Nitrogen partitioning in sheep offered three perennial ryegrass cultivars at two allowances in spring and autumn

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Abstract

The objective of this study was to determine the nitrogen (N) partitioning in sheep fed freshly cut diploid high-sugar (water-soluble carbohydrate, WSC) ryegrass (HSG), tetraploid ryegrass (TRG) and conventional diploid ryegrass (CRG) offered at 1.1 and 1.8 times maintenance energy requirements (MEm) in spring and in early autumn. Overall, N intake was higher for sheep fed HSG compared with sheep fed CRG (P=0.03), with sheep fed TRG being intermediate. Faecal-N output was higher for HSG than for TRG and CRG (P<0.001), urinary-N output was higher for TRG than for HSG, which were both higher than CRG (P<0.001) and N-retention in the body was higher for CRG compared with TRG (P<0.001), with HSG being intermediate. Relative to N intake, urinary-N output was higher (P<0.001) and faecal-N output (P<0.001) was lower in TRG compared to CRG and HSG with bigger differences among cultivars in spring than in autumn. Faecal-N output, urinary-N output and N-retention were higher at 1.8 × MEm than at 1.1 × MEm (P<0.001) and higher in autumn than in spring. Feeding CRG and HSG resulted in reduced urinary-N excretion relative to N intake compared with TRG in both spring and autumn and forage WSC intake was one of the main explanatory variables for differences in urinary-N as proportion of N intake.

Keywords: high-sugar ryegrass; tetraploid; cultivar; nitrogen balance; sheep

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the most common grass species sown in New Zealand (NZ). However, its high nitrogen (N) content in intensive farming systems, especially in spring and autumn, results in a low N use efficiency (NUE; i.e., animal product N/N intake) in grazing animals and consequently high N excretion into the environment (Kolver & Muller 1998). Grass with more readily available water-soluble carbohydrates (WSC) relative to slowly fermentable neutral detergent fibre (NDF) or crude protein (CP) might improve the N-to-energy balance in the rumen, improve NUE and reduce N excretion (Edwards et al. 2007). Perennial ryegrass cultivars differ in nutritional qualities in terms of CP, NDF and WSC, and thus, cultivar choice may improve NUE and reduce N excretion from ruminants. Perennial ryegrass with a higher WSC has been bred by plant breeders (Humphreys 1989). The difference in WSC between these cultivars and conventional ryegrass cultivars (CRG) in NZ is normally higher in spring than in autumn and the increased WSC can substitute either CP or NDF or both (Cosgrove et al. 2009; Cosgrove et al. 2014). Tetraploid perennial ryegrasses (TRG) have an extra chromosome pair, compared with diploid ryegrasses, and as a result have larger cells (Smith et al. 2001), and often have lower NDF and higher WSC concentrations than CRG (Cosgrove et al. 2007; Hume et al. 2010). Therefore, both high WSC ryegrass (HSG) and TRG might improve NUE and reduce N excretion compared with CRG. The objective of this study was to determine the N-balance in sheep fed freshly cut HSG, TRG, and CRG offered at two feeding levels in spring and in early autumn.

Materials and methods

Animals and feeding

The N-partitioning data presented here are part of a research programme to determine both N and methane emissions from sheep fed different ryegrass cultivars (Jonker et al. 2014). Two trials were carried out at Grasslands Research Centre (AgResearch Ltd., Palmerston North, NZ; 40.36° S, 175.61° E) in spring (23 September-1 October, 2013) and early autumn (24 March-10 April, 2014) using 48 Romney wethers in each season. The wethers for the spring trial were born during October 2012 and for the autumn trial during August 2013. The animal experiments reported here were reviewed and approved by the Grasslands Animal Ethics Committee (Palmerston North, NZ; approval #13004) and animals were cared for according to the Code of Ethical Conduct (1999). The sheep were stratified by weight and randomly allocated to graze a single plot of either Alto (CRG), AberMagic (HSG) or Base (TRG) perennial ryegrass (each with AR1 endophyte), sown as monocultures. The sheep grazed their respective cultivar *ad libitum* [~6 cm surface swart height according Frame (1993)] for at least 14 d (diet acclimatisation) before being moved indoors and fed cut forage of the same cultivar. Sheep within cultivar were grouped by weight and randomly sub-divided into two group pens of eight sheep per cultivar. Each cultivar was fed at either 1.1 or 1.8 × MEm according to CSIRO (2007) resulting in six dietary treatments. Vegetative ryegrass accumulated to approximately 3000-3500 kg DM/ha (rising plate meter, Farmworks Systems Ltd., Fielding, NZ) was cut daily (around noon) at around 7 cm above ground level. 65% of the total daily feed allowance was fed at around

15:30 h and the remaining 35% stored refrigerated at 4°C until feeding the following morning around 8:30 h. Forage subsamples were collected daily between 13:00 and 14:00 h in triplicate and dried at 105°C for 48 h to determine the dry matter (DM) content. During the N-balance period, another daily sample was taken, freeze-dried, ground through a 1-mm screen, pooled per cultivar and scanned by FeedTECH (AgResearch Ltd., Palmerston North, NZ) using near infrared reflectance spectroscopy (NIRS; model MPA; Bruker Optics, Ettlingen, Germany) with calibration curves for ash, lipids, NDF, ADF, ADL and WSC (Corson et al. 1999). The N concentration in feed was analysed using a Variomax CN Analyser (Elementar Analysensysteme GmbH, Hanau, Germany) at Lincoln University (Lincoln, NZ) to calculate N intake (IN).

Nitrogen balance measurements

Five sheep per treatment with similar live weight (33.5±1.73 kg in spring; 35.5±1.53 kg in autumn) were moved into metabolism crates three days before the N-balance measurements and harnesses fitted for attachment of faecal collection bags. The metabolism crates had a mesh floor with a funnel tray underneath for urine collection into a plastic bucket. Daily, 100 mL of 6 M sulphuric acid was added to the bucket to minimize urinary ammonia volatilization. Refusals, faeces and urine were collected in the afternoon for five consecutive days before feeding grass from a new pasture cut. Refusals were dried at 65°C for 48 h to determine DM content. Faeces and urine were weighed daily and aliquots (10% for faeces and 1% for urine) per sheep pooled and stored in the freezer at -20°C and the rest discarded. Faeces were freeze-dried after the trial and ground through a 1-mm screen. The N concentration of faeces (FN) and urine (UN) were determined as described above for feed. Body retained-N (RN) was calculated in g/d as: IN – FN – UN. One sheep fed HSG at 1.1×MEm in spring was removed from the trial during the collection period because DMI decreased drastically and the sheep had diarrhoea.

Statistical analysis

Animal data were analysed separately for the two seasons with cultivar, intake level and their interaction as fixed effects and for the overall data set, with cultivar, intake level, season and all interactions as fixed effects. Individual sheep were the experimental unit (n=5). The analysis was performed in GenStat (16th edition; VSN international, Hemel Hempstead, UK) using general ANOVA and the Tukey statement at $P<0.05$ was used for multi-treatment comparison. Linear regression analysis with separate regression lines for each cultivar was performed for urinary-N, UN/IN and UN/FN with dietary and intake parameters in GenStat. Probability values ($P<0.05$) for intercept and slope between the cultivars were used to determine if the final model required separate or parallel regression lines for cultivar according to the methods of Payne et al. (2009). There was no statistical replication for pasture nutritional composition because laboratory analyses were performed on samples within cultivar pooled across N-balance days. Therefore, comparisons of nutritional composition among cultivars and between seasons in this paper are descriptive only.

Results

Grass quality

The concentration of WSC was more than 50 g/kg DM higher in HSG than in CRG and TRG in spring, and similar among the three cultivars in autumn; the forage WSC concentrations were higher in spring than in autumn (Table 1). HSG had a lower NDF concentration in both spring and autumn than CRG and TRG, while CP concentration was variable among the three cultivars in the two seasons. The concentrations of ash, CP, NDF, ADF and ADL were higher in autumn than in spring.

Table 1 Nutritional composition of conventional diploid ryegrass (CRG), high-sugar diploid ryegrass (HSG) and tetraploid ryegrass (TRG) fed during the nitrogen balance period (5-d pool) in spring and in autumn. CP, crude protein; WSC, water-soluble carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

		DM (g/kg)	Ash	Lipid	CP	WSC (g/kg DM)	NDF	ADF	ADL	WSC/CP	WSC/NDF ratios
Spring	CRG	144	87	27	152	253	467	244	25	1.7	0.5
	HSG	166	80	28	140	304	418	227	24	2.2	0.7
	TRG	189	96	26	160	229	458	246	21	1.4	0.5
Autumn	CRG	177	120	27	207	133	631	298	33	0.6	0.2
	HSG	183	122	22	223	135	580	300	36	0.6	0.2
	TRG	160	128	25	238	128	611	290	25	0.5	0.2

Nitrogen intake, excretion and balance

Overall, N intake was higher for sheep fed HSG compared with sheep fed CRG ($P=0.02$), with sheep fed TRG being similar to HSG and CRG fed sheep. N intake was higher in autumn than in spring ($P<0.001$) (Table 2). The differences between cultivars in N intake were mainly apparent in autumn when dry matter intake (DMI) and grass N concentrations were higher in HSG fed sheep compared with CRG fed sheep. Faecal-N output was higher for HSG than for TRG in both seasons ($P<0.05$) and higher overall compared with CRG ($P<0.001$). Overall, urinary-N output was higher for TRG than for HSG, and both were higher

Table 2 Dry matter (DM) and nitrogen (N) intake and N partitioning in sheep fed one of three ryegrass cultivars (CRG, conventional diploid ryegrass; HSG, high-sugar diploid ryegrass; TRG, tetraploid ryegrass) offered at 1.1× or 1.8× metabolisable energy requirements for maintenance (MEm), in spring and autumn, and the data combined overs seasons. SED, standard error of the difference.

		DM intake	Intake N (IN)	Faecal N (FN)	Urine N (UN)	Retained N (RN)	N balance			
Season	Parameter	(kg/d)	(g/d)				FN/IN	UN/IN	RN/IN	UN/FN
Spring	CRG	0.82[a]	19.9	5.9[b]	9.2[a]	4.8[b]	29.8[b]	46.6[a]	23.6[b]	1.58[a]
	HSG	0.91[b]	20.4	6.3[b]	10.3[a]	3.8[ab]	30.8[b]	51.1[a]	18.1[ab]	1.67[a]
	TRG	0.76[a]	19.5	5.3[a]	11.6[b]	2.6[a]	27.2[a]	61.5[b]	11.4[a]	2.27[b]
	SED	0.029	0.66	0.21	0.49	0.65	1.00	2.58	2.70	0.111
	1.1×MEm	0.69[a]	16.6[a]	4.8[a]	9.5[a]	2.3[a]	28.9	57.8[b]	13.3[a]	2.03[b]
	1.8×MEm	0.97[b]	23.3[b]	6.9[b]	11.3[b]	5.2[b]	29.6	48.4[a]	22.0[b]	1.65[a]
	SED	0.023	0.54	0.17	0.40	0.53	0.78	2.11	2.20	0.090
Autumn	CRG	0.78	25.8[a]	8.1[ab]	17.6[a]	0.1	31.5[b]	68.7[a]	-0.2	2.21[a]
	HSG	0.82	29.1[b]	8.8[b]	19.8[b]	0.6	30.4[ab]	68.8[a]	0.8	2.27[a]
	TRG	0.74	28.3[ab]	7.8[a]	20.8[b]	-0.3	27.6[a]	74.5[b]	-2.1	2.71[b]
	SED	0.033	1.12	0.33	0.78	0.76	1.23	2.27	2.81	0.102
	1.1×MEm	0.67[a]	23.7[a]	7.0[a]	17.7[a]	-1.0[a]	29.4	74.8[b]	-4.2[a]	2.57[b]
	1.8×MEm	0.89[b]	31.8[b]	9.5[b]	21.1[b]	1.2[b]	30.3	66.5[a]	3.2[b]	2.23[a]
	SED	0.027	0.92	0.27	0.64	0.62	1.01	2.27	2.30	0.083
Overall	CRG	0.80[a]	22.9[a]	7.0[a]	13.4[a]	2.5[b]	30.7[b]	57.6[a]	11.7[b]	1.90[a]
	HSG	0.86[a]	24.8[b]	7.5[b]	15.1[b]	2.2[ab]	30.6[b]	60.0[a]	9.4[b]	1.97[a]
	TRG	0.75[b]	23.9[ab]	6.6[a]	16.2[c]	1.1[a]	27.4[a]	68.0[b]	4.6[a]	2.49[b]
	SED	0.022	0.65	0.20	0.46	0.50	0.78	1.72	1.95	0.075
	1.1×MEm	0.68[b]	20.1[a]	5.9[a]	13.6[a]	0.7[a]	29.2	66.3[b]	4.6[a]	2.30[b]
	1.8×MEm	0.93[a]	27.6[b]	8.2[b]	16.2[b]	3.2[b]	29.9	57.4[a]	12.6[b]	1.94[a]
	SED	0.018	0.53	0.16	0.38	0.41	0.64	1.40	1.59	0.061
	Spring	0.83[a]	20.0[a]	5.9[a]	10.4[a]	3.7[b]	29.3	53.1[a]	17.7[b]	1.84[a]
	Autumn	0.78[b]	27.7[b]	8.2[b]	19.4[b]	0.1[a]	29.8	70.7[b]	-0.5[a]	2.40[b]
	SED	0.018	0.53	0.16	0.38	0.41	0.64	1.40	1.59	0.061

[abc]Means in a column with different superscripts are significantly different (P <0.05).

[1]Urine volume and concentration were not adjusted for the 100 mL sulphuric acid added.

than CRG (P<0.001). Faecal-N and urinary-N excretions were higher at 1.8× than 1.1× MEm (P<0.05) and higher in autumn than the spring (P<0.05). Overall, retained-N (RN) in the body was higher for CRG than TRG (P<0.001), with HSG being intermediate, and higher at 1.8× than 1.1× MEm (P<0.001), and in spring than in autumn (P<0.001). However, liveweight change over the N-balance period was negative in both seasons for all cultivars at both intake levels and the ranking of liveweight change was different to RN ranking (data not shown). N-retention is a calculated pool which includes true RN or mobilization as well as sampling and analytical errors.

Overall, as a proportion of N intake, FN was lower (P<0.001) and UN higher (P<0.001) for TRG than HSG and CRG, resulting in a higher UN/FN ratio (P<0.001) for TRG than HSG and CRG (Table 2). FN/IN was similar for both intake levels and both seasons, while UN/IN and UN/FN ratio were higher for 1.1×MEm than 1.8×MEm, and higher in autumn than spring (P<0.001).

Total WSC intake explained 82% of variation in UN/IN and the intercept and slope were significantly lower for HSG compared with CRG and TRG (Fig. 1). Nitrogen and NDF intake did not relate to UN/IN (adj-R^2<2%). The WSC/CP and WSC/NDF ratios alone explained 56% and 53% of variation in UN/IN, respectively, with a descending slope. Similar trends were found for WSC intake, WSC/CP and WSC/NDF with UN/FN.

Discussion

Perennial ryegrass in NZ pastoral systems often contains CP concentrations above animal requirements and with high rumen degradability (Sun et al. 2010), which causes an oversupply of N relative to energy for microbes in the rumen (Tamminga et al. 1994). The excess CP in the rumen is deaminated releasing carbon skeletons, which can be utilized for energy by microbes, and ammonia-N, which is largely absorbed across the rumen wall and a large part excreted into urine, especially when low energy

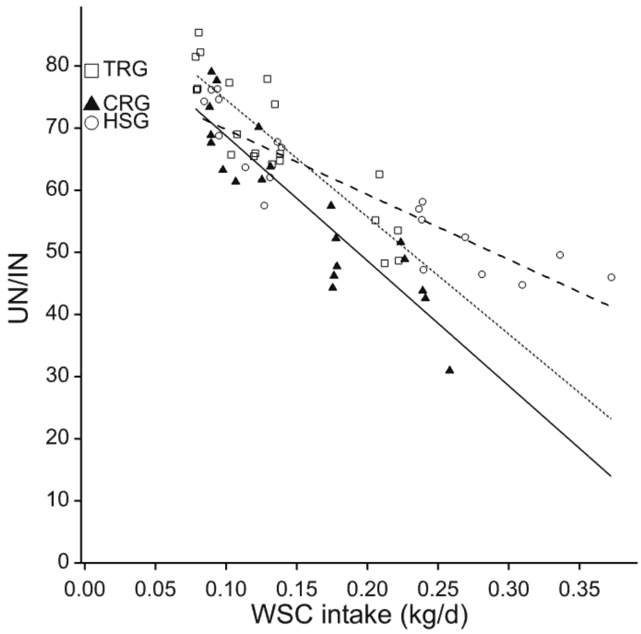

Figure 1 Linear regression of urinary nitrogen/nitrogen intake (UN/IN) on water-soluble carbohydrate (WSC) intake for conventional diploid ryegrass (CRG; solid line), high-sugar diploid ryegrass (HSG; intermitted line) and tetraploid ryegrass (TRG; dotted line) (adj-R^2=82%; SE 5.2); CRG vs. HSG, Constant P=0.051, Slope P<0.01; CRG vs. TRG, Constant P=0.341, Slope P=0.684.

supply in the rumen limits microbial protein synthesis (Van Duinkerken et al. 2005). Reducing the total CP concentration or improving energy availability in the rumen through increased WSC at the cost of CP or NDF in ryegrass might improve NUE and reduce N excretion (Edwards et al. 2007; Keim & Anrique 2011). The main objective of this experiment was to determine if ryegrass WSC, NDF and CP composition could affect N balance in sheep.

Overall, HSG had a higher WSC and lower NDF than CRG and TRG, which was consistent with previous studies in NZ for HSG and CRG (Cosgrove et al. 2007; Pacheco et al. 2009; Cosgrove et al. 2014). However, in previous studies tetraploid ryegrass (Italian and perennial) had a similar WSC concentration to HSG and higher than CRG, while NDF was lower in TRG compared with HSG and CRG (Cosgrove et al. 2007; Pacheco et al. 2009; Hume et al. 2010). The differences in WSC, NDF and CP composition among the cultivars were much larger in spring than in autumn in this study, which was also consistent with previous findings (Cosgrove et al. 2009; Cosgrove et al. 2014). As a consequence, there was a larger variation in WSC/CP and WSC/NDF ratios among the three cultivars in spring than in autumn. The overall lower forage CP concentration in combination with the more variable WSC/CP and WSC/NDF in spring were expected to result in bigger differences in N balance among the cultivars compared with autumn as discussed by others (Edwards et al. 2007; Pacheco et al. 2009). Indeed, there were significant interactions between cultivar and season for UN/IN and UN/FN with bigger differences between HSG and CRG in spring than in autumn, while urinary-N excretion was highest for TRG in both seasons. Increasing CP concentration of the diet is usually one of the main explanatory variables for increasing total urinary-N excretion (Kohn et al. 2005; Spek et al. 2013) and CP concentration was highest for TRG in both periods, which might explain the higher urinary-N excretions for TRG compared with CRG and HSG. However, total N intake was similar for TRG compared with both CRG and HSG in both seasons, which means that relatively more urinary-N was excreted per unit of N intake in sheep fed TRG. In contrast, faecal-N excretion was lower for sheep fed TRG compared with sheep fed CRG and HSG resulting in a higher apparent total tract N digestibility of 73% for TRG fed sheep compared with 69% for CRG and HSG fed sheep. This suggests that the TRG fed sheep had a higher digestible N intake compared with CRG fed sheep, but similar to HSG fed sheep. However, WSC intake ranking was lowest in TRG in both seasons which might have limited the N capture by microbes in the rumen.

The UN/IN and UN/FN decreased with increasing WSC intake and increasing grass WSC concentration relative to CP and NDF. Similar trends were found by Cheng et al. (2013) in sheep fed lucerne pellets sprayed with cane sugar and urea to create diets with different WSC/CP ratios. Also, regression analysis of multiple studies with dairy cows grazing ryegrass revealed that forage WSC/CP ratio was inversely related to UN/IN ratio (Edwards et al. 2007).

Nitrogen in urine is more prone to nitrate leaching, ammonia volatilisation and N_2O emissions than N in faeces, and therefore, reducing the UN/IN and UN/FN ratios might reduce these N losses into the environment (Luo et al. 2010). The higher UN/IN and UN/FN in TRG compared with CRG and HSG might result in higher N_2O emissions. This may also offset the lower CH_4 emissions found in the same trials for sheep fed TRG compared with sheep fed CRG (A. Jonker and GP Cosgrove, unpublished). Sheep fed HSG had lower CH_4 emissions compared with CRG (similar to TRG) (Jonker et al. 2014) and together with the similar proportional N excretions to CRG should result in overall lower greenhouse gas emissions from sheep fed HSG. However, the nutritional composition of TRG used in this study was atypical in having low WSC and high NDF and this may have affected the ranking of N excretions for this cultivar. Additionally, emissions per unit of animal product (average daily gain) and per unit of land (liveweight gain/ha) need to be considered before making recommendations.

Feeding CRG and HSG resulted in reduced urinary-N excretion relative to N intake compared with TRG in both spring and autumn, and forage WSC intake was one of the main explanatory variables for differences in urinary-N to N intake and faecal-N in this study.

Acknowledgements

This project was funded by the Sustainable Land Management and Climate Change Fund administered by the Ministry for Primary Industries (Wellington, NZ). Dr. Long Cheng was funded by AGMARDT Post-Doctoral Fellowship. The authors thank Grant Taylor, Fiona Smith, Phoebe Stewart-Sinclair and Sarah Lewis (AgResearch) for their help with animal handling and sample processing, and staff of Aorangi Research Farm (AgResearch) for managing and supplying cut pasture and sheep.

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