lower in CH (23 bites/minute) group than other groups (average 32 bites/minute). Heifers offered CH and PA+CH urinated almost twice as often as those on other treatments (Table 2).

**Table 1** Pre- and post-grazing herbage mass and ingested chemical composition of 100% pasture (PA), 100% chicory (CH), 100% plantain (PL), 50% pasture + 50% chicory (PA+CH), and 50% pasture + 50% plantain (PA+PL) grazed by heifers[1].

| | CH | PA+CH | PA | PA+PL | PL |
|---|---|---|---|---|---|
| Pre-grazing herbage mass (kg DM/ha) | 2005 ± 79.0 | 2259 ± 82.6 | 2464 ± 73.0 | 2405 ± 176.3 | 2270 ± 119.9 |
| Post-grazing herbage mass (kg DM/ha) | 842 ± 47.1 | 827 ± 22.6 | 1034 ± 76.1 | 1130 ± 63.5 | 1158 ± 40.6 |
| Dry matter (%) | 10.6 ± 0.08 | 19.3 ± 0.52 | 14.3 ± 0.82 | 20.1 ± 0.38 | 11.6 ± 0.40 |
| Metabolisable energy (MJ ME/kg DM) | 13.9 ± 0.33 | 11.5 ± 0.14 | 11.2 ± *0.18* | 12.0 ± *0.21* | 11.9 ± *0.54* |
| Neutral detergent fibre (g/kg DM) | 245 ± 48.3 | 290 ± 19.6 | 376 ± 17.9 | 325 ± 8.3 | 240 ± 25.5 |
| Nitrogen (g/kg DM) | 28.6 ± 1.72 | 34.1 ± 1.74 | 30.2 ± 2.03 | 28.7 ± 1.29 | 31.2 ± 3.06 |

[1] value for each treatment group: Mean ± SEM

**Table 2** Grazing and urination behaviour during the first six hours of fresh herbage allocation, and spot sample measured urinary nitrogen concentration, estimated urinary nitrogen (N) excretion and urinary output of heifers grazed on 100% pasture (PA), 100% chicory (CH), 100% plantain (PL), 50% pasture + 50% chicory (PA+CH), and 50% pasture + 50% plantain (PA+PL).

| | CH | PA +CH | PA | PA +PL | PL | LSD | P value |
|---|---|---|---|---|---|---|---|
| Grazing (mins/6 hrs) | 255 | 249 | 226 | 240 | 245 | 24.5 | 0.22 |
| Idling (mins/6 hrs) | $93^{a}$ | $89^{a}$ | $63^{b}$ | $90^{a}$ | $90^{a}$ | 20.8 | 0.032 |
| Ruminating (mins/6 hrs) | $12^{c}$ | $23^{bc}$ | $71^{a}$ | $30^{b}$ | $25^{bc}$ | 16.2 | <0.001 |
| Bite rate (times/minute) | 23 | 28 | 34 | 34 | 33 | 9.46 | 0.076 |
| Urination (times/6 hrs) | $4.5^{b}$ | $6.2^{a}$ | $3.1^{c}$ | $2.8^{c}$ | $2.9^{c}$ | 1.18 | <0.001 |
| Urinary N excretion (g/day)[1] | 67.0 | 74.2 | 69.8 | 72.4 | 79.9 | 14.72 | 0.423 |
| Urinary output (kg/day)[2] | 59.3 | 66.4 | 65.3 | 61.1 | 51.2 | 19.11 | 0.499 |
| Urinary N concentration (%) | 0.19 | 0.28 | 0.21 | 0.24 | 0.28 | 0.107 | 0.351 |

[1] Estimated from plasma urea nitrogen and live weight (Khon et al. 2005). [2] Estimated by creatinine and live weight (Chizzotti et al. 2008). [a-c] Means within the same row with different superscripts differ ($P < 0.05$)

## Discussion

A feature of the results was that heifers from CH and PA+CH treatments urinated more frequently than heifers from other treatments. The reason for this is unclear, but it may be related to a diuretic effect on urination behavior when animal is offered with feed containing high level of minerals. The higher mineral content in the feed increases urination frequency and excretion (Ledgard et al. 2015). Chicory is known to have a higher content of potassium, calcium, sodium, zinc, and molybdenum than does grass (Crush & Evans, 1990; Rumball et al. 1997). A further explanation for urination differences is variation in water intake. Calculated water intake from forage based on forage DM%, and pre- and post-grazing herbage mass measurements gave values of 55, 26, 37, 22, 42 litres for CH, PA+CH, PA, PA+PL, and PL. These values do not align with urination frequency, with highest was from PA+CH, which had a relatively low water intake from forage. However, this analysis does not take account of water consumed from trough, which may compensate for the low water intake from the forage.

No difference in estimated UO, UN and also urinary-N concentration measured independently from spot sample. With higher urination frequency in CH and PA+CH, it appears that UO and UN per urination event were lower in CH and PA+CH than in other treatments. This represents an opportunity to increase the spread of urine patches and N loading on soil and contribute to the reduction of nitrate leaching (Williams and Haynes, 1994). However, caution is needed to interpret the estimated UN and UO in this study, as diurnal variation in UO and UN were observed by Betteridge et al. (2013).

The lower NDF content of chicory and plantain compared to pasture in this study is similar to the report of Gregorini et al. (2013). Lower NDF content may be one of the variables contributing to greater time ruminating on PA than in other treatments in this study. This is because of Gregorini et al. (2013) suggested that cows grazing on chicory and plantain had higher mastication activity compared to cows grazing on pasture, which may reduce particle size of the feed, and rumination time. This may be further explained by greater leaf mass in CH than PA (McCoy et al. 1997), which is partly supported by the lower bite rate from heifers grazed on CH than those on PA in this study. Previous research (Laca et al. 1992; Bryant et al. 2012) shows a bigger bite size requires more mouth handling and mastication before swallowing, and is associated with a slower bite rate.

In conclusion, data from this study indicate that heifers fed on CH and PA+CH urinated more often without increasing UO and UN. Therefore, chicory may potentially reduce N loading and subsequent nitrate leaching from soil through smaller urine patches and more frequent urination events.

## Acknowledgements

We acknowledge the support of the AGMARDT Postdoctoral Fellowship to author Dr Long Cheng, and Pibulsongkram Rajabhat University visiting research scholarship for Associate Professors Yingluck Moonsan and Preecha Moonsan. Thanks also go to Helen Hague, David Jack, Daniel Dash, Adam Caldwell, Jeffrey Curtis, Sarah Taylor and Alan Marshall from Lincoln University for pasture preparation, animal management and sampling. Dr Racheal Bryant (Lincoln University) and Dr Pablo Gregorini (Dairy NZ) are appreciated for providing useful discussion.

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