Liveweight gain and urinary nitrogen excretion of lambs grazing diverse (plantain, Italian ryegrass and red clover) or ryegrass-white clover pasture in autumn

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Abstract

The objective of this study was to investigate the effect of grazing a diverse pasture of plantain, Italian ryegrass, and red clover versus a ryegrass-white clover pasture on the liveweight gain (LWG) and urinary nitrogen (N) excretion of ram lambs over an 82-day experimental period in autumn. Eighty Coopworth ram lambs (8 months old) were allocated to two replicates of two pasture treatments (n = 20), a diverse pasture containing plantain, Italian ryegrass, and red clover (Diverse) and conventional ryegrass-white clover pasture (RGWC). Unfasted live weight (LWT) of lambs was recorded on seven occasions during the autumn experimental period. Apparent dry matter intake (DMI) was estimated as the difference between pre- and post-grazing herbage mass plus daily herbage growth. On two occasions, urine and blood samples were collected from each lamb to determine concentration of urinary N and plasma urea N (PUN). Liveweight gain (P=0.008) and feed conversion efficiency (P=0.022) were greater in Diverse than RGWC treatment (240 vs 160 g/day and 111 vs 86 g LWT per kg DMI, respectively). Urinary N and PUN concentration were similar between treatments; however, when urinary N excretion (g/d) was estimated as a function of PUN, lambs grazing Diverse pasture excreted less (P=0.003) urinary N per 100 g LWG than those grazing RGWC pasture. The proportion of N intake retained in LWG was greater (P=0.008) for lambs grazing Diverse (11.0%) than those grazing RGWC (9.0%). The results indicate that there is a potential to achieve target LWT of lambs in a shorter time and with less N loss to the environment by using a diverse mix containing plantain, Italian ryegrass and red clover in comparison to ryegrass-white clover mix in autumn.

Keywords: Growing lambs; liveweight gain; urinary nitrogen; diverse pasture; ryegrass

Introduction

The New Zealand pastoral farming system is primarily based on grazed pastures comprising perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). However, such swards have seasonal variations in production and nutrient composition, limiting animal production during summer and autumn (Burke et al. 2002). A diverse sward mix that includes legumes and herbs has been reported to provide a more-even distribution of dry matter (DM) production and feed quality throughout the grazing season than the conventional ryegrass-white clover sward (Sanderson et al. 2007; Nobilly et al. 2013). Therefore, diverse swards are known for better supporting high rates of animal production, particularly under dryland systems during summer and autumn, compared to ryegrass-white clover (Cranston et al. 2015). For example, average liveweight gain (LWG) was 50% and 40% greater for weaned lambs grazing on a diverse mix sward than those grazing ryegrass-white clover sward in summer (Somasiri et al. 2015) and autumn (Somasiri 2014), respectively. However, most of the studies comparing performance of lambs grazing diverse versus conventional sward mixes were conducted under a dryland North Island system, and whether any production benefits are expected under an irrigated Canterbury system is unclear.

The pastoral livestock system in New Zealand has been linked to adverse environmental impact due to ongoing intensification (Di & Cameron 2002). The environmental impact mainly includes the contamination of ground waters and surface waterways as a result of nitrate leaching and global warming as a result of greenhouse gas emissions. The urine patch of grazing ruminants is the major source of nitrate leaching, which occurs as a result of imbalance between the nitrogen (N) load per urine patch and herbage capability for N uptake (Di & Cameron 2007). Establishing a pasture sward comprised of plant species varying in functional traits such as growth activity during the season (i.e., Italian ryegrass *Lolium multiflorum* Lam. with greater growth at low temperatures), rooting depth, and profile of bioactive components (i.e., plantain *Plantago lanceolata* L. as a deeper rooted forage with N availability-altering compounds) could be used as a strategy to reduce nitrate leaching. The mechanisms to achieve reduced nitrate leaching from diversified pasture include increasing the uptake of N from the root zone (Moir et al. 2013; Woods et al. 2016; Maxwell et al. 2019) and reducing urinary N excretion, as observed from dairy cattle (Totty et al. 2013; Box et al. 2017; Cheng et al. 2017) and sheep (Al-Mamun et al. 2008) fed a forage-based diet with or without plantain. Therefore, we hypothesised that feeding lambs on a diverse pasture sward of plantain, Italian ryegrass and red clover during autumn would improve live weight (LWT) performance and reduce urinary N excretion per kg LWG compared to lambs fed a perennial ryegrass-white clover pasture sward. The objective of this study was to thus, evaluate LWT performance and urinary N excretion of ram lambs grazing either plantain, Italian ryegrass and red clover (Diverse) pasture or perennial ryegrass-white clover (RGWC) pasture.

Materials and methods

This is part of a longer-term study established in 2016 at Lincoln University's Ashley Dene Research and Development Station, located near Burnham, New Zealand

(−43.65 °N, 172.33 °E) with the approval of the Lincoln University Animal Ethics Committee (AEC application # 2017-05). This manuscript presents data collected during the autumn experimental period from 07 March to 28 May 2019.

Experimental design and management

The 3.6 ha experiment site was comprised of twelve 0.3-ha rectangular paddocks, split into two blocks of six paddocks in a randomised complete block design. Each block contained three paddocks of Diverse pasture and three paddocks of RGWC pasture. Swards were regularly irrigated under a lateral irrigator from November to March, however, no irrigation was applied to these paddocks during the autumn experimental period reported on here.

The 80 ram lambs used in this experiment lineate from the Lincoln University Coopworth flock. From twin-bearing and mixed-age Coopworth ewes, 60 ram lambs were born on the Diverse and RGWC pasture paddocks, and 20 others were born on non-experiment paddocks in Spring (September) 2018 at Ashley Dene. Ewes and lambs continuously grazed each of the 12 paddocks, stocked with 4 – 6 ewes and their twin lambs per paddock, until weaning. After weaning, ram lambs were allocated into four groups of 20 lambs based on LWT (34.6±5.40 kg; mean ± SD) and previous exposure to herbage type; ram lambs born and reared on Diverse or RGWC pasture remained on this pasture type post-weaning. Each grazing block of six paddocks had two lamb groups (n = 20 lambs), with each group rotationally grazing three paddocks of Diverse pasture or three paddocks of RGWC pasture. Lambs were shifted to the next paddock within the three paddock rotation, when the post-grazing residual target range (900 – 1200 kg DM/ha) was met. During the current autumn study, each lamb group grazed one paddock for an average of 12.3±0.81 days before being shifted to the next paddock. At the start of the current autumn study on 7th March 2019, all lambs were weighed and LWT was similar between lambs on Diverse (44.2±6.21 kg) and RGWC (43.2±5.80 kg) pasture. The similar LWT for lambs at weaning, and at the start of this study, suggest that the pre-weaning exposure of lambs to forage type had no effect on LWG of lambs during this study. On 3rd May, due to reduction in herbage growth and subsequent feed availability, each pasture treatment was destocked by reducing the number of lambs in each group from 20 down to 12 lambs. Removed lambs were selected by LWT, with the heaviest lambs taken off the trial.

Pasture establishment

The two pasture sward types were established in October 2016: Diverse pasture (plantain *Plantago lanceolata* L. cv. Tonic, Italian ryegrass *Lolium multiflorum* Lam. cv. Asset, red clover *Trifolium pratense* L. cv. Relish) and RGWC pasture (perennial ryegrass *Lolium perenne* L. Prospect, white clover *Trifolium repens* L. cv. Tribute). Prior to establishment, the twelve paddocks were sprayed out with glyphosate-based herbicide and then cultivated in early October 2016. In late October 2016, paddocks were heavy rolled, seed mixtures were broadcast over sown, chain harrowed and heavy rolled again. Italian ryegrass, plantain and red clover were each sown at 10, 5 and 6 kg/ha respectively for the six paddocks sown in the diverse sward mix. The ryegrass-white clover mix was sown at rates of 20 and 5 kg/ha respectively.

Herbage measurements

Pre- and post-grazing herbage mass in each paddock were assessed 1-2 days before grazing and immediately after grazing respectively, using an electronic rising-plate meter (RPM; Jenquip F150 Electronic Pasture Meter, Fielding, New Zealand). An average of 50 random RPM readings were recorded by walking along a zig-zag transect through the paddock. The RPM measurements were then calibrated against a total of 21 pre-grazing (3-4 per paddock) and 14 post-grazing (2-3 per paddock) 0.2 m^2 quadrat cut to ground level from each sward site. The cut herbage was dried at 60°C for 48 hours to quantify DM weight per quadrat. To measure regrowth of herbage during period of grazing per paddock, two 0.5-m^2 cages were pegged down in two representative areas of each paddock, and herbage regrowth (kg DM/day) was measured as the difference between herbage mass readings taken before and after grazing events, divided by number of grazing days.

Herbage samples were collected from each paddock before grazing to determine chemical and botanical composition for each sward. The herbage (bite size) was cut with clippers above grazing height (2-3 cm above ground) along a diagonal line across the paddock. Approximately six to seven pluck samples were collected per paddock, grouped and subsampled. One subsample was separated into each herbage species, dried at 60°C for 48 hours, dry weight of each component was recorded and proportion of each species was determined as a % of the whole sward DM. A second subsample was dried at 60°C for 48 hours, ground to 1 mm, and analysed for the chemical compositions using near-infrared spectrophotometry (Foss NIRSystems 5000, FOSS NIRSystemsInc, USA). Metabolisable energy (ME) of feed was estimated based on the equation [ME (MJ/kg) = digestible OM content × 0.016 (g/kg of DM)]. Herbage N (%) was calculated by dividing the crude protein content by 6.25.

Animal measurement

Apparent dry matter intake (DMI) was calculated per lamb group using the following equation:

Apparent DMI (kg/day) =
[(Pre-grazing herbage mass – post-grazing herbage mass) + herbage growth during grazing]/ number of grazing days

Lamb apparent DMI (kg/lamb/day) was then calculated by dividing group DMI by number of lambs grazed per paddock.

During the current autumn experimental period, unfasted LWT of lambs was recorded on seven occasions (7th of March, 4th and 17th of April, and 3rd, 14th, 24th and 28th of May), before lambs were shifted to graze the next paddock. All lambs were grouped in a portable yard located on the laneways adjacent to the paddocks and were individually weighed using Prattley scale crate and a Tru-test ID3000 scale head and matching wand (Bluetooth ID reader).

On two occasions (17th April and 28th May) lambs from each treatment were grouped in a portable yard located on the laneways adjacent to the paddocks and were individually sampled for urine and blood. The lamb's nasal and oral passages were obstructed by holding a plastic cup tightly over the restrained animal's mouth and nose. A urine sample was generally produced within 5-10 s of airway obstruction. Obstruction of the lamb's airways was halted as soon as approximately 10-20 ml of mid-stream urine was obtained. Urine was acidified to a pH <4 to minimise volatilisation. All samples were then frozen to -20°C until analysis. Blood samples were collected from the jugular vein using 10-ml EDTA Vacuette tubes. Blood samples were placed on ice immediately after collection and later centrifuged at 3,000 × g for 15 min at 4°C. Plasma was collected into clean tubes, frozen to -20°C until analysis. Urine was analysed for N concentration using a Variomax CN Analyser (Elementar Analysensysteme GmbH, Germany). Plasma urea and urine urea concentration were determined using a kinetic UV and colorimetric assay (RX Daytona Randox, Antrim, Northern Ireland). Urinary N excretion (g/day) was estimated using the following equation: Urinary N (g/day) = 1.2 × PUN (g/l) × LWT (kg), was reported for sheep in the study of Kohn et al. (2005). The N retained in LWG was calculated assuming 1 kg of LWG contains 29 g of N as reported for sheep in the study of Kohn et al. (2005).

Statistical analysis

GenStat 18th Edition was used for analysis. All animal measurements were analysed using one-way ANOVA with sward type as treatment and the mean value averaged across lambs in each group/replicate being the experimental unit (two experimental units per treatment). Paddock was used as the replicate for the analysis of herbage chemical composition. Results were declared significant at P<0.05, and tendency was declared at P<0.1.

Results

Pre- and post-grazing herbage mass and chemical composition of the Diverse and RGWC pasture swards are presented in Table 1. Herbage contents of neutral (P<0.001) and acid (P=0.002) detergent fibre were lower in Diverse than RGWC (35.8 vs 46.3 and 23.9 vs 27.0 % of DM, respectively). High quality herbage was maintained during this study with an average ME content of 11.4 MJ/kg DM (11.3 and 11.6 MJ/kg DM for RGWC and Diverse respectively). The Diverse pasture sward comprised 17.1±5.1% plantain, 41.6±6.7% Italian ryegrass and 15.3±4.2% red clover (26% weeds), while the RGWC sward comprised 63.2±6.6% ryegrass, 11.8±3.9% clover (25% weeds).

Table 1 Pre- and post-grazing herbage mass (kg DM/ha), chemical composition (% of DM) and metabolisable energy content (MJ/kg DM) of RGWC (perennial ryegrass-white clover) and Diverse (plantain, red clover, and Italian ryegrass) swards in autumn at Ashley Dene Research and Development Station, Canterbury, New Zealand.

	RGWC	Diverse	SEM	P value
Pre-grazing mass	2063	1877	211.9	0.597
Post-grazing mass	1235	938	145.9	0.287
Neutral detergent fibre	46.3	35.8	0.93	<0.001
Acid detergent fibre	27.0	23.9	0.54	0.002
Water soluble carbohydrate	13.8	14.6	1.00	0.576
Nitrogen	3.1	3.2	0.15	0.498
Metabolisable energy	11.3	11.6	0.12	0.132

Table 2 Apparent intake, initial and final live weight, liveweight gain, and feed conversion efficiency (FCE) of lambs grazing RGWC (perennial ryegrass-white clover) and Diverse (plantain, red clover, and Italian ryegrass) swards in autumn at Ashley Dene Research and Development Station, Canterbury, New Zealand.

	RGWC	Diverse	SEM	P value
Dry matter intake (kg DM/lamb/day)	1.8	2.1	0.09	0.155
Metabolisable energy intake (MJ/lamb/day)	20.7	24.6	0.89	0.089
Nitrogen intake (g/lamb/day)	56.3	68.4	1.36	0.024
Initial live weight (kg)	43.2	44.2	0.41	0.224
Final live weight (kg)	55.8	63.0	0.69	0.017
Liveweight gain (kg/lamb/day)	0.16	0.24	0.010	0.008
FCE (g live weight/kg DM intake)	86.0	111.0	2.69	0.022

Apparent DMI of both pasture types was similar (P=0.155; average 1.9 kg DM/lamb/day; Table 2). However, ME intake tended to be greater (P=0.089) and N intake was greater (P=0.024) for lambs grazing Diverse pasture compared to those grazing RGWC pasture (Table 2). Change in lamb LWT over the current autumn experimental period is presented in Figure 1.

Initial LWT was similar (P=0.224) between treatments, but final LWT was greater (P=0.017) for lambs grazing the Diverse sward compared to those grazing the RGWC sward (Table 2). The LWG (P=0.008) and feed conversion efficiency (FCE; P=0.022) were greater for lambs on Diverse pasture than those on RGWC pasture (Table 2).

Pasture type did not affect lamb urinary concentration of N, urea, or concentration of plasma urea N (Table 3). Similarly, estimated urinary N excretion (g/day) was similar

Figure 1 Liveweight change of lambs grazing RGWC (--●--; perennial ryegrass-white clover) and Diverse (-■-; plantain, red clover, and Italian ryegrass) pasture in autumn at Ashley Dene Research and Development Station, Canterbury, New Zealand. Bars indicate SEM.

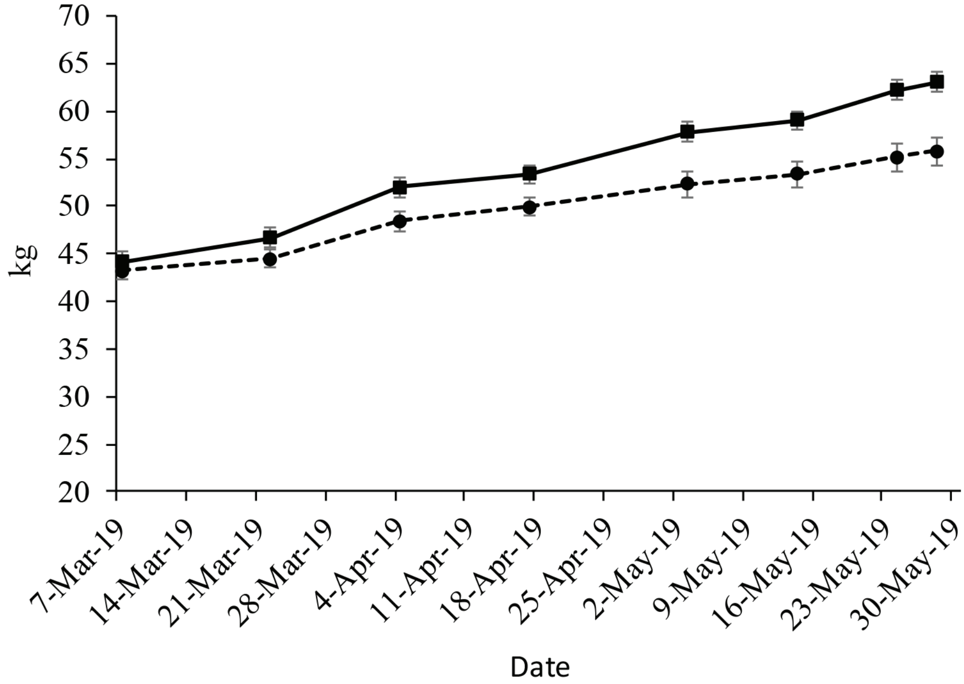

Table 3 Blood and urine metabolites, urinary nitrogen (N) excretion, and N retained in liveweight gain (LWG) of lambs grazing RGWC (perennial ryegrass-white clover) and Diverse (plantain, red clover, and Italian ryegrass) swards in autumn at Ashley Dene Research and Development Station, Canterbury, New Zealand.

	RGWC	Diverse	SEM	P value
Plasma urea N concentration (mmol/l)	20.0	19.9	0.62	0.94
Urine N concentration (%)	0.61	0.57	0.086	0.709
Urine urea concentration (mmol/l)	178	194	25.8	0.687
Urinary N excretion (g/day)	17.8	19.7	0.68	0.193
Urinary N (% of N intake)	31.7	28.7	0.46	0.046
Urinary N (g/100 g LWG)	11.3	8.3	0.11	0.003
Retained N (g/day)	5.0	7.5	0.16	0.008
Retained N (% of N intake)	9.0	11.0	0.06	0.002

(P=0.193) between treatments (Table 3). However, urinary N as % of N intake (P=0.046) and urinary N excreted per 100 g LWG (P=0.003) were both lower for lambs grazing Diverse pasture compared to lambs grazing RGWC pasture (Table 3). The N retained in LWG (g/day; P=0.008) and proportion of N intake retained in LWG (P=0.008) were both greater for lambs grazing Diverse pasture compared to lambs grazing RGWC pasture (Table 3).

Discussion

The objective of this study was to quantify LWG and urinary N excretion of ram lambs grazing either a diverse pasture sward of plantain, Italian ryegrass, and red clover or a perennial ryegrass-white clover pasture sward during the time of decreasing herbage supply and increased risk of nitrate leaching (i.e., autumn) in Canterbury.

The average LWG that accumulated over the autumn period of this study was approximately 50% greater for lambs grazing Diverse pasture compared to lambs grazing the ryegrass-white clover sward (240 vs 160 g LWG/day for Diverse and RGWC respectively). This result is comparable to the 40% increase in LWG reported by Somasiri (2014) for lambs grazing a mixed sward of herbs and clover (chicory, plantain, red clover and white clover) in comparison to ryegrass-white clover during autumn, but less than the >100% increase in LWG reported by Golding et al. (2011) for post-weaning lambs grazing a herbs-clover mix sward compared to lambs grazing ryegrass-white clover. The differences in the magnitude of effect of diverse sward versus a conventional ryegrass-white clover sward on lamb LWG between studies could be attributed to the differences in sward quality between pasture types in each study. The herbs-clover mix sward grazed by lambs in the study of Golding et al. (2011) contained 28% more ME than did the ryegrass-white clover (11.4 vs 8.9 MJ of ME/kg DM for herbs-clover mix and ryegrass-white clover mix respectively), while the ME content of Diverse and RGWC swards in this study was similar (average 11.4 ±0.12 MJ of ME/kg DM).

Despite the ME content of herbage and apparent DMI being similar between pasture treatments in this study, apparent ME intake tended to be greater for lambs on Diverse than those on RGWC pasture, which may partially explain the greater LWG observed in Diverse compared to RGW treatment. Italian ryegrass was reported to contain higher levels of water-soluble carbohydrate and lower levels of neutral detergent fibre than perennial ryegrass (King et al. 2012). Intake or grazing selectivity of lambs for individual pasture species were not measured in this study; however, there is a possibility that the lambs on the Diverse pasture were more selective to the highly digestible pasture species than were their counterparts in the RGWC pasture. The apparent N intake was also greater for lambs in Diverse compared to RGWC treatments (68 and 56 g N/day, respectively); however, the apparent N intake observed was unlikely to be limiting performance of lambs grazing RGWC compared to Diverse pasture. Apparent N intake in both treatments was well above the requirements of weaned lamb (43 g N/day) grazing good-quality pasture (ME = 11.6 MJ/kg DM) for 320 g LWG per day (AA Council 1990). Therefore, the difference in apparent N intake observed between the treatments in this study is unlikely to explain the greater LWG observed in Diverse compared to RGWC treatment.

Sorbitol, a sugar alcohol molecule with six carbons derived from glucose present in plantain herbage, which comprised 17% of Diverse pasture treatment, may have influenced the ability of lambs to gain weight. Sorbitol was reported to alter *in vitro* fermentation pattern (increase propionate concentration and reduce acetate to propionate ratio) and improve LWG and FCE of bulls when fed at 50 g per day (Geay et al. 1992). Plantain contains a greater proportion of sorbitol than does ryegrass herbage (42.1 vs 0% of water-soluble carbohydrate respectively; Jiang et al. 2019), suggesting that lambs on Diverse pasture may have ingested larger quantities of sorbitol than did their counterparts on RGWC pasture. This may explain the greater FCE observed from Diverse compared to RGWC pasture. More work is required to investigate the effect of sorbitol from plantain on LWG and rumen function of lambs.

Nitrogen intake was reported as the major driver for urinary N output in ruminants; both have been positively correlated with each other (Castillo et al. 2000; Kebreab et al. 2009). In this study, however, estimated urinary N excretion was similar between the treatments (average 18.8 g N/day), despite N intake being greater in lambs from Diverse than those from RGWC treatment. The similar urinary N output observed was supported by similar urinary N concentration, urinary urea concentration and PUN concentration results between treatments. This suggests that lambs grazing Diverse pasture containing plantain were more efficient in utilising N compared to those grazing RGWC pasture. Proportion of N intake retained in LWG was greater, and proportion of N intake excreted in urine and urinary N excreted per 100 g of LWG were lower for lambs grazing Diverse compared to those grazing RGWC pasture. Several studies have reported the role plantain has in reducing urinary N excretion (g/day) and concentration, in cattle (Totty et al. 2013; Box et al. 2017; Cheng et al. 2017) and sheep (Al-Mamun et al. 2008), with New Zealand research suggesting plantain as an alternative forage species to ryegrass to mitigate on-farm N loss to water (Totty et al. 2013; Box et al. 2017; Cheng et al. 2017). Furthermore, the greater LWG, FCE and N utilisation observed in lambs grazing Diverse compared to RGWC pasture may also be attributable to both the Italian ryegrass and red clover components which comprised 42% and 15% of the Diverse pasture respectively. Italian ryegrass has higher WSC levels than perennial ryegrass (King et al. 2012), and red clover contains polyphenol oxidase (PPO) which protects plant protein from degradation in the rumen by protecting leaf proteins in the form of protein-bound polyphenols (PBP) (Lee 2014).

It was important in this study to estimate urinary N excretion (g/day) of lambs as a function of physiological factor (i.e., PUN) rather than N intake, to reflect the expected effect of the Diverse pasture on N digestion and utilisation. However, it is worth noting that the estimation of urinary N excretion in this study was based on one PUN spot sample taken per day which may have not covered the expected diurnal variation in the PUN concentration of lambs. This may have affected the accuracy of PUN to give a quantitative reference measure for urinary N excretion, but could be used to rank the treatments (Al-marashdeh et al. 2015). Further research is required to investigate the N balance in sheep fed diverse pasture containing plantain cultivars similar to that used in this study (cv. Tonic).

Conclusion

The results of this study indicated that under a Canterbury irrigated farming system, offering ram lambs a diverse pasture mix containing plantain, Italian ryegrass and red clover in autumn is effective for improving LWG and FCE, and reducing urinary N excreted per unit of LWG in comparison to a ryegrass-white clover mix sward.

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