Urinary nitrogen excretion from cows at different stage of lactation grazing different ryegrass cultivars during spring or autumn

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ABSTRACT

The effects of season (spring or autumn) and stage of lactation (early or late) on the partitioning of dietary nitrogen (N) between milk and urine were measured in experiments in which cows grazed forages with putative differences in the ratio of water soluble carbohydrates (WSC) to crude protein (CP). Sixty cows (n = 30 for each stage of lactation) grazed one of three forages during spring and autumn. Forage treatments consisted of a 'standard' perennial ryegrass (STG) and two ryegrasses with high WSC concentrations: a perennial 'high sugar' ryegrass (HSG), and an annual ryegrass (IRG). In spring, grass type had a significant effect on the estimated urinary N excretion. Both the HSG and STG resulted in greater N excretion compared to the IRG, due to the low CP concentration in this forage in one of the grazing periods. In autumn, cows grazing STG excreted less urinary N than both HSG and IRG. In both seasons, cows in early lactation secreted more N into milk but were not different in N excretion. In early lactation cows, the extra N in milk was only a small proportion of the potential N intake arising from higher CP content in autumn grasses.

Keywords: nitrogen utilisation; dairy cows; pasture; high sugar grasses.

INTRODUCTION

'High sugar' grasses have been proposed as means for improving the efficiency of utilisation of protein in grazing systems (Edwards *et al*., 2007; Moorby *et al.*, 2006; Tas *et al*., 2006) because of their potential to express a greater water soluble carbohydrate (WSC) to crude protein (CP) ratio (Edwards, *et al*., 2007).

In New Zealand, the role of high sugar grasses has been investigated in a series of experiments involving trials in spring and autumn (Cosgrove *et al*., 2007a; Pacheco *et al*., 2007a; Tavendale *et al*., 2006) using spring-calving cows. In these trials, changes in productivity (Cosgrove *et al*., 2007a) or efficiency of nitrogen (N) utilisation have been documented (Pacheco *et al*., 2007a) even though the responses have not always been directly associated with increase in WSC concentration (Cosgrove *et al*., 2007a). However, the confounding effects of stage of lactation and season means that results reported are a combination of the effects of seasonal changes in pasture composition and the physiological changes in the cow as lactation advances.

The objective of the experiments described in this manuscript was to distinguish between the effects of season of the year affecting the chemical composition of the forage eaten, and the stage of lactation by using cows from two different calving groups. In this way, we expect to determine if stage of lactation or seasonal characteristics of the forage are responsible for the different responses in productivity and efficiency previously reported. Ultimately, this knowledge is important for designing management strategies to improve the utilisation of N in pastoral dairying. This manuscript describes the measurements in N partitioning between milk and urine, conducted in the season 2007-08.

MATERIALS AND METHODS

The experiment was carried out with the approval of the Grasslands Animal Ethics Committee in Palmerston North.

Sixty mixed-age, Friesian cows from spring- or autumn-calving herds with 30 in each calving group were used for two grazing trials at Massey University's Dairy Farm No. 4, situated on the outskirts of Palmerston North (40°19'S, 175°37'E) during October to December 2007 (Spring) and May to June 2008 (Autumn). For each season a balanced cross-over design was used in which groups of 20 cows comprising 10 spring-calving cows and 10 autumn-calving cows were allocated to each of three grasses with potentially contrasting concentrations of water soluble carbohydrates (WSC) and crude protein (CP). The grasses were two diploid cultivars of perennial ryegrass (*Lolium perenne*, cvs. AberDart, a UK 'high sugar grass': (HSG) and Impact, a New Zealand control (STG), and one tetraploid cultivar of annual ryegrass *Lolium multiflorum* (cv. Grasslands Moata (IRG). Treatments were balanced in terms of days in milk, live weight, condition score and parity. A description of the animals at the beginning of each season is presented in Table 1.

TABLE 1: Description of experimental animals at the beginning of the grazing trials in spring 2007 and autumn 2008.

Season	Stage of lactation[1]	Days in milk	Parity	Body condition score	Live weight (kg)
Spring	Early (Spring calving)	57 ± 12	4 ± 1	4.1 ± 0.3	501 ± 48
	Late (Autumn calving)	216 ± 7	3 ± 1	4.6 ± 0.2	511 ± 41
Autumn	Early (Autumn calving)	37 ± 9	4 ± 1	3.7 ± 0.6	478 ± 39
	Late (Spring calving)	246 ± 15	4 ± 1	3.9 ± 0.6	500 ± 49

[1]For the spring calving cows, the two seasons were part of the same lactation. For the autumn calving cows, the two seasons were part of two consecutive lactations.

TABLE 2: Mean ± standard deviation of the forage composition variables for the 'standard' ryegrass Impact (STG), the "high sugar" perennial ryegrass AberDart (HSG), and the annual ryegrass Moata (IRG) for the grazing trials in spring 2007 and autumn 2008. CP = Crude protein, WSC = Water soluble carbohydrate, NDF = Neutral detergent fibre.

Grass type	Forage composition variables(g/kg DM)				
	CP	WSC)	Lipid	Ash	NDF
Spring trial 2007					
STG	184 ± 35	194 ± 33	29 ± 3	97 ± 9	451 ± 19
HSG	190 ± 20	216 ± 25	29 ± 2	93 ± 5	433 ± 26
IRG	178 ± 38	217 ± 31	30 ± 5	93 ± 11	437 ± 36
Autumn trial 2008					
STG	265 ± 10	144 ± 26	45 ± 3	118 ± 5	377 ± 33
HSG	272 ± 11	160 ± 27	46 ± 2	117 ± 4	338 ± 18
IRG	280 ± 14	139 ± 36	46 ± 2	122 ± 4	356 ± 31

Each of the two grazing trials used a balanced cross-over design with the three grasses grazed in a pre-determined sequence during three consecutive 14-day periods. In each period, each treatment group (grass type x stage of lactation where n = 10) was further split into two subgroups (n = 5 each), and each subgroup assigned to one of two mirror-image cross-over sequences. This was necessary to balance the 3 x 3 cross-over design so that in the grass allocation sequence each group followed each other an equal number of times. Each group of cows was offered an equal allowance of grass twice daily, comprised of 40% of the total daily allocation following the morning milking and 60% following the afternoon milking. The pre-grazing pasture mass (kg DM/ha) was estimated using a rising plate meter, and after subtracting the desired residual (1,600 kg DM/ha) the area required was calculated such that each cow could consume 18 kg DM/d. More details of the grass treatments and method of allocation have been published elsewhere (Cosgrove *et al*., 2007a; Tavendale *et al*., 2006).

Within each experimental period, matching grass, milk and urine samples were collected for each group of animals. Grass samples were collected and their composition analysed by near infrared reflectance spectroscopy (FeedTECH™, Palmerston North, New Zealand). Forage samples were collected in the morning at 08:00 h and at 16:00 h. During each 14-day period, milk yields were recorded daily and samples were taken for milk composition analysis on four occasions (two times weekly), each sampling including two consecutive milkings (evening and morning). Milk protein, fat and lactose concentrations were determined using a Foss Fourier Transform infrared analyser (TestLink, Hamilton, New Zealand). Daily yields of milk protein were calculated from measured milk yield and protein concentration in milk. Samples of milk were proportionally pooled within each group of five cows, stored frozen and subsequently skimmed and analysed for milk urea as described by Pacheco *et al*. (2007b). Milk urea N (MUN) concentration was calculated on a molar basis by multiplying the molar concentrations of milk urea by two. In the second week of each 14-day period, and coinciding with the milk sampling days, four spot samples (an evening and a morning pair on two occasions) of urine were collected for determination of urinary N excretion using the nitrogen and creatinine concentration in the urine and the live weight (LW) of the cows at the beginning of each period. The urinary N excretion (g/d) was estimated as (21.9 (mg/kg) × LW (kg) × (1/urinary creatinine (mg/kg))) × urine N (g/kg), using the methods described by Pacheco *et al.* (2007b). Dry matter intake was estimated using the

TABLE 3: Estimated intakes of nitrogen (N), its partition between urine and milk and the milk urea N concentrations in milk for the 'standard' ryegrass Impact (STG), the "high sugar" perennial ryegrass AberDart (HSG), and the annual ryegrass Moata (IRG) for the grazing trials in spring 2007 and autumn 2008. SED = Standard error of difference. N = Nitrogen.

Effect	Spring 2007		Autumn 2008[1]		Autumn 2008[2]	
	Mean	SED	Mean	SED	Mean	SED
Nitrogen intake (g/d)						
STG	408[a]	6	522[a]	9	529[a]	6
HSG	409[a]		545[b]		548[b]	
IRG	374[b]		550[b]		---	
Significance	**		**		**	
Early lactation	431[a]	10	593[a]	14	586[a]	13
Late lactation	363[b]		486[b]		491[b]	
Significance	**		**		**	
Urinary N excretion (g/d)						
STG	215[a]	4	276[a]	18	291[a]	11
HSG	222[a]		336[b]		332[b]	
IRG	147[b]		343[b]		---	
Significance	**		**		**	
Early lactation	204	27	315	16	312	15
Late lactation	185		322		311	
Significance	NS		NS		NS	
Milk N secretion (g/d)						
STG	109[ab]	1	101	3	106	2
HSG	110[b]		107		112	
IRG	106[a]		107		---	
Significance	*		NS		**	
Early lactation	122[a]	4	120[a]	4	123[a]	4
Late lactation	95[b]		90[b]		95[b]	
Significance	**		**		**	
Milk urea nitrogen (mmol/L)						
STG	9.0[a]	0.4	16.1[a]	0.5	16.4	0.4
HSG	9.0[a]		17.9[b]		17.5	
IRG	5.5[b]		17.4[b]		---	
Significance	**		**		NS	
Early lactation	8.1	0.3	16.7[a]	0.4	16.4[a]	0.4
Late lactation	7.6		17.6[b]		17.5[b]	
Significance	NS		*		*	

Different letters within a column indicate values for each variable that are significantly different between effect in each season.
[1]Includes data from Periods 1 and 3 only. [2]Includes data from all three periods, excluding IRG data.

sum of the metabolisable energy (ME) required for maintenance and measured milk production, divided by the ME content of the forage eaten, as described by Pacheco *et al*. (2007a).

Statistical analysis of the milk protein, milk urea and urinary nitrogen excretion data was conducted using the MIXED procedure in SAS (2002) with period, grass type, stage of lactation and their interactions as fixed effects, cow as a random effect and a simple variance covariance structure. Each grazing trial; spring 2007 and autumn 2008, was analysed separately.

RESULTS

Each grass contained less CP and more WSC per unit of dry matter in spring compared to autumn (Table 2). For HSG and STG, the decline was approximately 2.5 percent units, from approximately 210 to 185 g CP/kg DM. By comparison the IRG exhibited a more pronounced decline in CP of 7 percent units; from 210 to 140 g CP/kg DM. Lipid and, to a lesser extent, ash were reduced in similar proportion to CP, whilst NDF and WSC remained relatively constant across periods.

These changes were associated with the dry conditions experienced in the Manawatu region during the spring in 2007.

Estimated N intakes were different for each grass type (P <0.01), with the IRG having the lesser estimated intakes, and consequently less urinary N excretion. The decline observed in CP concentration across the grazing periods was paralleled by significant reductions in the amount of urinary N excreted, concentration of milk urea N and yield of milk N with a significant period effect (P <0.01). The IRG had smaller yields of milk N (106 versus 110 and 109 g/d for HSG and STG, respectively, P = 0.01). The markedly smaller estimate of urinary N excretion for IRG during spring is the result of the value estimated during Period 3 (~ 90 g urine N, compared with >170 g for the other two grasses in the same period, when the CP for IRG was only 140 g per kg DM. Together with the reduction in urinary N excretion, the yield of milk N was smaller for IRG (89 g/d milk N) than for the other two grasses (~102 g/d milk N) in the same period. Significant differences between stage of lactation were observed for estimated nitrogen intake (P <0.01) and secretion of N into milk (P <0.01).

The results obtained from the analysis of spot-samples of urine, were supported by the analysis of MUN in milk from pooled samples (Table 2). The small MUN value for IRG in Period 3 of 3.6 versus 7.6 and 8.3 mmol/L for STG and HSG, respectively, is in agreement with the lower concentration of CP in the grass and the low estimates of urinary N excretion.

In autumn, IRG did not grow enough in the second period to sustain grazing. The cows were removed from this treatment and it was left to recover whilst the other two grasses were grazed. The IRG recovered after the rest and the third period in autumn was conducted with the three grasses. Thus, data for this experiment was analysed as two sets; one set included data for all three periods for HSG and STG, and the other set included data for all grasses in Periods 1 and 3, to allow for estimation of IRG effects (Table 3).

Similar to the spring trial, there was a significant period effect on estimated N intake, however in contrast to spring there was no decline in CP of the sampled forages as the trial progressed during autumn. Grass type had a significant effect on urinary N excretion, with STG resulting in less excretion of urinary N (P <0.01). Milk N secretion was significantly greater for HSG, but only when data from the three periods were included in the analysis. When data from Period 2 was removed to allow comparisons including IRG, there was still a numerical difference between HSG and STG, but it failed to attain statistical significance (P = 0.11). In similar fashion to the spring trial, cows in early lactation in autumn had greater estimates (P <0.01) of N intake and milk N secreted. In spite of the greater N intake estimated for the early lactation cows, the milk urea N concentrations (P <0.05) were greater for the cows in late lactation.

DISCUSSION

Grasses in late spring had less CP than in autumn, which is a normal pattern documented for dairy pastures (Litherland & Lambert, 2007). During the trial in spring 2007 all three grasses were in the low range of the 18 – 24% CP recommended as necessary for forage-fed lactating cows of 18% to 24% CP (Kolver, 2000). In spring, the significant effect of grass on milk N secretion is most likely driven by the reduced yield from IRG in Period 3. The smaller yield of milk N for the IRG suggests that the CP supply from this grass was insufficient to sustain milk production. The similar yields of milk N measured in spring and autumn, when animals of comparable stage of lactation consumed forage with approximately 27% CP, suggests that CP in the range of 17.5 to 21% was still adequate for the HSG and STG groups in spring. In autumn, the greater milk N secretion and urinary N excretion from HSG compared to STG was consistent with the estimated greater N intake from HSG compared to STG. This highlights that greater productivity does not translate to reductions in urinary N excretion if the main driver for more production is an increase in dry matter intake of a protein-rich forage, as HSG was in autumn. Increases in WSC may occur at the expense of CP or NDF in the forage (Vibart *et al*., 2009). Increases in WSC that are accompanied by a reduction in NDF, as seen in earlier studies (Cosgrove *et al.*, 2007a), can result in higher dry matter intakes due to reduced physical filling of the rumen (Moorby *et al.,* 2006). This higher intake, combined with high protein concentration, may erode the potential environmental benefits of the extra WSC.

It is not possible with the current analysis to identify if the amount of milk N differed significantly between spring and autumn, but it is evident that a 1.5 fold increase in the estimated N intake in autumn did not translate into more milk N. Although the actual amounts of N secreted into milk appear similar between the two seasons, the efficiency of transfer of N from the forage into milk was greater in spring at about 30%, than in autumn at about 20%, regardless of grass type or stage of lactation. Thus, the greater efficiencies reported in our previous trials comparing seasons (Pacheco *et al*., 2007a) appear to be more related to the seasonal characteristics of the forages, rather than differences inherent to the stage of lactation of the cows.

The use of creatinine concentrations to estimate urinary output resulted in estimates that are in

agreement with theoretical values expected for forage diets (Pacheco & Waghorn, 2008). Creatinine concentrations in urine spot samples have been used elsewhere to successfully detect differences in urinary N excretion from dairy cows fed different amounts of protein and carbohydrate (Gressley & Armentano, 2007). Estimation of urinary output using creatinine concentration in urine will depend on the constant assumed to represent the rate of excretion of creatinine per unit of live weight. In order to use an estimate relevant for the New Zealand conditions, we have assumed a constant excretion of 21.9 mg creatinine per kg of live weight per day, calibrated from an indoor experiment in which total urine collection was performed from 15 lactating, pasture fed dairy cows (D. Pacheco, Unpublished data). The validity of the estimates of urinary N excretion is supported in our experiments by measurements of the concentrations of urea N in pooled milk samples. In both seasons, milk urea concentrations reflected the estimates of urinary N excretion, with both the urinary N excretion and the milk urea concentration changing by similar order of magnitude between grasses and stages of lactation.

The amount of N retained in lactating cows is small relatively to N intake, and the majority of absorbed N is partitioned between milk and urine. (Pacheco & Waghorn, 2008). Thus, increasing the amount of N partitioned to be able to capture more than 100 g/d of N into milk and tissue, would appear to be the most effective way to minimise urinary N excretion. In the present work, more N was captured in milk of cows in early lactation regardless of the season of the year. However, the extra 26 to 29 g/d of N secreted in milk of cows in early lactation, compared to those in late lactation, is minuscule compared to the increase in intake of 127 to 187 g/d of N that occurs in autumn as a result of the greater CP concentrations in the grass. Thus, we conclude that unless the amount of N incorporated into milk can be increased beyond what can currently be achieved, the most effective way to minimise urinary N excretion is through a reduction in N intake.

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