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High sugar ryegrass and methane emissions

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EXECUTIVE SUMMARY

Some prior studies conducted in the United Kingdom indicated that a high-sugar ryegrass would reduce methane production. Subsequently both a review article and modelling studies suggested mechanisms by which higher water soluble carbohydrate could influence the rumen environment and reduce methane.

This 3-year programme was conducted to test the hypothesis that a high sugar perennial ryegrass, selected for higher concentrations of water soluble carbohydrate (WSC) would reduce methane emissions compared with a control, non-selected perennial ryegrass.

To test this hypothesis 3 cultivars of perennial ryegrass were compared: a high sugar diploid perennial, a control diploid perennial (negative control) and a tetraploid perennial (positive control). Replicate plots of these cultivars were sown at the AgResearch Aorangi Research Station in autumn 2013, and used for supplying cut herbage for indoor and for grazing trials.

Three periods of experimental measurements were undertaken indoors in early spring 2013, early autumn 2014 and late spring 2014, followed by field experiments in the same seasons. Each period included measurements of methane production indoors in calorimeters, measurement of nitrogen balance indoors in metabolism crates, and a grazing study that included measurement of animal performance and methane emissions, using the SF6 technique and dry matter intake based on digesta markers (both long-chain alkanes and titanium dioxide).

For the indoor trials measurement of methane emissions was conducted on 48 wether sheep over 2 days, in two batches of 24 sheep, and nitrogen balance was conducted using 30 of the 48 sheep over 5 days. Grazing experiments were conducted in spring 2013 with measurements of average daily gain (ADG) and liveweight gain per hectare (LWG/ha) conducted over 84 days using 1-year old ewe hoggets, in autumn 2014 using 6-month old cryptorchid lambs with measurements conducted over 99 days and from late spring 2014 to early winter 2015 using 3-4- month-old cryptorchid lambs, with measurements conducted over 160 days.

Indoor studies used a factorial design with 2 levels of dry matter intake (1.1 and 1.8 x metabolisable energy requirements for maintenance) x 3 cultivars and 8 sheep per group for methane measurements and 5 sheep per group for nitrogen balance measurements.

The field study used a randomised complete block design with 3 cultivars x 4 replicates. Grazing management consisted of continuous variable stocking with 10 testers per group (15 per group in late spring-summer) and a variable number of grazers to maintain plots at a uniform sward surface height of 6 cm.

The WSC concentrations of the ryegrasses cut and fed indoors for methane and nitrogen balance measurements in spring 2013 were highest for HSG (330 g WSC/kg DM), intermediate for the diploid control (260 g WSC/kg DM) and lowest for the tetraploid (220 g WSC/kg DM). In spring 2014 the concentrations were 345, 320 and 308 g WSC/kg DM for the tetraploid, the high-sugar ryegrass and the diploid ryegrass respectively. Cultivars were similar to each other in WSC concentration in autumn. The mean concentrations were 254, 65, 314 g/kg DM in spring 2013, autumn 2014 and spring 2014, respectively.

Overall, methane production measured in calorimeters was lowest for the tetraploid control (13.9 g/d), intermediate for the high-sugar ryegrass (15.9 g/d) and highest for the diploid control (17.6 g/d). Methane yield was similar for the high-sugar and tetraploid ryegrasses (19.4 and 18.4 g/kg DMI, respectively) and both were lower than the diploid control (20.8 g/kg DMI), but there was no apparent relationship of methane yield and ryegrass WSC concentration.

Overall, only faecal nitrogen excretion was affected by ryegrass cultivar. Faecal N excretion (g N/d) as a proportion of N intake (g N/d) was lower for the tetraploid control (31.7%) compared with the high-sugar and diploid control ryegrasses (36.3%). In spring and autumn, there were differences among cultivars in urinary N excretion which reflected differences in N intake rather than differences in N utilisation. Urinary N excretion (g/d) had a strong relationship with dietary N concentration that was independent of N intake (R^2=0.79), and also strong negative relationships with dietary WSC intake R^2=0.79 and WSC/CP ratio (R^2=0.75).

In the methane measurement periods in each season of the field study the concentration of water soluble carbohydrate (determined in hand pluck samples) was higher in the HSG compared with the diploid and tetraploid controls by 39 g WSC/kg DM, 26 g WSC/kg DM and 35 g WSC/kg DM in spring, autumn and late spring-summer, respectively. Over the whole grazing season differences among cultivars (determined in samples from grazing exclosure cages) were comparable with these, except for spring when the smaller difference of 16 g WSC/kg DM was not significant.

For ADG the ranking of cultivars differed in each measurement season, with no significant differences in spring, the tetraploid (positive) control significantly greater than the other two cultivars in autumn and the HSG significantly greater than the diploid (negative) or tetraploid controls.

Despite the different ranking, when ADG was related to organic matter digestibility there was a moderately strong relationship (R^2=0.68); ADG increased by 0.5 g/d for every 1 g/kg DM increase in organic matter digestibility. Attributes which increase OMD, which could include WSC (and tetraploidy), result in higher ADG. There was no relationship between ADG and WSC.

There were no significant differences among cultivars in LWG/ha.

For dry matter intake estimated during the methane measurement periods, the cultivars ranked differently in the different seasons. The tetraploid control resulted in the highest or equal highest DMI in 5 out of 6 measurement periods, and the HSG was highest equal with the tetraploid in 3 out of 6 periods. The diploid control often resulted in the lowest or lowest equal DMI, and it was never the highest.

Methane production (g CH_4/hd/d) did not differ among cultivars in any season, however, methane yield (g CH_4/kg DMI; with DMI based on the mean of the alkane and titanium estimates) was lowest for the tetraploid control (15.4 g CH_4/kg DMI), intermediate for the HSG (17.6 g CH_4/kg DMI) and highest for the diploid control (20.5 g CH_4/kg DMI). This ranking of cultivars for methane yield is similar that measured indoors. There was no relationship between methane yield and ryegrass WSC concentration.

Methane emissions intensity (g CH_4 produced per g of daily gain) did not differ significantly among cultivars in any measurement period. The mean intensity was lower in autumn than in

either spring or late spring-summer, suggesting the composition of ryegrass in autumn was used more efficiently for higher ADG without a corresponding increase in emissions.

Total herbage accumulation, and average stocking rate did not differ among cultivars in any season. Consequently, DMI for the entire grazing season derived from herbage accumulation and average stocking rate did not differ among cultivars. Estimates using this method were comparable with those from the alkane marker method in spring and late spring-summer, but were higher in autumn.

An HSG and two control diploid perennial ryegrass cultivars growing in Waikato, Manawatu, Canterbury and Southland were sampled for WSC at every grazing over two consecutive years to determine the extent to which expression of WSC (and so the potential to influence methane emissions) might be influenced by growing environment. With the exception of the second growing year in Manawatu, the HSG had significantly higher concentrations of WSC than the controls. The mean increment in WSC for the HSG compared with the controls was 32, 26, 39 and 42 g WSC/kg DM for Waikato, Manawatu, Canterbury and Southland, respectively. These increases are similar to those measured in the field experiments in Manawatu, and suggest that effects of an HSG on methane emissions and animal performance would probably be similar across different regions.

In summary, methane yield was affected by ryegrass cultivar. Indoors, the HSG and the tetraploid (positive control) had similar methane yield that was 9% lower than the diploid (negative) control. In the field, methane yield from the tetraploid was 25% lower, and the HSG 14% lower than the diploid control. These effects could not be attributed specifically to water soluble carbohydrate.

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is the most common grass species sown in New Zealand (NZ). However, methane (CH4) emissions in pastoral systems in NZ are high (MfE, 2013) and the high nitrogen (N) content of ryegrass in intensive grazing systems, especially in autumn, results in a low N use efficiency (NUE; i.e. animal product N/N intake) in grazing animals. Consequently N excretion into the environment is high (Kolver and Muller, 1998) and this N is prone to nitrate leaching, ammonia volatilisation and nitrous oxide emissions (N_2O) (Luo et al., 2010).

Ryegrass with a higher WSC can potentially alter rumen microbial fermentation, synchrony of energy and protein in the rumen, total energy supply to the animal and consequently productivity (Edwards et al., 2007). Methane originates mainly from rumen fermentation and increased WSC concentration in ryegrass can change rumen fermentation patterns (Lee et al., 2003) which may consequently affect methane emissions. Methane emissions are largely driven by dry matter intake (DMI) (Sun et al., 2012) with methane mitigation strategies often being more effective at a high DMI (Moe and Tyrrell, 1979). However, a true methane mitigation strategy should be effective at any DMI level. Ryegrass 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). Recently, Janssen (2010) proposed a theoretical framework which explains how a higher concentration of WSC could reduce methane formation. Factors such as lower rumen pH, higher rumen hydrogen concentrations and faster rate of passage of digesta, conditions that more WSC would favour, could be expected to reduce methane emissions. The contributing effect of constituents other than just WSC is important in determining the outcome of higher WSC on methane emissions. Mathematical models have been used to more generally predict the outcome for greenhouse gas emissions of various changes in structural and soluble carbohydrates and protein (Ellis et al. 2011, 2012). When methane output is expressed as MJ/d or per unit of gross energy intake these models predict higher emissions as WSC increases, particularly so if WSC increases at the expense of reduced crude protein rather than at the expense of reduced fibre. However, when expressed on an intensity basis i.e. methane emitted per unit of milk or meat produced, these models predict lower emission intensity particularly if higher WSC is offset by lower fibre content because this may lead to higher dry matter intake and so higher animal production.

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 and greenhouse gas (GHG) emissions 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, resulting in increased cell content relative to cell walls (Smith et al., 2001). Therefore, both high WSC ryegrass (HSG) and TRG might improve NUE and reduce N excretion and GHG emissions compared with CRG.

The objective of this project was to determine the effects of high-sugar ryegrass, selected to express a higher concentration of WSC, on the greenhouse gas emissions by sheep. The hypothesis tested was that a higher concentration of water soluble carbohydrate would improve rumen fermentation and nitrogen utilisation and reduce methane emissions.

MATERIAL AND METHODS

There were three major components to this project

Measurement of methane emissions by sheep under controlled conditions in calorimeters, and under field conditions with grazing sheep (Indoor and Grazing Trials)
Measurement of sheep average daily gain and liveweight gain per hectare to provide animal production data for the calculation of methane emissions on an intensity basis (Animal Performance)
Field sampling from grazed ryegrass pastures over two years at four sites throughout New Zealand, to provide data on seasonal and regional variation in concentrations of water soluble carbohydrate and associated constituents, in high-sugar and control ryegrass cultivars (Regional Sampling)

The indoor and grazing trials, including animal performance measurements, each used the same HSG and diploid and tetraploid control cultivars. For the regional sampling the same HSG and diploid control were used and in addition an extra diploid control was used (cv. Prospect).

Three indoor trials and three grazing trials were performed with sheep offered the control diploid (CRG) the high-sugar ryegrass (HSG) or the tetraploid control (TRG) in each trial. The indoor trials consisted of methane measurements in respiration chambers and N balance measurements using sheep in metabolism crates, repeated in different seasons. The grazing trial included measures of pasture growth, sheep growth performance and estimates of methane emission using the SF_6 tracer method. The animal experiments reported here were reviewed and approved by the AgResearch Grasslands Animal Ethics Committee (Palmerston North, NZ; approval #s 13004, 13385) and animals were cared for according the AgResearch Code of Ethical Conduct.

Experimental plots

The three cultivars of perennial ryegrass used, each containing AR1 endophyte, were a high-sugar diploid perennial (*cv.* Abermagic; flowering date +15 days compared with Nui), a conventional diploid perennial ryegrass (*cv.* Alto; +14 days) and a tetraploid perennial ryegrass (*cv.* Base, +25 days). The design consisted of a randomised complete block, with four replications. Plot size of each cultivar was identical within block, while plots size ranged from 0.56 to 0.67 ha across blocks.

Each cultivar was sown in monoculture at 20 kg seed/ha into a cultivated seedbed in autumn 2013. Pastures were fertilised after sowing with 250 kg/ha of Cropmaster 15 (15-10-10-8) in May 2013, and with 300 kg/ha superphosphate (0-9-0-11) in July 2013, as part of farm fertiliser policy at Aorangi. Lime was applied at 2.5 t/ha in summer 2014. Nitrogen fertiliser was applied at 75 kg urea/ha 2-3 times during each grazing period. Between grazing periods, plots were stocked with sheep and occasionally cattle to maintain the continuously-stocked sward state.

INDOOR TRIALS

Animals

Three indoor trials were carried out at Grasslands Research Centre (AgResearch Ltd., Palmerston North, NZ) in spring 2013 (23 September-1 October), autumn 2014 (24 March-10 April) and late spring 2014 (3-21 November) using 48 Romney wethers in each season. The wethers for the spring 2013 trial (32.5 ± 2.0 kg) were born during October 2012 and for the autumn and spring 2014 trials during August 2013 with mean ± standard-deviation live-weights of 32.5 ± 2.0 kg, 37.5 ± 0.8 kg and 54.7 ± 3.3 kg, respectively, at the start of the trial. Each trial allowed for at least 14 days grazing the respective cultivars for adaptation, followed by five

days in group pens, eight days in metabolism crates with 5 days of nitrogen-balance measurement, and 4-5 days in respiration chamber crates, with 2 days of methane measurement.

Pasture feeding

The sheep were stratified by weight and randomly allocated to graze CRG, HSG or TRG in a single block (described above). 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 offered at either 0.7 or 1.0 kg DM/d (1.1 and 1.8 x metabolisable energy requirements for maintenance) resulting in six dietary treatments per season. Vegetative ryegrass accumulated to approximately 3000-3500 kg DM/ha (rising plate meter, Farmworks Systems Ltd., Fielding, NZ) and was cut daily (around noon) at around 7 cm above ground level. Sixty-five% of the total daily feed allowance was fed 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.

Methane measurements

Methane emissions and dry matter intake (DMI) measurements were performed after acclimatisation of at least five days indoor housing and 2-3 days in individual metabolism crates before entering 24 open-circuit respiration chambers for two consecutive days (Pinares-Patiño et al., 2012). The 48 sheep had to enter the chambers in two sub-groups of 24 and the 24 chambers are run in three clusters of eight chambers per gas analyzer. Sheep on each treatment were present in each cluster and each group. Chamber doors were opened twice daily for ~15 min (0800 h and 1530 h) for refusal collection, excreta removal and feeding. No measurements were performed during this periods and data was interpolated by taking the average of the last 12 values (~30 min) before opening the door. Dry matter intake was determined in the last two days of acclimatization and during the CH_4 measurement period. The DM content of refusals was determined by drying an aliquot at 65°C for 48 h. During the chamber days, a subsample of each diet offered was taken and pooled per diet in the freezer at -20°C. These samples were freeze-dried, ground through a 1 mm screen and analyzed as described below.

Four hours after morning feeding, on the day before the sheep entered the respiration chambers, a rumen sample was taken from each sheep via stomach tubing. The rumen samples were later analysed for volatile fatty acid (VFA) composition by gas chromatography as

described by Sun et al. (2012). The ratio between VFAs that result in a net hydrogen production:net hydrogen reduction (AB/PV) in the rumen was calculated as (Acetate + Butyrate)/(Propionate + Valerate) (Demeyer, 1991).

Nitrogen balance and digestibility measurements

Five sheep per treatment with similar live weight 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. Four hours after morning feeding, on day 2 and 4 of the N balance period, a rumen and blood sample were collected for later rumen VFA (as described above) and ammonia analysis and plasma urea concentration. Branch chain fatty acids (*iso*-butyrate + *iso*-valerate) and ammonia (NH_4) are defined as by-products of protein fermentation in the rumen.

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 below. Body retained-N (RN) was calculated in g/d as: IN – FN – UN. One sheep fed HSG at 0.7 kg DM/d in spring 2013 and one sheep fed HSG at 1 kg DM/d in spring 2014 were removed from the respective trial during the collection period because DMI decreased drastically and the sheep had diarrhoea.

Sample analysis

These samples were freeze-dried, ground through a 1 mm screen and analyzed by the Nutrition Laboratory of Massey University (Palmerston North, NZ) according to procedures of the Association of Official Analytical Chemists (AOAC, 1990) for ash (AOAC #942.05), crude fat (AOAC #991.36), crude protein (CP; AOAC #968.06), neutral detergent fiber (NDF) assayed with heat stable α-amylase, acid detergent fiber (ADF) and acid detergent lignin (ADL) in sulphuric acid (Robertson and Van Soest, 1981). The N concentration in feed, faeces and urine was analysed using a Variomax CN Analyser (Elementar Analysensysteme GmbH, Hanau, Germany) at Lincoln University (Lincoln, NZ). Water soluble carbohydrates (WSC) were analysed after extraction in 80% ethanol and using anthrone as the colorimetric agent (Jermyn, 1956). *In vitro* digestibility was determined by the neutral detergent cellulose method described

in Roughan and Holland (1977) corrected with *in vivo* standards by the Nutrition Laboratory of Massey University.

GRAZING TRIALS

Grazing trials with sheep grazing CRG, HSG or TRG were conducted at the AgResearch Aorangi Research Farm (near Palmerson North), with measurement periods conducted in spring 2013 (26 September – 18 December; 84 days), autumn 2014 (22 April- 30 July; 99 days) and late spring-summer 2014/15 (160 days). The latter period was interrupted by drought conditions in summer, and all sheep were removed from plots for 2 weeks in February because sward surface height (SSH) declined below the target of 6 cm (see description under Measurements below). Once removed these sheep were held for 1 week as a single group on a ryegrass pasture of a non-trial cultivar, and then separated back into three treatment groups (replicate groups within treatment were still combined) and given 1 week for readaptation to their assigned cultivar before being returned to their treatment and replicate plots.

Animals and stocking management

For measurements in spring 2013, one-year old ewe hoggets were used (n=120; 42.0 ± 3.33 kg initial LW); in autumn 2014 6-month old cryptorchid lambs (n=120; 30.7 ± 1.37 kg LW) and in spring 2014 3-month old newly weaned cryptorchid lambs were used (n=180; 28.7 ± 1.53 kg LW), on which measurements of ADG were based. For each season, sheep were weighed and allocated to 12 groups (three treatments replicated four times) stratified by LW. In each season an additional pool of cohorts from the same flock was identified, from which put-and-take grazers could be drawn (described below).

A continuous, variable stocking method was used. For spring 2013 and autumn 2014 each plot was stocked with 10 'tester' sheep, and 15 sheep for late spring-summer 2014/15 15, on which measurements of ADG were based. In addition, a variable number of 'put-and-take' (P&T) sheep were used. Decisions to add or remove P&T animals were based on changes in SSH from the target of 6 cm. Liveweight gain/ha for the grazing period was calculated from the total number of grazing days (total number of sheep each day × number of days) × ADG of the tester sheep. For late spring-summer of 2014/15, 15-month old heifers (346 ± 13.4 kg LW) were used as P&T grazers in addition to sheep to give greater capacity to control SSH

during the period of high pasture accumulation rates when ryegrasses were flowering. Each heifer was considered equivalent to 5 lambs for calculating grazing days and liveweight gain per ha. Overall, heifers accounted for 15% of total grazing days and lambs 85%.

Animal and pasture measurements

Pasture: sward surface heights were recorded by taking 25 readings per plot twice weekly using a sward stick (Hill Farming Research Organisation, UK, see Frame (1993) for description of methodology). To collect representative samples of the herbage offered, three (four in spring-summer 2014/15) grazing exclosure cages (1.0 × 0.5m) were randomly placed in each plot and after 2 weeks herbage accumulation in spring and 3-4 weeks in autumn (and one interval of 5 weeks in summer 2015) a sample was cut to the mean SSH of that plot recorded when the cage was placed. After each sampling each cage was relocated to a new site within the plot (no pre-trimming). The sample from each cage was composited into a single sample for the plot and was immediately frozen in liquid nitrogen and stored frozen. These samples were collected six times during spring 2013, five times during autumn 2014, and seven times during spring-summer 2014/15.

Animal: for each grazing period sheep were introduced to plots and after 1 week of adaptation to grazing the assigned cultivar, the sheep were weighed again directly off pasture (full liveweight), and after a 24 hour fast (empty liveweight. At the end of the grazing period, full and empty liveweights were again recorded. Average daily gain was calculated from the difference between start and end full liveweight divided by the duration of the grazing period. For spring-summer 2014/15 the calculation of ADG included the 2-week period when drought forced the removal of lambs from plots.

Methane emissions estimated by the SF6 technique

During each season, two sub-periods of SF_6 measurements were performed with 48 sheep to estimate methane emissions. Four sheep per cultivar per plot were grouped (16 per cultivar) into one plot per sub-period and another four for the second sub-period resulting in 32 sheep measurements per cultivar per season. The principles of the SF_6 technique are based on a known SF_6 gas release rate from a permeation tube, dosed into the rumen, and continuous subsample collection of exhaled breath into an evacuated canister. The CH_4 emissions are estimated from the SF_6:CH_4 ratio in the canister and the SF_6 permeation rate, with corrections for background values of SF_6 and CH_4 (Lassey et al., 2011). Permeation tubes containing the SF_6 tracer were prepared by NIWA (Wellington, NZ, as described in Lassey et al. (2011) before the first season. Permeation rate was determined by weighing the tubes at weekly intervals over at least eight

weeks (linear regression; Table 1), before orally dosing the tubes in each sheep at 6-7 days before the first breath sample collections. Sheep were slaughtered after each season and permeation tubes recovered, re-calibrated and used in the next season. For the last sub-period (HSG_P09), new calibrated permeation tubes were used because there were not enough properly functioning tubes left after the first two seasons.

Table 1 The mean ± standard deviation SF_6 permeation rate (mg/d)[1] of permeation tubes dosed in sheep before each measurement period (HSG_P02 to P09), gas concentrations in background canisters in each period and % of samples excluded before statistical analysis based on criteria detailed by Lassey et al. (2011).

	Spring 2013		**Autumn 2014**		**Summer 2014**	
	Period 1	**Period 2**	**Period 3**	**Period 4**	**Period 5**	**Period 6**
SF_6 permeation rate (mg/d)[1]	0.96±0.11	0.91±0.18	0.81±0.14	0.76±0.19	0.88±0.18	1.05±0.09
Background canister						
SF_6 (ppt)	8.0±0.4	8.6±0.2	8.3±1.1	8.9±0.6	10.2±1.5	8.0±0.4
CH_4 (ppm)	2.4±0.2	3.2±0.5	3.5±1.3	3.4±0.2	4.7±0.4	4.0±1.0
CO_2 (ppm)	477±44	470±69	454±11	522±30	580±35	586±78
% of samples excluded[2]	12	6	25	15	50	13

[1]SF_6 permeation rate was determined weekly weighing the permeation tubes for at least eight weeks and fitting the weight loss over time to a linear regression (R^2>0.99).

[2]Samplings were mainly excluded because of blockage or leaking of the sampling line or extremely low gas concentrations relative to the background.

During the measurement period, air was sampled adjacent to the nostrils (flow restricted through a 10 cm crimped capillary tube) and accumulated in 1.7 L evacuated yoke shaped collection canisters placed around neck of each sheep (Lassey et al., 2011). The canisters were changed every 24 h for 5-6 days. An additional four canisters were placed upwind near the three treatment plots to measure background gas concentrations (Table 1), which were also replaced daily. Sample collection canisters were pressurised with compressed air and sub-sampled into triplicate vials using an piston developed by NIWA (Lassey et al., 2014), which were analysed by gas chromatography for CH_4, CO_2 and SF_6 concentrations by NIWA as described in detail by Lassey et al. (Lassey et al., 2014). The daily CH_4 production was calculated as follows:

$$CH_4\ (g/d) = SF_6 \text{ permeation rate (mg/d)} \times \frac{CH_4 \text{ sample - } CH_4 \text{ background (µmol/mol)}}{SF_6 \text{ sample - } SF_6 \text{ background (pmol/mol)}} \times \frac{16}{146} \times 1000$$

where, 16 and 146 are the molecular weight (g/mol) of CH_4 and SF_6, respectively, and the multiplier 1000 accounts for the different units in the equation for CH_4 and SF_6. The CO_2 production was calculated with the same equation, but with the molecular weight of CO_2 (44 g/mole).

The criteria for ensuring sample integrity are described in Lassey et al. (2011), to identify (and reject) samples associated with leaks and blockages, or low concentrations of SF_6 and CH_4 samples relative to background air. Outliers were identified based on Z-scores as used by Grainger et al. (2007).

Estimation of dry matter intake

Daily dry matter intake (DMI) was estimated in this trial using two independent methods 1) titanium oxide (TiO_2) (Glindemann et al., 2009) and 2) natural long-chain alkanes which are components of the plant leaf cuticular waxes (Dove and Mayes, 1992), as external markers to estimate total faecal DM output and in combination with diet DM digestibility was used to estimate DMI as:

$$\text{DMI (kg DM/d)} = \text{Faecal DM output (kg DM/d)} / (1 - \text{digestible DM; g/kg})$$

Faecal output estimated using TiO_2 and long-chain alkanes as external indigestible markers involves orally dosing gelatine capsules containing measured amounts of each marker starting at least 5 days before the start of faecal collection, to allow each marker to reach an equilibrium concentration in the digestive tract and in excreta, and continuing for a further 5 days till the end of the breath sample collection. The gelatine capsule containing 2.5 g TiO_2 and 35 mg of synthetic C32 and C36 alkanes was dosed once a day (only C36 was used during the spring measurement periods because synthetic C32 was not available at the time). Before the start of marker dosing, a background faecal sample was taken from each sheep to adjust for background TiO_2 naturally present in faeces. Faecal grab samples were collected around 0900-1000 h starting at the same time as breath sample collections and lasted 5-6 days. Grab samples were stored in the freezer at -20°C till after the experiment, and then all faecal samples freeze-dried, ground through a 1 mm screen, all faecal samples within sheep (per period) proportionally

pooled. These samples were analysed for DM (AOAC 930.15) and TiO_2 concentration by colorimetric analysis after acid digestion, by Massey University Nutrition Laboratory (Palmerston North, NZ). Then, faecal DM output was calculated as:

Faecal DM output (kg DM/d) = Dose of TiO_2 (g/d) / TiO_2 in faeces (g/kg DM; corrected for background TiO_2)

During the faecal grab sampling period, a representative pasture samples was collected daily around 1100 h from each plot. After the trial, the pasture samples were pooled per plot, freeze-dried, ground through a 1 mm screen and analysed for DM, ash, CP, lipids, NDF, ADF, ADL, WSC and *in vitro* DM digestibility as described above, and natural and synthetic alkanes C31, C32, C33, C35 and C36.

In addition to the methodology details that apply to both markers described above, the alkane marker methods involves some specific differences. An important assumption in this technique is that the faecal recoveries (the amount of the alkane recovered in faeces as a proportion of the amount dosed) of the dosed synthetic and the naturally present alkane of adjacent chain length (i.e. C32 and C31 and C33, or C36 and C35) are equal (it does not matter if the recoveries are less than 100%). In the first indoor trial where intake and faecal output were measured the opportunity was taken to dose those sheep with alkanes and TiO2, to measure the faecal recoveries for sheep eating the same cultivars as used in the grazing trial. Where these recovery values for alkanes of adjacent chain length in the indoor were not equal, faecal concentrations of the synthetic dosed alkane measured in the grazing trial were adjusted to the concentration that would have been recorded had the recovery been the same as the natural alkane. In this study, the recovery of the synthetic even-chain alkanes were lower than for the natural odd-chain alkanes.

Statistical analysis

There was no statistical replication for indoor pasture nutritional composition because laboratory analyses were performed on samples within cultivar pooled across N-balance or respiration chamber days. Therefore, comparisons of nutritional composition among cultivars and between seasons in this paper are descriptive only. All other data were analysed by Linear Mixed Models in GenStat (17th edition; VSN international, Hemel Hempstead, UK).

- Methane emissions and rumen fermentation data in respiration chambers were analysed with cultivar, feed DM offer and their interaction as fixed effects and sheep and measurement day within sheep as random effects (Table 3).
- N-balance, digestibility and rumen and plasma parameters were analysed with cultivar, feed DM offer and their interaction as fixed effects and sheep as random effect (Table 3, 4, 5).
- Methane emission and rumen fermentation data from grazing sheep by the SF_6 method were analysed with cultivar, season and their interaction as fixed effects and sheep and block within season as random effects (Table 6).
- For the field study methane production and yield were analysed by ANOVA separately for each measurement period. For these measurement periods 4 sheep from each replicate group were pooled into a single group of 16 sheep for the 10-day duration and the individual animal was used as the experimental unit. For animal performance and pasture growth and chemical composition determined using grazing exclosure cages cultivar effects were determined by ANOVA using plot as the experimental unit.
- Within each regional site nitrogen fertiliser (main plot), cultivar (split-plot) and nitrogen x cultivar effects were determined by split plot ANOVA.

Simple regression in excel was used to determine the relationship between x and y parameters related to N balance. Significance was declared at $P<0.05$ and trends at $P<0.10$.

REGIONAL SAMPLING

This study was based on an existing network of trial sites. This Species Interaction trial contributes to the development of the Forage Value Index (Chapman et al. 2013), a joint initiative of DairyNZ and the NZ Plant Breeding and Research Association.

Sites

Trials were located in Waikato at DairyNZ Scott Farm, Newstead (latitude 37.8° S), in Manawatu at Massey University No 1 Dairy (40.4° S), in Canterbury at Lincoln University Research Dairy Farm (43.7° S) and in Southland at AgResearch Woodlands Research Farm 46.4° S). The Waikato and Lincoln sites are managed by DairyNZ and the Manawatu and Southland sites by AgResearch. Summary climate statistics for each site were extracted from the NIWA Virtual Climate Station network (Tait *et al*. 2006).

Experimental design and treatments

At each site the Species Interaction trial consists of two levels of nitrogen (N) fertiliser application x two levels of white clover x eight cultivars of perennial ryegrass x five replications, laid out in a split-plot design (160 plots in total). For the study described here, three cultivars were selected for sampling from the low N x no clover main plots and samples were taken from three of the five replications (nine plots). The low N treatment received 50 kg N/year applied as two applications of urea at 55 kg N each, in autumn and spring. The Canterbury site received 100 kg N/year, reflecting higher rates typically used under irrigation in this region. Main plots were individually fenced but cultivars within plots were grazed in common. The cultivars selected for sampling were the high sugar diploid perennial ryegrass cultivar Abermagic with AR1 endophyte (89% endophyte infection based on a grow-out test) and control diploid perennial ryegrass cultivars Alto and Prospect (each with AR37 endophyte; 85% and 75% endophyte infection, respectively). Trials in Waikato, Manawatu and Canterbury were sown in autumn 2012, and in Southland in November 2012. Sampling commenced in December 2012, and March 2013 in Southland, and samples were collected each time the plots were grazed over the next 12-month period (10 samplings in Waikato, Manawatu and Southland and 11 in Canterbury).

Trial management

Plots at each site were grazed by lactating dairy cows (dry dairy cows or dairy-beef animals of equivalent liveweight in Southland) whenever herbage mass of all cultivars within the plot reached the range 2500-3300 kg DM, to a target residual of 1600 kg DM/ha, as measured using a rising plate meter (FarmWorks Precision Farming Systems Ltd, Feilding).

Measurements and analyses

Just prior to each grazing subsamples of the ryegrasses were cut between 1300 – 1500 h from an area of approximately 100 mm x 80 mm at 10 sites randomly within each plot using hand or electric shears to 4 cm above ground level and composited into a single sample. These samples, comprising about 100 g fresh weight for each plot, were immediately frozen in liquid nitrogen, stored frozen and subsequently freeze-dried and ground to pass a 1mm sieve. Subsamples of this material were analysed using near infrared reflectance spectroscopy (feedTECH, AgResearch Grasslands, Palmerston North) to predict the concentrations of water soluble carbohydrates, crude protein (CP) and neutral detergent fibre (NDF).

Data analysis

Within site, the ryegrass cultivar effect (2 d.f.) was determined by ANOVA (SAS), using the replicate x cultivar interaction (4 d.f.) as error term. Site effects (3 d.f.) and site x cultivar interactions (6 d.f.) were tested using the replicate within site interaction. Seasonal effects were not statistically compared.

RESULTS & DISCUSSION

INDOOR TRIAL

Pasture composition

Crude protein concentration and fibre fractions (NDF, ADF and ADL) were higher in autumn 2014 than in both spring seasons (Table 2). TRG had and higher CP concentration in spring 2013 and autumn 2014 compared with HSG and CRG. Pasture WSC concentration averaged 254, 65, 314 g/kg DM in spring 2013, autumn 2014 and spring 2014, respectively, and was higher for HSG than TRG and CRG in spring 2013 only.

Methane emissions

There were slight, but significant differences in DMI among cultivars in spring 2013 (P=0.001) and autumn 2014 (P=0.03) with sheep fed TRG having overall lower DMI (P=0.003) (Table 3). The differences in DMI were mainly cause by slight differences in forage DM resulting in slightly different amounts of grass being offered from each cultivar, which was especially apparent in spring 2013. For autumn 2014 and spring 2014 an attempt was made to manage feed DM offer by microwaving grass samples to a constant weight to estimate the DM content of the grass, which reduced variation in DMI among cultivars. In general, TRG had a lower DM concentration than HSG and CRG.

Table 2 Nutritional composition of conventional diploid ryegrass (CRG), high sugar diploid ryegrass (HSG) and tetraploid ryegrass (TRG) fed to sheep in respiration chambers (methane measurements) or metabolism crates (N-balance) in three seasons.

	Cultivar	DM	Ash	CP	Fat	NDF	ADF	ADL	WSC
		(g/kg)				(g/kg DM)			
Spring 2013									
Chambers	CRG	167	96	134	39	443	261	16	249
	HSG	166	91	142	41	408	251	16	311
	TRG	130	114	156	44	427	261	18	202
N-balance	CRG	166	102	158	42	401	225	13	270
	HSG	189	88	150	41	377	210	12	354
	TRG	144	112	173	45	403	239	15	236
Autumn 2014									
Chambers	CRG	183	133	206	45	485	362	52	65
	HSG	178	144	227	41	460	252	30	65
	TRG	163	151	232	44	454	250	24	66
N-balance	CRG	177	145	209	45	501	294	32	49
	HSG	184	144	219	41	489	277	31	55
	TRG	160	161	244	43	467	266	28	46
Spring 2014									
Chambers	CRG	209	90	141	42	411	217	13	308
	HSG	207	87	141	42	400	204	14	302
	TRG	193	95	126	39	382	206	10	330
N-balance	CRG	237	85	119	35	408	217	13	308
	HSG	234	80	114	36	403	213	13	342
	TRG	218	92	106	33	388	217	12	357

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; WSC, water soluble carbohydrates.

A lower DMI is related to increased CH_4 per unit of intake, independent of treatment effect (Sun et al., 2012) and therefore the overall 7% lower CH_4 yield (P=0.002) for sheep offered 1.0 vs. 0.7 kg DM/d is as expected. Overall, methane yield was 6% higher (P=0.04) in spring 2013 than spring 2014, with autumn 2014 intermediate, and methane yield was overall lowest for TRG, intermediate for HSG and highest for CRG (P<0.001) with 17.6, 19.0 and 20.8 g/kg DMI, respectively. Interestingly, differences among cultivars were apparent at both DM levels offered, while treatment effects on CH_4 yield are normally higher at higher intake levels (Blaxter and Clapperton, 1965; Sauvant and Giger-Reverdin, 2009).

There was an interaction (P<0.001) between cultivar and season for methane yield (g/kg DM intake) which was 9% lower (P=0.007) in spring 2013 for sheep fed HSG compared with sheep fed CRG, with TRG fed sheep intermediate, 18% lower (P<0.001) in autumn 2014 for sheep fed TRG compared with sheep fed CRG, with HSG fed sheep intermediate, and 18% and 28% lower (P<0.001) in spring 2014 for sheep fed TRG compared with sheep fed HSG and CRG, respectively (Table 3). The CH_4 per unit of DMD (g/kg DMD) followed similar trend as CH_4

yield (g/kg DMI) as DMD was similar among the three cultivars, except in spring 2013 when TRG had a higher (P=0.04) DMD than HSG and CRG (Table 3).

Table 3 Average dry matter intake (DMI) methane emissions, rumen (acetate + butyrate)/(propionate + valerate) ration (AB/PV)[1] and total tract DM digestibility[2] of sheep offered conventional diploid ryegrass (CRG), high sugar diploid ryegrass (HSG) or tetraploid ryegrass (TRG) in spring 2013, autumn 2014, spring 2014 and overall seasons.

	Cultivar					DM offer (kg/d)			
	CRG	**HSG**	**TRG**	**SED**	**P-value**	**0.7**	**1.0**	**SED**	**P-value**
Spring 2013									
DMI (kg/d)[1]	0.82	0.87	0.74	0.034	**0.001**	0.67	0.94	0.028	**<0.001**
CH4 (g/d)	16.6	15.8	14.8	0.52	**0.003**	14.0	17.4	0.45	**<0.001**
CH4 (g/kg DMI)	20.6	18.8	20.4	0.68	**0.007**	21.1	18.8	0.46	**<0.001**
AB/PV	2.78	2.47	2.78	0.086	**<0.001**	2.77	2.58	0.070	**0.010**
DMI (kg/d)[2]	0.81	0.90	0.76	0.030	**<0.001**	0.69	0.95	0.025	**<0.001**
DMD (% of DM)	79.1	79.8	82.8	1.53	**0.038**	83.6	77.5	1.25	**<0.001**
CH_4 (g/kg DMD)	26.1	22.4	24.8	0.89	**0.002**	25.8	23.1	0.73	**0.001**
Autumn 2014									
DMI (kg/d)[1]	0.82	0.78	0.78	0.019	**0.031**	0.67	0.91	0.015	**<0.001**
CH4 (g/d)	16.5	14.9	13.0	0.36	**<0.001**	12.8	16.8	0.30	**<0.001**
CH4 (g/kg DMI)	20.3	19.4	16.7	0.48	**<0.001**	19.2	18.4	0.40	0.060
AB/PV	3.98	4.00	4.22	0.103	**0.043**	4.10	4.03	0.084	0.372
DMI (kg/d)[2]	0.78	0.82	0.74	0.033	0.107	0.67	0.89	0.027	**<0.001**
DMD (% of DM)	64.9	65.8	66.0	1.24	0.664	66.3	64.8	1.01	0.159
CH_4 (g/kg DMD)	31.3	30.0	25.6	1.17	**<0.001**	29.2	28.7	0.96	0.593
Spring 2014									
DMI (kg/d)[1]	0.92	0.90	0.90	0.019	0.319	0.73	1.08	0.016	**<0.001**
CH4 (g/d)	19.6	17.9	16.4	1.12	**<0.001**	14.1	19.7	0.91	**<0.001**
CH4 (g/kg DMI)	21.5	20.0	18.4	1.36	**<0.001**	19.2	18.2	1.11	0.359
AB/PV	2.81	2.74	2.48	0.215	0.285	2.78	2.58	0.175	0.273
DMI (kg/d)[2]	0.95	0.85	0.92	0.055	0.153	0.76	1.05	0.045	**<0.001**
DMD (% of DM)	80.1	79.4	79.0	0.93	0.456	80.6	78.3	0.76	**0.007**
CH_4 (g/kg DMD)	27.1	26.9	23.7	0.95	**0.001**	27.8	24.0	0.78	**<0.001**
Overall									
DMI (kg/d)[1]	0.86	0.85	0.80	0.017	**0.003**	0.69	0.98	0.014	**<0.001**
CH4 (g/d)	17.6	15.9	13.9	0.45	**<0.001**	13.7	17.9	0.37	**<0.001**
CH4 (g/kg DMI)	20.8	19.4	18.4	0.55	**<0.001**	19.8	18.5	0.45	**0.003**
AB/PV	3.23	3.11	3.22	0.160	0.686	3.25	3.12	0.130	0.307
DMI (kg/d)[2]	0.84	0.86	0.81	0.029	0.230	0.70	0.97	0.024	**<0.001**
DMD (% of DM)	75.0	75.3	75.4	1.98	0.979	76.0	74.5	1.62	0.377
CH_4 (g/kg DMD)	28.2	26.8	24.7	0.87	**<0.001**	27.6	25.5	0.71	**0.003**

[1]DMI, CH_4 (g/d and g/kg DMI) and AB/PV data were collected during respiration chamber measurements (n=48).
[2]DMI, DMD and CH_4 (g/kg DMD) data were collected during the N balance measurements (n=30). CH4 was extrapolated by multiplying CH4 (g/kg DMI) from chamber by DMI during the N balance period.

Nitrogen balance and digestibility

Nitrogen intake was similar for sheep fed CRG, HSG or TRG in spring 2013 and autumn 2014, while being higher (P=0.02) for CRG in spring 2014 compared with HSG and TRG (Table 4). Faecal N output was higher (P<0.03) for sheep fed HSG in spring 2013 and autumn 2014 compared with sheep fed CRG and TRG and similar among cultivars in spring 2014. Urine N output was highest for sheep fed TRG in spring 2013 and autumn 2014 and similar among cultivars in spring 2014. Nitrogen intake, FN and UN were higher at 1.0 than 0.7 kg DM/d offer in all seasons, except NI in spring 2013 and UN in spring 2014, and these parameters were in general higher in autumn than in either spring. 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 also overall in this study, UN (g/d) had a strong regression with dietary N (g/kg DM) (R^2=0.79), but also a strong negative regression with dietary WSC (R^2=-0.79) and WSC/CP ratio (R^2=-0.75). Total N intake (g/d) had a moderate/strong relationship with of FN (R^2=0.53), UN (R^2=0.55) and FN+UN (R^2=0.72). Retained N (g/d) was similar for sheep fed any of three cultivars in any of three seasons and at both feed DM offer levels.

As a % of NI, FN/NI tended to be lower (P<0.10) in autumn 2014 and spring 2014 and was lower (P<0.03) in spring 2013 and overall for TRG compared with CRG and HSG and FN/NI was lower at 0.7 vs. 1.0 kg DM/d, indicating that dietary N was more digestible in TRG and at 0.7 kg DM/d offer (Table 4). FN/UN followed similar trends as FN/NI because UN/NI was similar among cultivars in all seasons, except in autumn was higher for TRG than CRG, with HSG intermediate.

Relationship of rumen and plasma characteristics with N balance parameters

Plasma urea is a strong indicator of UN excretion (Kohn et al., 2005) and BCFA and NH_4 are end products of protein fermentation in the rumen (Allison, 1978; Tamminga, 1979) and can therefore be used as indicators of protein metabolism and excretion. Ruminal BCFA and NH_4 and plasma urea were higher (P<0.01) for TRG in spring 2013 and lower (P<0.10) in spring 2014 than for CRG and HSG. In autumn 2014, plasma urea was higher (P<0.001) and ruminal NH_4 tended to be higher (P=0.08) for HSG than for CRG and TRG. Plasma urea and BCFA had a strong relationship with UN and UN + FN (g/d; R^2>0.67 and 0.58, respectively), while NH_4 had a moderate relationship with UN and UN + FN (R^2<0.38). There were only moderate to weak relationships between plasma or rumen parameters and FN and RN. As for UN, ruminal BCFA and plasma urea had a strong relationship with pasture N (R^2>0.65) and WSC (R^2>-0.77) concentrations and WSC/CP ration (R^2>-0.66). Therefore, plasma urea and rumen BCFA appear good indicators of dietary CP and WSC and excretion of UN and UN + FN that can be

used if UN and FN are not measured, as is normally the case in grazing studies. Plasma urea had a strong relationship with rumen BCFA (R^2=0.74) and moderate relationship with rumen NH_4 (R^2=0.49).

Table 4 Nitrogen balance in sheep offered conventional diploid ryegrass (CRG), high sugar diploid ryegrass (HSG) or tetraploid ryegrass (TRG) in spring 2013, autumn 2014, spring 2014 and overall seasons.

	Cultivar					DM offer			
	CRG	**HSG**	**TRG**	**SED**	**P-value**	**0.7**	**1.0**	**SED**	**P-value**
Spring 2013									
N intake (NI; g/d)	17.1	17.8	19.7	1.52	0.218	17.1	19.3	1.24	0.072
Faecal N (FN; g/d)	5.9	6.3	5.3	0.05	**<0.001**	4.8	6.9	0.18	**<0.001**
Urine N (UN; g/d)	9.2	10.3	11.6	0.50	**<0.001**	9.5	11.3	0.41	**<0.001**
Retained N (RN; g/d)	2.0	1.2	2.8	1.34	0.409	2.8	1.1	1.10	0.166
FN (% of NI)	35.0	38.3	26.9	3.38	**0.004**	28.0	38.8	2.76	**<0.001**
UN (% of NI)	54.4	62.0	60.8	5.96	0.328	56.0	62.1	4.87	0.266
RN (% of NI)	10.7	-0.3	12.3	8.94	0.221	16.0	-0.9	7.30	**0.039**
UN/FN	1.58	1.67	2.27	0.113	**<0.001**	2.03	1.65	0.092	**<0.001**
Autumn 2014									
N intake (NI; g/d)	22.7	25.8	26.0	2.15	0.245	22.9	26.8	1.76	**0.039**
Faecal N (FN; g/d)	8.1	8.8	7.8	0.33	**0.024**	7.0	9.5	0.27	**<0.001**
Urine N (UN; g/d)	17.6	19.8	20.8	0.78	**0.001**	17.7	21.1	0.64	**<0.001**
Retained N (RN; g/d)	-3.0	-2.8	-2.6	1.57	0.969	-1.7	-3.8	1.28	0.107
FN (% of NI)	38.1	34.6	30.2	3.59	0.106	30.5	38.1	1.28	**0.015**
UN (% of NI)	82.0	77.9	80.8	6.87	0.826	77.3	83.1	5.61	0.313
RN (% of NI)	-20.2	-12.5	-11.0	10.20	0.633	-7.8	-21.3	8.33	0.120
UN/FN	2.21	2.27	2.71	0.102	**<0.001**	2.57	2.23	0.083	**<0.001**
Spring 2014									
N intake (NI; g/d)	18.1	15.5	15.6	1.00	**0.020**	13.8	19.1	1.00	**<0.001**
Faecal N (FN; g/d)	6.2	5.8	6.0	0.41	0.466	4.7	7.2	0.33	**<0.001**
Urine N (UN; g/d)	9.4	7.8	7.0	1.29	0.182	7.9	8.2	1.05	0.768
Retained N (RN; g/d)	2.5	2.0	2.7	1.52	0.841	1.1	3.7	1.24	**0.050**
FN (% of NI)	33.7	37.7	38.1	1.94	0.071	34.6	38.4	3.77	**0.026**
UN (% of NI)	53.4	55.1	46.1	9.70	0.634	57.1	46.0	7.92	0.166
RN (% of NI)	12.7	7.3	15.8	10.53	0.724	8.2	15.6	8.60	0.380
UN/FN	1.41	1.45	1.23	0.285	0.687	1.53	1.19	0.233	0.157
Overall									
N intake (NI; g/d)	19.3	19.9	20.4	1.41	0.713	17.9	21.8	1.15	**0.001**
Faecal N (FN; g/d)	6.74	7.01	6.37	0.35	0.196	5.51	7.90	0.29	**<0.001**
Urine N (UN; g/d)	12.1	12.9	13.1	1.46	0.768	11.8	13.7	1.20	0.121
Retained N (RN; g/d)	0.6	-0.1	1.0	1.10	0.643	0.7	0.3	0.90	0.647
FN (% of NI)	35.6	37.0	31.7	1.99	**0.025**	31.1	38.5	1.62	**<0.001**
UN (% of NI)	63.2	65.8	62.6	5.55	0.831	63.7	64.0	4.54	0.974
RN (% of NI)	1.2	-2.8	5.7	6.65	0.436	5.2	-2.5	5.43	0.175
UN/FN	1.73	1.81	2.07	0.159	0.089	2.05	1.70	0.130	**0.008**

Table 5 Rumen branch chain fatty acids (BCFA) and ammonia (NH_4) and plasma urea in sheep offered conventional diploid ryegrass (CRG), high sugar diploid ryegrass (HSG) or tetraploid ryegrass (TRG) in spring 2013, autumn 2014, spring 2014 and overall seasons.

	Cultivar					DM offer (kg/d)			
	CRG	**HSG**	**TRG**	**SED**	**P-value**	**0.7**	**1.0**	**SED**	**P-value**
Spring 2013									
BCFA (%)	1.52	1.42	1.87	0.140	**0.009**	1.63	1.58	0.115	0.633
NH_4 (mM)	2.10	2.40	6.54	0.606	**<0.001**	4.22	3.14	0.495	**0.050**
Urea (mmol/L)	3.3	3.7	4.8	0.19	**<0.001**	3.8	4.0	0.16	0.747
Autumn 2014									
BCFA (%)	3.52	3.58	3.69	0.179	0.619	3.60	3.60	0.146	0.995
NH_4 (mM)	9.51	10.07	11.07	0.665	0.080	10.42	10.01	0.543	0.458
Urea (mmol/L)	7.8	10.2	9.7	0.46	**<0.001**	9.4	9.1	0.37	0.419
Spring 2014									
BCFA (%)	1.63	1.95	1.44	0.181	**0.030**	1.97	1.38	0.147	**<0.001**
NH_4 (mM)	6.19	8.14	3.32	1.644	**0.025**	7.12	4.64	1.343	0.077
Urea (mmol/L)	3.6	3.6	2.8	0.37	0.086	3.6	3.0	0.30	0.060
Overall									
BCFA (%)	2.22	2.36	2.33	0.272	0.880	2.42	2.19	0.222	0.289
NH_4 (mM)	5.93	7.03	6.98	1.035	0.507	7.36	5.93	0.845	0.099
Urea (mmol/L)	4.9	5.9	5.8	0.76	0.334	5.7	5.4	0.62	0.616

GRAZING TRIAL

Sward surface height

During spring 2013 each cultivar was maintained at a similar SSH, although SSH was marginally below the target of 6 cm during October and increased above the target from early November, and particularly so during December (Figure 1a). In autumn 2014, the tetraploid control had a lower SSH than the other two cultivars during May, but from then on all three cultivars were above the target until the termination in late July (Figure 1b). For 2014/15, cultivars were similar in SSH until early February, although SSH had declined to 4 cm when lambs were temporarily removed. Plots recovered to 7 cm SSH during spelling, but the tetraploid control again had lower SSH than the other cultivars during March, yet recovered to be similar to the other cultivars by the end of April.

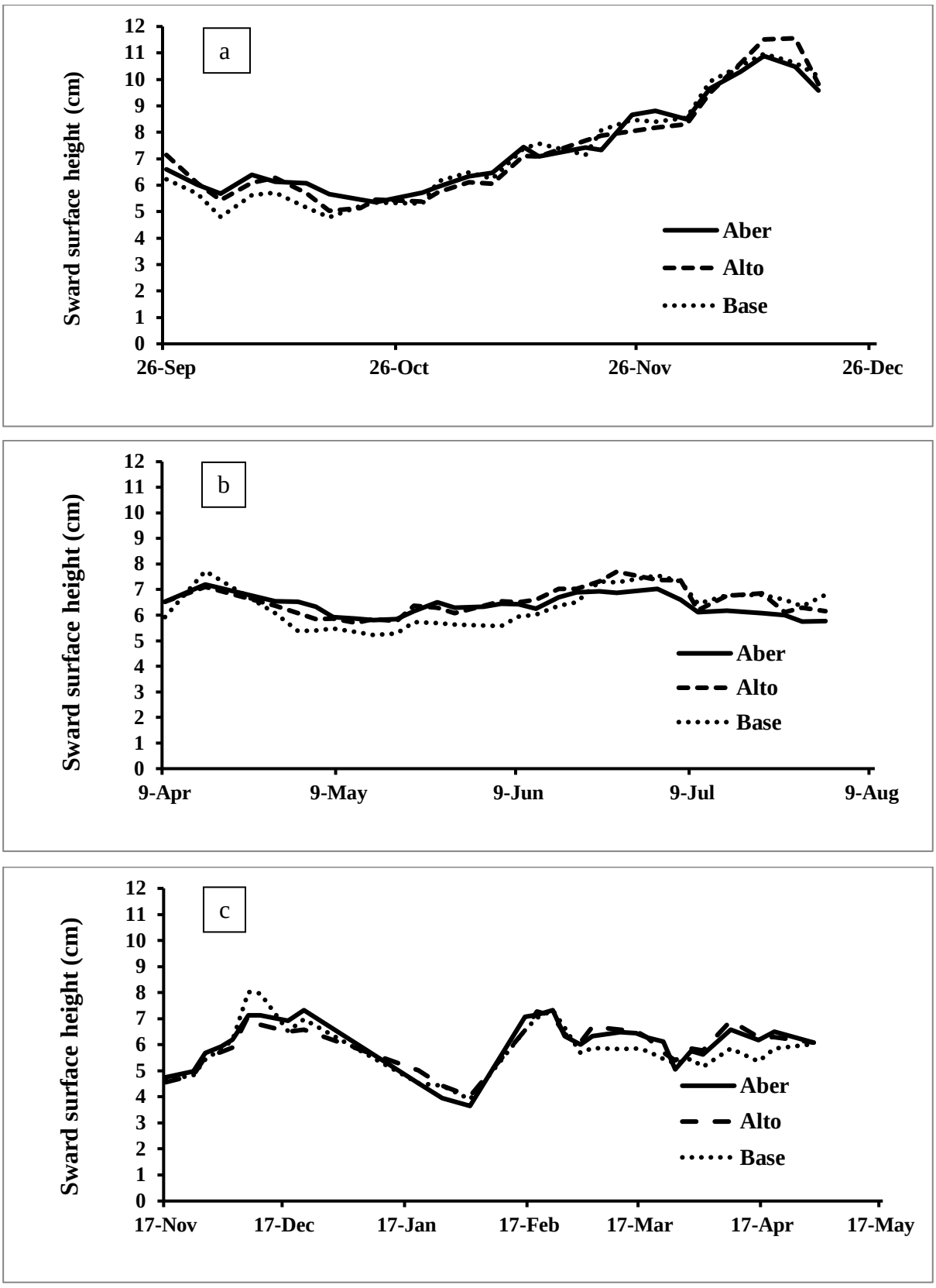


Figure 1 The sward surface height of three cultivars of perennial ryegrass, a diploid control cultivar (Alto), a diploid high water soluble carbohydrate (WSC) cultivar (Abermagic), or a tetraploid control cultivar (Base) under continuous variable stocking by sheep during measurement periods in spring 2013 (a), autumn 2014 (b) and late spring-summer 2014/15 (c).

Ryegrass chemical composition

In spring 2013 the cultivars did not differ significantly in concentrations of WSC or CP, but the HSG and the tetraploid control were lower in NDF than the diploid control (Table 6). In autumn 2014 the HSG had a significantly higher concentration of WSC (P=0.009) and significantly lower concentration of CP (P=0.02) than either of the controls and had a lower concentration of NDF (P=0.001) compared with the diploid control. In late spring-summer, the HSG had a higher concentration of WSC (P=0.017) and lower concentration of NDF (P=0.0004), than either of the control cultivars.

Table 6 The effects of three cultivars of perennial ryegrass, a diploid control cultivar (Alto), a diploid high water soluble carbohydrate cultivar (Abermagic), or a tetraploid control cultivar (Base), on the mean concentrations (g/kg DM) of water soluble carbohydrate (WSC), crude protein (CP) and neutral detergent fibre (NDF) during measurements periods in spring 2013, autumn 2014 and late spring-summer 2014/15.

		Ryegrass cultivar				
		Abermagic	Alto	Base	$P_{0.05}$	$LSD_{0.05}$
Spring 2013	WSC	196	182	178	0.71	29.4
	CP	216	213	225	0.68	33.3
	NDF	434b[1]	458a	440b	0.02	15.0
Autumn 2014	WSC	186a	155b	162b	0.009	10.5
	CP	252b	267a	274a	0.02	13.7
	NDF	416b	439a	416b	0.001	14.8
Late spring-summer 2014/15	WSC	251a	221b	213b	0.017	24.3
	CP	175	177	189	0.100	14.2
	NDF	462c	497a	485b	0.0004	10.1

[1] Means within row followed by different letters differ significantly P<0.05

Animal performance

There were no significant differences among cultivars in sheep ADG (mean of 164 g/hd/d) or in LWG/ha (427 kg LWG/ha) in spring 2013 (Table 2). In autumn 2014, lambs grazing the tetraploid control had higher ADG (206 g/d) compared with those grazing the HSG or diploid control (179 g/d; P=0.04), but this was offset by lower stocking rate (data not shown), such that LWG/ha did not differ among cultivars (mean 335 kg/ha). In late spring-summer 2014/15, ADG was higher (P=0.003) for sheep grazing the HSG (133 g/d) than for either of the controls (mean of 119 g/d), but this did not translate to significantly higher LWG/ha.

Table 7 The effects of three cultivars of perennial ryegrass, a diploid control cultivar (Alto), a diploid high water soluble carbohydrate cultivar (Abermagic), or a tetraploid control cultivar (Base), on average daily gain (ADG; g/d) and liveweight gain per hectare (LWG/ha; kg/ha) of sheep during measurements periods in spring 2013, autumn 2014 and late spring-summer 2014/15.

		Ryegrass cultivar				
		Abermagic	Alto	Base	$P_{0.05}$	$LSD_{0.05}$
Spring 2013	ADG[1]	170	158	164	0.62	37.3
	LWG/ha[2]	478	381	421	0.17	108.3
Autumn 2014	ADG	179b[3]	179b	206a	0.04	22.8
	LWG/ha	329	339	337	0.89	55.2
Late spring-summer 2014/15	ADG	133a	120b	118b	0.003	6.7
	LWG/ha	500	434	446	0.28	97.6

[1] Means of ADG are for the sheep used as 'testers'

[2] Means of LWG/ha are calculated for ADG of testers and the stocking density of testers plus put-and-take sheep

[3] Means within row followed by different letters differ significantly $P<0.05$

Dry matter intake

Dry matter intake during each methane measurement periods was estimated using two, independent digesta markers, plant cuticular wax long-chain alkanes (Table 8) and titanium dioxide (Table 9). For the latter method, the mean of estimates based on using the natural C31 and C33 alkanes in conjunction with the dosed, synthetic C32 alkane and the natural C35 alkanes in conjunction with the dosed C36 alkane are presented. While there was variation among the three different alkanes in the estimates of dry matter intake the means are presented for simplicity, and because it was considered that collectively they provide a more robust estimate. It should be noted that for measurement periods in spring, estimates of DMI were based only on C35 and C36 alkanes; no synthetic C32 was available at that time and so estimates of DMI based on natural C31 and C33 alkanes could not be calculated. There were significant differences among cultivars in both periods of measurement in each season. The DMI for Base was higher than for Abermagic or Alto during spring and similar to Abermagic in autumn and late spring-summer. With the exception of period 2 in autumn the DMI for Base was higher than for Alto. Base was always the highest or equal highest ranked cultivar

for DMI, and with the exceptions of period 1 in spring and period 2 in autumn, Alto was always the lowest or lowest equal ranked cultivar .

Table 8 Dry matter intake and *in vivo* digestibility of three cultivars of perennial ryegrass, a diploid control cultivar (Alto), a diploid high water soluble carbohydrate cultivar (Abermagic), or a tetraploid control cultivar (Base), estimated using natural herbage cuticular wax alkanes (C31, C33 and C35) and dosed synthetic alkanes (C32 and C36).

			Ryegrass cultivar				
Season	Period	Item	Abermagic	Alto	Base	P	$LSD_{0.05}$
Spring	Period 1	DM Intake	1.48b	1.71b	2.13a	0.002	0.36
		Digestibility	749b	760b	782a	0.002	18.3
	Period 2	DM Intake	1.56b	1.16c	1.89a	<0.001	0.27
		Digestibility	750a	692b	758a	<0.001	25.4
Autumn	Period 1	DM Intake	1.54a	1.09b	1.55a	0.004	0.299
		Digestibility	812a	801b	784c	<0.001	8.4
	Period 2	DM Intake	1.31b	1.51a	1.34ab	0.103	0.196
		Digestibility	815	823	828	0.477	20.3
Summer	Period 1	DM Intake	1.14a	0.90b	1.15a	0.004	0.154
		Digestibility	780a	734b	771a	<0.001	13.8
	Period 2	DM Intake	1.43ab	1.27b	1.52a	0.081	0.220
		Digestibility	625	642	640	0.198	20.7

For both periods in spring, the estimates of DMI based on titanium dioxide were very high (2.2 – 3.6 kg DMI/hd/d) and approximately two-fold expected. Although the liveweight of these sheep was heavier than the sheep used in either autumn or in late spring-summer, the estimates represent approximately 4.4% – 7.2% of liveweight, more than could physically be consumed. The reasons for this over-estimate of DMI in spring is unclear, especially when the same method produced apparently reasonable estimates in autumn and late spring-summer that were more consistent with expected DMI for the size of sheep used, and consistent with the alkane-based estimates.

The titanium-based estimates indicated that DMI was highest for Base in period 2 in spring and in both periods of late spring-summer higher than Alto, consistent with the alkane-based estimates. Differences among cultivars were not significant in period 1 of spring, nor in autumn. The consistency between the two methods of estimating DMI was greatest in late-spring summer.

Table 9 Dry matter intake (kg DM/hd/d) of three cultivars of perennial ryegrass, a diploid control cultivar (Alto), a diploid high water soluble carbohydrate cultivar (Abermagic) or a tetraploid control cultivar (Base), during two measurement periods in each of spring, autumn and late spring-summer, estimated using titanium dioxide.

		Ryegrass cultivar				
Season	Period	Abermagic	Alto	Base	P	$LSD_{0.05}$
Spring	Period 1	2.21	2.61	2.60	0.403	0.678
	Period 2	2.69b	2.78b	3.63a	0.024	0.722
Autumn	Period 1	1.51	1.08	1.20	0.151	0.450
	Period 2	1.76	1.72	1.71	0.964	0.396
Summer	Period 1	1.16a	0.92b	1.25a	0.020	0.228
	Period 2	1.44b	1.28b	1.85a	0.005	0.342

Digestibility

In addition to estimating dry matter intake, the alkane methodology allows for estimates of *in vivo* digestibility based on the herbage natural odd-chain alkanes in the grazed pasture and in the faeces. This is independent of the dosed synthetic even-chain alkanes. While this method still uses a sample of grazed herbage that applies to the whole plot (i.e. not to the individual sheep) and so does not account for individual differences in diet selection, it does account or individual differences among sheep in apparent digestibility (by comparison, estimates of DMI using titanium dioxide apply an *in vitro* estimate of digestibility to all sheep in the group). Differences among sheep in diet selection are considered to be a minor source of error given that each cultivar was grown in monoculture. There was no opportunity for selection of different species and by maintaining pastures at a uniform sward surface height sheep were presented with leafy swards for grazing, from which their diet would have been homogenous.

As described above for DMI, differences among cultivars in digestibility were not consistent (Table 8). Base was significantly higher digestibility than Alto in spring, and in period 1 in summer. However, Base had the lowest digestibility in period 1 in autumn. With the exceptions of spring period 1 when Abermagic had lower digestibility than Base and period 2 in autumn when it had higher digestibility, Abermagic was similar to Base.

Digestibility tended to be highest in autumn (overall mean 811 g/kg DM) compared with spring (749 g/kg DM) or summer (699 g/kg DM). Digestibility was particularly low in period 2 in summer when the mean of all cultivars was 636 g/kg DM. This was in late January, and

coincided with the very dry and warm conditions, and virtual cessation of herbage growth. Swards were grazed down to approximately 4 cm (Figure 1), well below the target sward surface height of 6 cm and at that time visual observation indicated they contained significant proportions of dead material.

Relationship of animal performance with herbage intake and composition

Comparisons among cultivars in nutritive value, expressed through ADG, and herbage accumulation rates, expressed through LWG/ha, were the experimental variables of primary interest in this study. Even though estimates of DMI (Tables 8 and 9) apply to the 5 day periods of measurement in each season, and average daily gains apply to the full duration of each grazing season, there was some indication of consistency across seasons in the effects of cultivar on ADG and DMI. The cultivar Alto tended to rank lower than the other cultivars (particularly Base) in DMI, digestibility and ADG, even though the effects were not always significant. Lower daily intake of less digestible feed is expected to result in lower ADG.

A greater concentration of WSC in herbage may increase nutritive value but this effect is influenced by the changes in concentrations of other constituents. For example, if a greater concentration of WSC is offset by a lower concentration of structural carbohydrates this can lead to higher DMI, and so greater ADG (Lee *et al*. 2001), and if crude protein is low, the greater concentration of WSC can improve microbial protein capture in the rumen and so improve overall protein nutrition (Kingston-Smith & Theodorou 2000). The concentration of WSC was higher in the HSG than in the diploid control by 14 – 31 g WSC/kg DM, a difference comparable with some New Zealand studies conducted under cutting (Hume *et al*. 2010) or sheep grazing (Parsons *et al*. 2004), but lower than the additional 35 – 47 g WSC/kg DM in rotationally grazed dairy pastures at four sites differing in latitude (Cosgrove *et al*. 2014). While cultivars did not differ significantly in WSC (or CP) in spring 2013 (a possible explanation for this is discussed below), the significantly lower concentration of NDF in the high-WSC ryegrass is consistent with the expected difference in chemical composition of that compared with a control, and as the concentration of one constituent increases the concentration of others inevitably decreases (Cosgrove *et al*. 2009; Rasmussen *et al*. 2009). Within season there did not appear to be a consistent relationship between a greater concentration of WSC and higher ADG. However, the associated changes in NDF and CP may also have influenced the observed ADG response, as modelling studies have suggested for nitrogen emissions and milk yield (Ellis *et al*. 2011). In each season the concentration of CP in each of the three cultivars was above the recommended minimum level for growing sheep (National Research Council 2007), and so ADG would be more likely to have been

affected by differences in WSC and NDF than by differences in CP. Given the uncertain response to the aggregate effect of an increase in the concentration of WSC and a decrease in NDF, digestibility may be a simpler explanatory variable for assessing overall trends. Using the seasonal mean value of OMD (concentrations predicted using near infra-red reflectance spectroscopy) for each cultivar (this mean was based on herbage samples collected from grazing exclosure cages at 2-4 week intervals during each season of 84 days in spring, 99 days in autumn and 160 days in late spring-summer, and so represents the whole grazing season, not just the DMI measurement periods), across cultivars and seasons there was a clear, positive relationship between ADG and organic matter digestibility ($R^2 = 0.68$; Figure 3), although even this does not fully account for the observed ADGs. Overall, ADG increased by 0.5 g/d for each 1 g/kg DM increase in organic matter digestibility. Choosing cultivars selected for high WSC, or tetraploid cultivars, which also achieve higher organic matter digestibility, regardless of whether that results from a greater concentration of WSC or from a lower concentration of NDF, can be expected to result in higher daily gains. Other factors such as seasonal growing conditions that result in favourable chemical composition and higher organic matter digestibility will also increase daily gains. Higher WSC specifically, may be important when the herbage CP concentration is low, because under those conditions the additional WSC improves ruminal protein utilisation and so the availability of protein for liveweight gain

There was an equally strong relationship between neutral detergent fibre and ADG, however this relationship is not presented. Neutral detergent fibre is but one constituent, and such a relationship does not take into account the contributing effects of other constituents such as crude protein, WSC, lipids and ash, in the way OMD does.

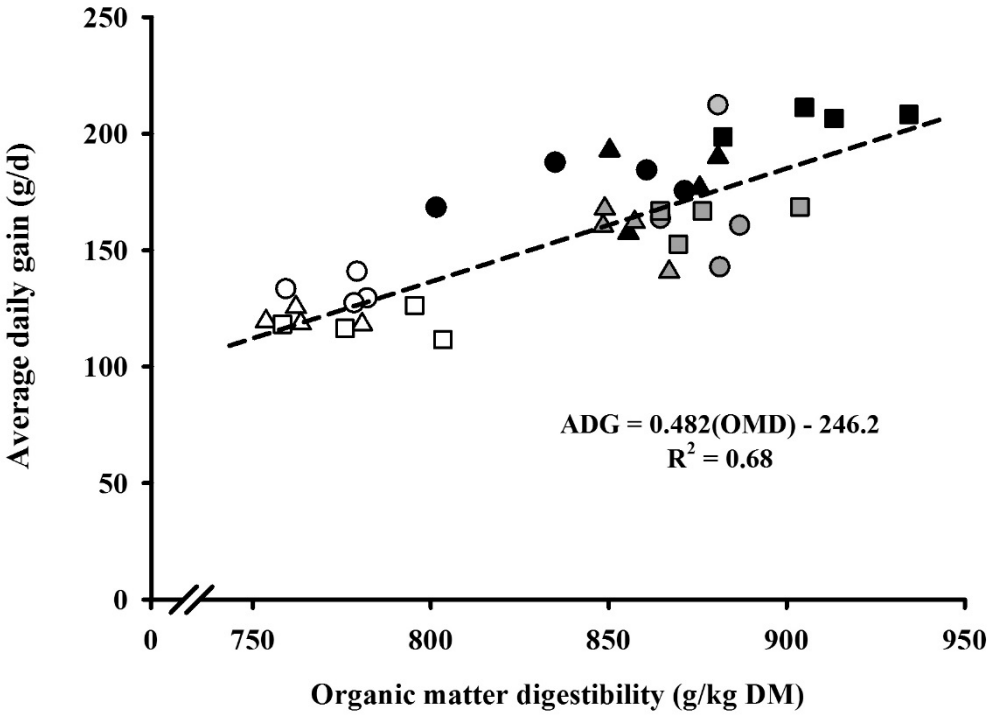


Figure 2 The relationship between organic matter digestibility of three cultivars of perennial ryegrass, a diploid control cultivar (triangles), a diploid high water soluble carbohydrate (WSC) cultivar (circles), or a tetraploid control cultivar (squares) and average daily gains of sheep grazing those cultivars during measurement periods in spring 2013 (grey symbols), autumn 2014 (solid symbols) and late spring-summer 2014/15 (open symbols).

Methane emissions during grazing using the SF6 method

During grazing trials, DMI estimated by both alkanes and TiO2 was higher in spring 2013 than autumn and summer 2014. Larger sheep were used in spring than autumn and summer 2014 and larger sheep are expected to have an higher DMI (CSIRO, 1990). Methane production was also higher in spring 2013 than autumn and summer 2014, which was expected because DMI is the main driver of CH_4 production (Blaxter and Clapperton, 1965; Sun et al., 2012). Overall, methane yield was lowest for TRG, intermediate for HSG and highest for CRG ($P<0.001$) with both methods of estimating intake, but more pronounced with alkanes than with TiO2 (Table 7), which is consistent with the ranking of the cultivars in the indoor trial (Table 3). Sun et al. (2013) also found a lower CH_4 yield for sheep fed winter forage rape compared with ryegrass dominated pasture, both in respiration chambers and while grazing using the SF_6 tracer method.

Ruminal BCFA and NH_4 are end-products of protein fermentation, which related to dietary N concentration and UN and UN + FN output in the indoor trial described above. In the grazing

trials there was an interaction between cultivar and season for ruminal BCFA and NH_4, with HSG fed sheep overall having lower ruminal BCFA and NH_4 than CRG and TRG fed sheep which was especially apparent in spring and summer for BCFA and in spring and autumn for NH_4. The WSC/CP ratio was higher for HSG compared with CRG and TRG in spring and summer, but similar to them in autumn (Table 10).

Ruminal BCFA was higher in autumn than in spring or summer, consistent with the higher concentration of N in the grass in autumn than in spring and summer, while NH_4 was also higher summer.

Table 10 The composition of the conventional diploid ryegrass (CRG), diploid high-sugar ryegrass (HSG) or tetraploid ryegrass (TRG) during SF_6 measurement periods in spring 2013, autumn 2014 and summer 2014.

	Spring			Autumn			Summer		
	CRG	HSG	TRG	CRG	HSG	TRG	CRG	HSG	TRG
Ash (g/kg DM)	112	106	119	111	104	116	101	96	113
Protein (g/kg DM)	208	202	228	327	319	340	205	171	213
Fat (g/kg DM)	51	48	47	52	48	53	43	41	45
NDF (g/kg DM)	386	388	359	362	367	320	440	462	425
ADF (g/kg DM)	212	207	195	196	187	183	220	217	212
ADL (g/kg DM)	17	20	14	15	19	20	20	21	20
WSC (g/kg DM)	237	276	237	132	166	148	177	209	171
DMD (g/kg DM)	727	726	736	742	737	752	706	696	714
DOMD (g/kg DM)	672	673	679	686	683	696	653	644	656
OMD (g/kg OM)	753	753	764	770	763	783	729	717	736
WSC/CP	1.14	1.37	1.04	0.40	0.52	0.44	0.86	1.22	0.81
WSC/NDF	0.61	0.71	0.66	0.36	0.45	0.46	0.40	0.45	0.40

NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent fibre; WSC, water soluble carbohydrates; DMD, dry matter digestibility; DOMD, organic matter digestibility on a dry matter basis; OMD, organic matter digestibility.

Table 11 Methane emissions from sheep grazing conventional diploid ryegrass (CRG), a diploid high sugar ryegrass (HSG) or tetraploid ryegrass (TRG) in spring 2013, autumn 2014 and summer 2014.

		Cultivar				Cultivar (C)		Season (S)		C×S	
	Season	**CRG**	**HSG**	**TRG**	**Mean**	**SED**	**P-value**	**SED**	**P-value**	**SED**	**P-value**
DMI (kg/d)	Spring 13	2.38	2.30	2.63	2.44	0.083	**<0.001**	0.083	**<0.018**	0.144	0.165
TiO2	Autumn 14	1.40	1.52	1.45	1.46						
	Summer 14	1.14	1.35	1.57	1.35						
	Mean	1.64	1.73	1.88							
DMI (kg/d)	Spring 13	1.47	1.52	2.01	1.67	0.052	**<0.001**	0.052	**<0.001**	0.090	**<0.001**
Alkane	Autumn 14	0.99	1.21	1.15	1.12						
	Summer 14	1.00	1.24	1.44	1.23						
	Mean	1.15	1.32	1.53							
Live weight (kg)	Spring 13	51.1	51.6	51.7	51.5	0.50	0.599	0.50	**<0.001**	0.87	0.874
	Autumn 14	38.7	39.1	39.6	39.1						
	Summer 14	36.5	36.0	36.3	36.3						
	Mean	42.1	42.3	42.5							
CH_4 (g/d)	Spring 13	25.8	26.9	27.7	26.8	0.83	0.655	0.83	**<0.001**	1.44	0.185
	Autumn 14	23.7	24.5	21.7	23.3						
	Summer 14	24.0	23.7	22.5	23.2						
	Mean	24.5	24.8	24.0							
CH_4 (g/kg DMI)	Spring 13	11.6	12.2	11.8	11.9	1.01	**<0.001**	1.01	**0.009**	1.74	0.102
TiO2	Autumn 14	19.2	17.3	16.2	17.5						
	Summer 14	22.1	18.5	15.3	18.7						
	Mean	17.6	16.0	14.4	17.4						
CH_4 (g/kg DMI)	Spring 13	18.4	19.3	14.6	21.6	1.01	**<0.001**	1.01	**<0.001**	1.74	0.097
Alkane	Autumn 14	20.4	25.4	19.1	19.9						
	Summer 14	18.9	25.6	15.3							
	Mean	23.4	19.2	16.3							
CH4 (g/kg LW)	Spring 13	0.51	0.52	0.54	0.52	0.024	0.474	0.024	**<0.001**	0.041	0.457
	Autumn 14	0.63	0.63	0.64	0.62						
	Summer 14	0.65	0.58	0.62	0.64						
	Mean	0.60	0.60	0.58							
AB/PV	Spring 13	3.56	3.42	3.26	3.42	0.067	**<0.001**	0.067	0.590	0.116	0.900
	Autumn 14	3.53	3.41	3.34	3.43						
	Summer 14	3.66	3.42	3.35	3.48						
	Mean	3.59	3.42	3.32							
BCFA (%)	Spring 13	2.7	2.3	3.0	2.7	0.12	**0.002**	0.12	**<0.001**	0.21	**0.045**
	Autumn 14	4.4	4.1	4.7	4.4						
	Summer 14	2.9	2.8	2.7	2.8						
	Mean	3.4	3.1	3.5							
NH_4 (mM)	Spring 13	6.3	4.8	7.3	6.1	0.66	**0.015**	0.66	**<0.001**	1.14	**0.007**
	Autumn 14	14.1	9.1	13.7	12.3						
	Summer 14	11.4	11.4	10.3	11.0						
	Mean	10.6	8.4	10.5							

Methane emissions intensity

Methane emissions intensity relates the quantity methane emitted (g/hd/d) to the animal production achieved at that level of emissions (average daily gain g/hd/d). While ADG used in this calculation relates to the entire grazing period in each season, different sheep were used for measurement of methane in periods 1 and 2, so the intensity (g CH_4/g ADG) was calculated and analysed separately for each period (Table 12). There were no significant differences among cultivars in emissions intensity during either measurement period of any season. There was a trend ($0.05<P<0.1$) for lower intensity for the tetraploid control (0.131 g CH_4/g ADG) compared with the HSG (0.202 g CH_4/g ADG) or the diploid control (0.178 g CH_4/g ADG) during period 1 in autumn. There was an indication of lower emissions intensity in autumn (0.148 g CH_4/g ADG) compared with spring (0.190 g CH_4/g ADG) or late spring-summer (0.200 g CH_4/g ADG). This appears to be due to the higher ADG in autumn, as methane production and methane yield were not higher than in spring or late spring-summer.

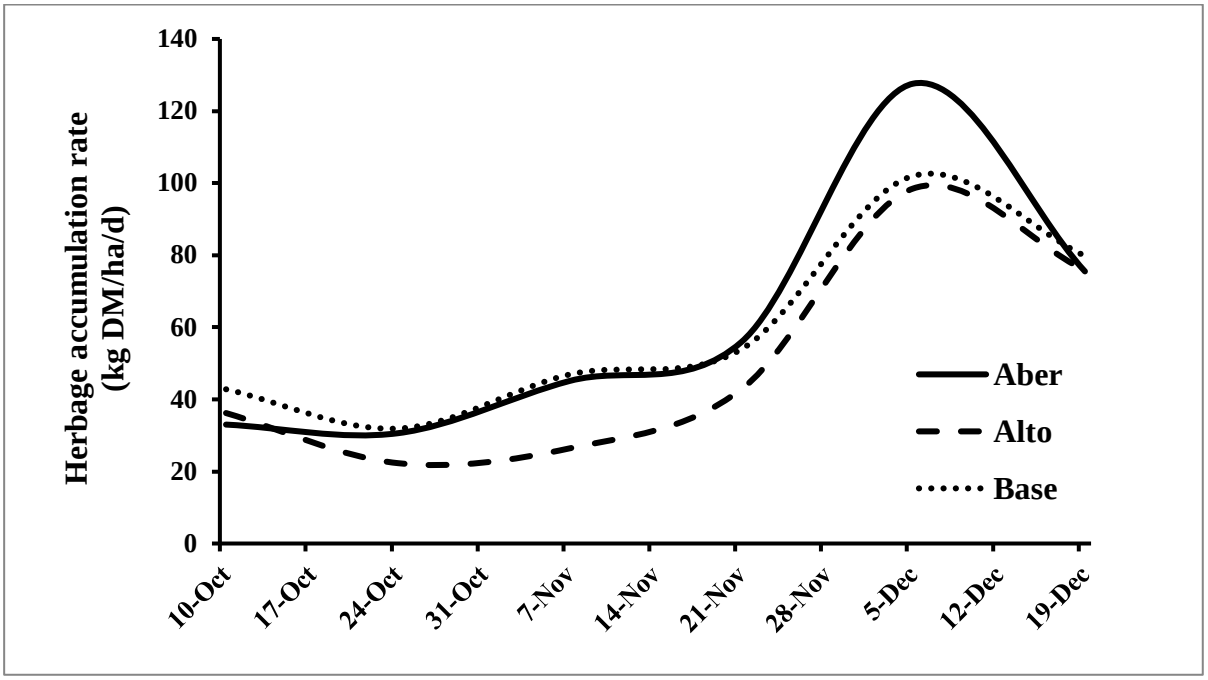
Table 12 Methane emissions intensity (g CH4/g ADG) of sheep grazing one of three cultivars of perennial ryegrass, a diploid high water soluble carbohydrate) cultivar (Abermagic), a diploid control cultivar (Alto) and a tetraploid control cultivar (Base), during measurement periods in spring, autumn and late spring-summer.

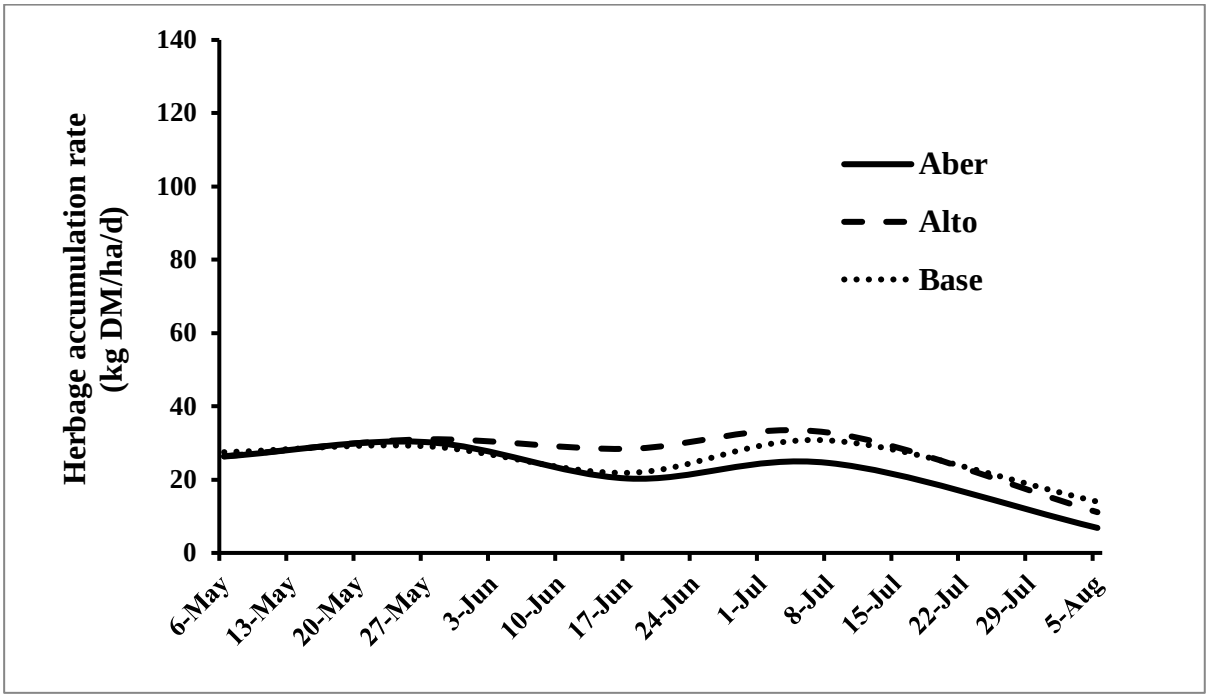
		Cultivar				
Season	Period	Abermagic	Alto	Base	P	$LSD_{0.05}$
Spring	Period 1	0.193	0.174	0.194	0.74	0.068
	Period 2	0.171	0.177	0.232	0.6	0.134
Autumn	Period 1	0.202	0.178	0.131	0.09	0.064
	Period 2	0.132	0.129	0.113	0.51	0.034
Summer	Period 1	0.156	0.199	0.167	0.14	0.047
	Period 2	0.215	0.234	0.233	0.54	0.038

Ryegrass herbage accumulation

The patterns of herbage accumulation during each season are shown in Figure 3a-c. There were no significant differences among cultivars in mean herbage accumulation rates in spring, autumn or summer. Herbage accumulation rates varied through each season of measurement in response to changing climatic conditions. Peak accumulation rates reached 80-100 kg DM/ha/d in late-spring 2013, 100-120 kg DM/ha/d in late-spring 2014 and approximately 30 kg DM/ha/d in autumn. Herbage accumulation rates were low at approximately 30 kg DM/ha/d in mid-spring 2013, and approximately 10 kg DM/ha/d as the autumn measurement period drew to an end in mid-winter and less than 20 kg DM/ha/d during the drought in mid-summer (herbage accumulation rate during this period included a period of recovery after the

drought to accumulate sufficient to harvest and measure; accumulation rate dropped effectively to zero during early February).





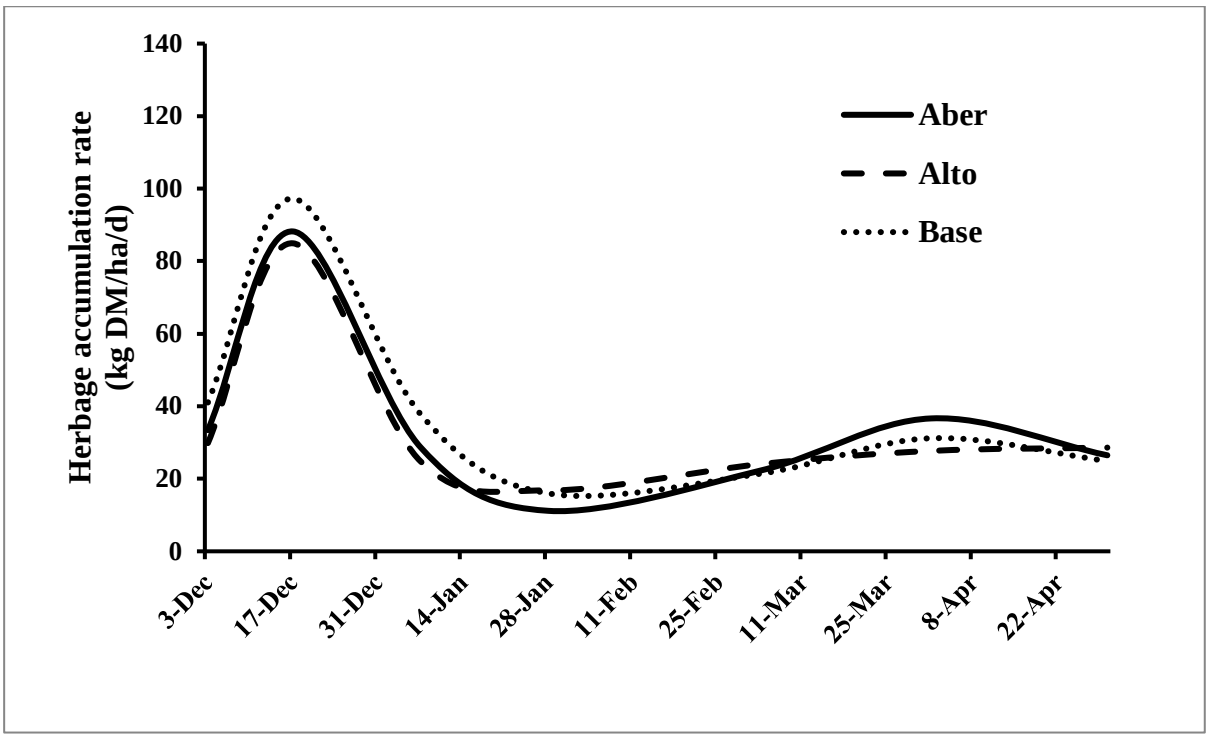
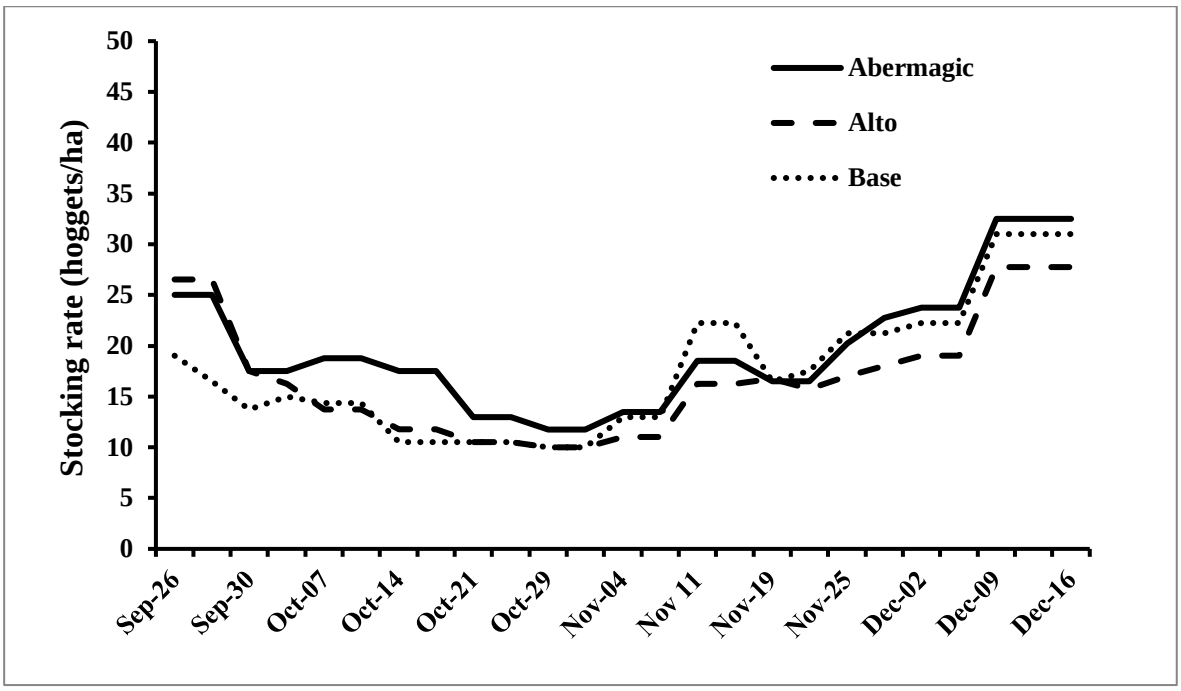
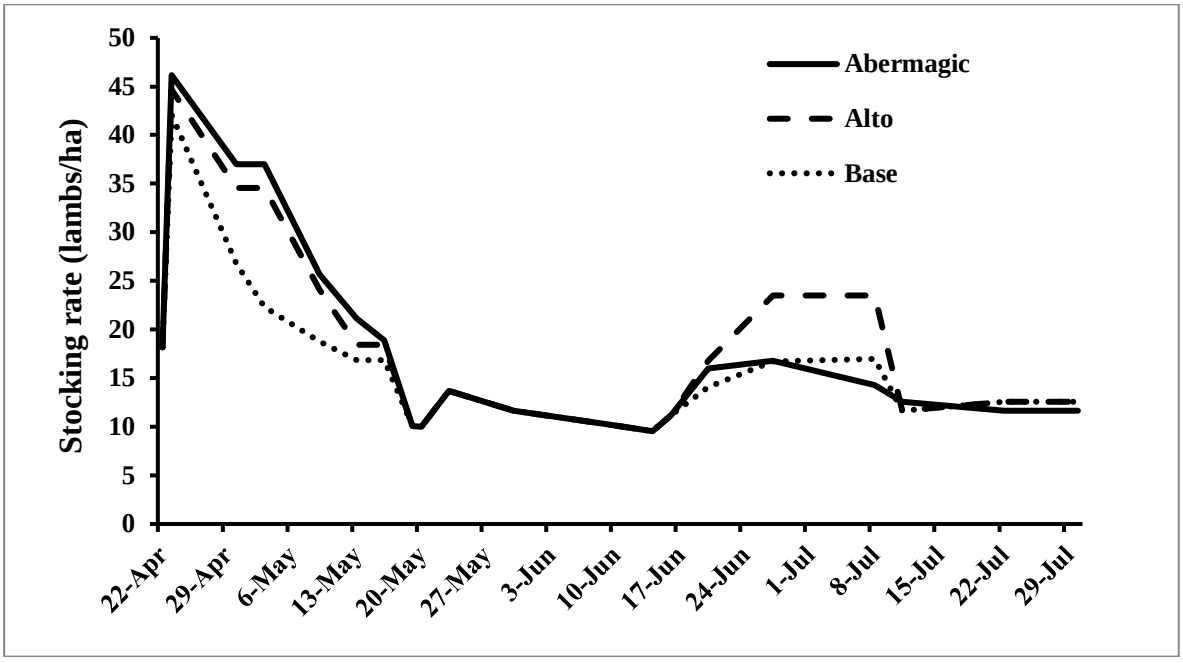


Figure 3 Herbage accumulation rate (kg DM/ha/d) of three cultivars of perennial ryegrass, a diploid high water soluble carbohydrate) cultivar (Abermagic), a diploid control cultivar (Alto) and a tetraploid control cultivar (Base), during measurement periods in spring (84 days), autumn (99 days) and late spring-summer (160 days).

Stocking rate

Stocking rate adjustments were made on the basis of changes in sward height, which reflect the balance between growth (herbage accumulation rate) and consumption (daily dry matter intake). The changes in herbage accumulation rate described above are reflected through stocking rates (Figure 4a-c). In spring 2013, stocking rates declined through mid-spring as herbage accumulation rates were low, but then increased through to mid-December. In autumn, stocking rates were high initially because sward surface height was above the target of 6 cm (Figure 1b), but were reduced rapidly through May and largely reflected herbage accumulation rates for the remainder of that measurement period. For late-spring 2014, stocking rates were low initially, again because sward surface height was below target, but were increased during the period of very high herbage accumulation rates during December, and were reduced as dry conditions reduced herbage accumulation rates and eventually forced the removal of all lambs from experimental plots in early February. Lambs were returned to plots in late February at low stocking rates until termination in May.


Stocking rate (hoggets/ha)
50
45
40
35
30
25
20
15
10
5
0
Abermagic
Alto
Base
Sep-26
Sep-30
Oct-07
Oct-14
Oct-21
Oct-29
Nov-04
Nov 11
Nov-19
Nov-25
Dec-02
Dec-09
Dec-16


Stocking rate (lambs/ha)
50
45
40
35
30
25
20
15
10
5
0
Abermagic
Alto
Base
22-Apr
29-Apr
6-May
13-May
20-May
27-May
3-Jun
10-Jun
17-Jun
24-Jun
1-Jul
8-Jul
15-Jul
22-Jul
29-Jul

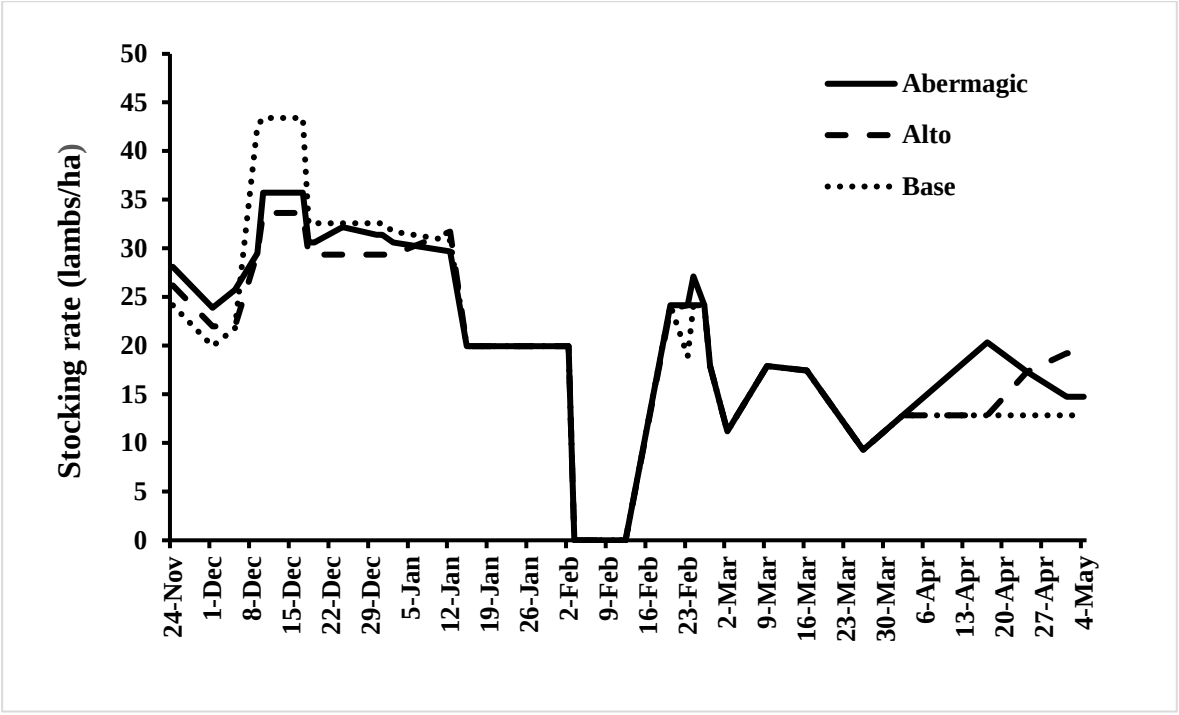
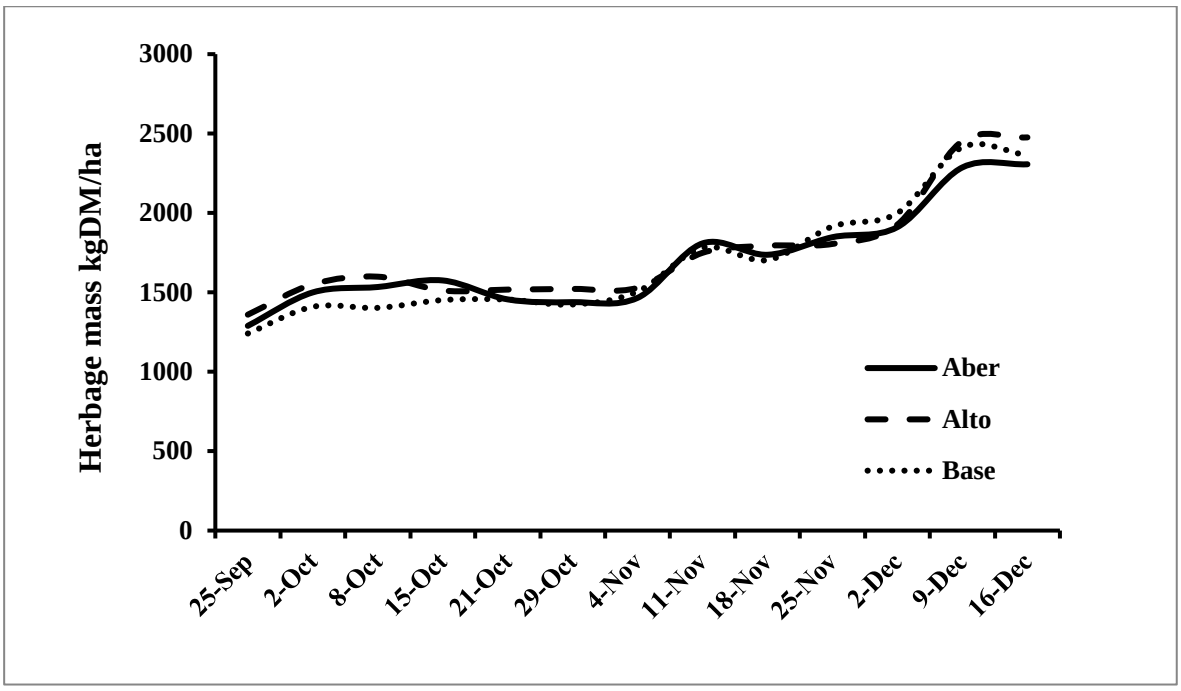
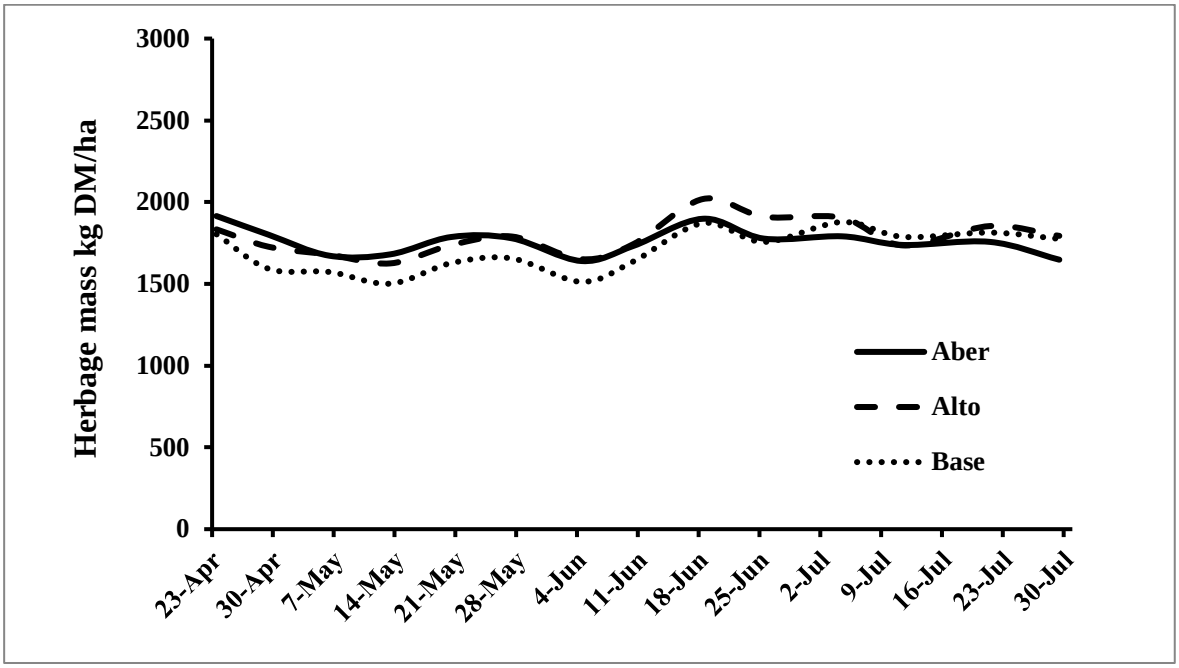


Figure 4 Stocking rate of sheep on three cultivars of perennial ryegrass, a diploid high water soluble carbohydrate) cultivar (Abermagic), a diploid control cultivar (Alto) and a tetraploid control cultivar (Base), during measurement periods in spring (84 days, using 1-year old ewe hoggets), autumn (99 days, using 6 month old lambs) and late spring-summer (160 days, using 3-4 month old lambs).

Herbage mass

Herbage mass also reflect sward conditions, although this was not used as the basis for stocking rate adjustments as sward surface height was. While herbage mass is a less sensitive measure than sward surface height, and is influenced by factors such as the dry matter content of the herbage, dead matter and the presence of reproductive stem, it indicates that swards were maintained at or above 1500 kg DM/ha throughout each measurement period (Figure 4a-c). High herbage mass (2000 – 2500 kg DM/ha) was apparent in Dec 2013 (end of spring measurement period) and Dec 2014 (start of late-spring summer measurement period).


Herbage mass kgDM/ha
3000
2500
2000
1500
1000
500
0
25-Sep
2-Oct
8-Oct
15-Oct
21-Oct
29-Oct
4-Nov
11-Nov
18-Nov
25-Nov
2-Dec
9-Dec
16-Dec
Aber
Alto
Base


Herbage mass kg DM/ha
3000
2500
2000
1500
1000
500
0
23-Apr
30-Apr
7-May
14-May
21-May
28-May
4-Jun
11-Jun
18-Jun
25-Jun
2-Jul
9-Jul
16-Jul
23-Jul
30-Jul
Aber
Alto
Base

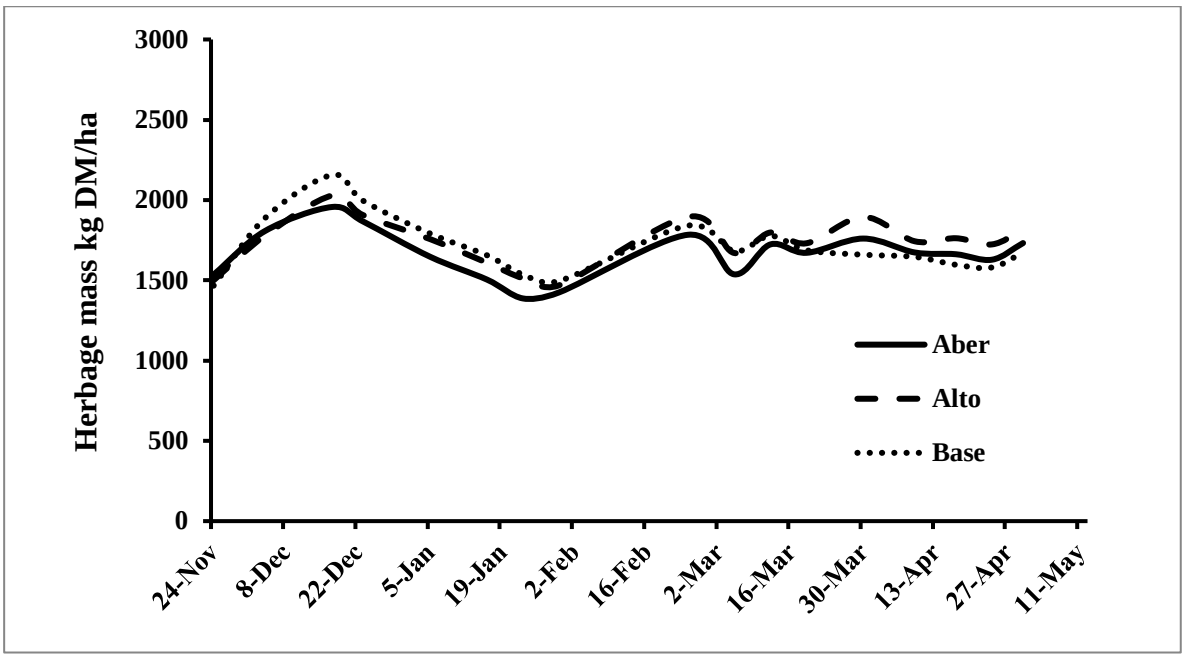


Figure 5 Herbage mass of three cultivars of perennial ryegrass, a diploid high water soluble carbohydrate) cultivar (Abermagic), a diploid control cultivar (Alto) and a tetraploid control cultivar (Base), during measurement periods in spring (84 days), autumn (99 days) and late spring-summer (160 days).

Ryegrass herbage accumulation

Total herbage accumulation for each measurement period was calculated from the sum of accumulation under the grazing exclosure cages cut at 2-4 weekly intervals. There were no significant differences among cultivars in total herbage accumulation in any measurement period (Table 13). The mean across cultivars of total herbage accumulation was 3700 kg DM/ha in spring (over 84 days), 2380 kg DM/ha in autumn (over 99 days) and 5150 kg DM/ha in summer (over 160 days). This represents mean herbage accumulation rates of 44 kg DM/ha/d, 24 kg DM/ha/d and 32 kg DM/ha/d during spring, autumn and late spring-summer, respectively.

Table 13 Total herbage accumulation of three cultivars of perennial ryegrass, a diploid high water soluble carbohydrate) cultivar (Abermagic), a diploid control cultivar (Alto) and a tetraploid control cultivar (Base), during measurement periods in spring (84 days), autumn (99 days) and later spring summer (160 days).

	Ryegrass cultivar				
Season	Aber	Alto	Base	P	$LSD_{0.05}$
Spring	4130	3110	3880	0.122	1035
Autumn	2140	2570	2440	0.363	653
Summer	5110	4930	5410	0.656	1173

Dry matter intake calculated from herbage accumulation

In addition to the digesta marker estimates, dry matter intake was also calculated from total herbage accumulation measured using grazing exclosure cages, and the total number of sheep grazing days (this is equivalent to average stocking rate when expressed on a daily basis by dividing by the number of days of stocking) during that season (Table 14). This estimates feed consumption per plot over the full duration of stocking, in contrast to the estimates for individual sheep using digesta markers over two 5 day periods in each season. There were no significant differences among cultivars in DMI in any season using this method. Estimates of DMI were high in autumn, compared with the digesta marker estimates (2.32 kg DM/hd/d compared with 1.4 and 1.5 kg DM/hd/d for the alkane and titanium estimates, respectively). For late spring-summer the pasture-based estimates were very similar to the marker estimates (mean DMI of 1.4, 1.3 and 1.3 kg DM/hd/d for alkane, titanium and pasture-based estimates, respectively). In spring, the pasture-based estimate was 1.4 kg DM/hd/d compared with 1.7 kg DM/hd/d for the alkane-based estimate (the titanium-based estimate was higher than can be explained, as was discussed earlier.

Table 14 Dry matter intake, calculated from total herbage accumulation (kg DM/ha) and average stocking rate (sheep/ha/day), of sheep grazing one of three cultivars of perennial ryegrass, a diploid high water soluble carbohydrate) cultivar (Abermagic), a diploid control cultivar (Alto) and a tetraploid control cultivar (Base), during measurement periods in spring (84 days), autumn (99 days) and late spring-summer (160 days).

	Ryegrass cultivar				
Season	Aber	Alto	Base	P	$LSD_{0.05}$
Spring	1.49	1.30	1.51	0.443	0.393
Autumn	2.14	2.33	2.49	0.336	0.512
Summer	1.32	1.34	1.44	0.627	0.283

REGIONAL PASTURE COMPOSITION

Cultivar effects

With the exception of year 2 in Manawatu where there was no significant difference among cultivars, Abermagic consistently had a significantly higher concentration of WSC than either the diploid or tetraploid controls (Tables 15, 16, 17 and 18). This greater concentration in Abermagic ranged from an additional 46 g WSC/kg DM in Southland in year 1, to 28 g WSC/kg DM in Waikato, also in year 1.

The higher concentration of WSC in Abermagic was offset by consistently lower concentrations of NDF than the diploid or tetraploid controls at each site. The differences among cultivars in CP was low and for the few instances where there were significant differences among cultivars in CP the concentrations were lower in Abermagic than in the diploid or tetraploid controls.

Nitrogen effects

With the exception of year 2 in Manawatu where the level of N fertiliser applied did not affect the concentration of WSC, higher applications of N fertiliser resulted in lower concentrations of WSC. This difference in WSC between levels of N was greatest in Canterbury (an additional 52 and 39 g WSC/kg DM in year 1 and 2, respectively, at the higher level of N) where the contrast between the levels of N fertiliser applied was also greatest (the high N treatment received 225 kg N/ha more than the low N treatment in Canterbury compared with a difference between N levels of 175 kg N/ha at the other 3 sites).

With the exception of Manawatu in year 2, the higher level of N fertiliser resulted in a greater concentration of CP and with the exception of Canterbury and Southland in year 1, lower concentrations of NDF.

Year effects

Concentrations are reported separately for year 1 and year 2, and they were not statistically compared. Differences between years were comparatively small and not consistent across the different sites. Only for Waikato did the differences in concentrations between year 1 and year 2 exceed 10% of the mean. At that site the concentrations of WSC (overall mean of 290 g WSC/kg DM in year 2 and 259 g WSC/kg DM in year 1) and NDF (overall mean of 481 g NDF/kg DM in year 1 and 418 g NDF/kg DM in year 2) were greater in year 2 than in year 1, and the concentration of CP was lower in year 2 compared with year 1 (145 g CP/kg DM in year 1 and 172 g CP/kg DM in year 2).

Region effects

The overall means across both years of the three main constituents (WSC, NDF and CP) differed little among the four regional sites. The concentrations of WSC were higher in Waikato (275 g WSC/kg DM) and lower in Manawatu (246 g WSC/kg DM) compared with the other two sites (269 and 265 g WSC/kg DM for Canterbury and Southland, respectively.

Crude protein concentrations were higher in Southland (181 g CP/kg DM) than in the other three regions (159, 166 and 163 g CP/kg DM for Waikato, Manawatu and Canterbury, respectively).

Table 15 The effect of ryegrass cultivar and level of nitrogen (N) fertiliser applied on the annual mean concentrations of water soluble carbohydrates (WSC), crude protein (CP), neutral detergent fibre (NDF), lipid and ash in three cultivars of perennial ryegrass for two years in Waikato (Year 1 = 2013, and Year 2 = 2014).

Region	Year	Effect		WSC	CP	NDF	Lipid	Ash
Waikato	1	Cultivar	Abermagic	278	172	399	24	78
			Alto	253	172	424	22	80
			Prospect	247	174	431	23	81
			P	0.0004	0.722	<0.0001	0.003	0.032
		Nitrogen	High N	240	193	404	23	85
			Low N	279	151	432	22	75
			P	0.0004	<0.0001	0.0007	0.532	<0.0001
	2	Cultivar	Abermagic	313	142	459	25	72
			Alto	278	147	494	24	76
			Prospect	278	145	490	24	77
			P	<0.0001	0.035	<0.0001	0.136	0.002
		Nitrogen	High N	284	157	468	25	77
			Low N	295	133	494	23	73
			P	0.142	0.008	0.014	0.008	0.069

Table 16 The effect of ryegrass cultivar and level of nitrogen (N) fertiliser applied on the annual mean concentrations of water soluble carbohydrates (WSC), crude protein (CP), neutral detergent fibre (NDF), lipid and ash in three cultivars of perennial ryegrass for two years in Manawatu (Year 1 = 2013, and Year 2 = 2014).

Region	Year	Effect		WSC	CP	NDF	Lipid	Ash
Manawatu	1	Cultivar	Abermagic	274a	160b	408b	25a	78b
			Alto	243b	162ab	441a	23b	81a
			Prospect	236b	166a	445a	23b	82a
				<0.0001	0.01	<0.0001	0.007	0.001
		Nitrogen	High N	242	172	424	23	82
			Low N	260	153	439	25	78
				0.009	0.043	0.035	0.044	0.024
	2	Cultivar	Abermagic	253	172	446	28	79
			Alto	234	172	464	26	82
			Prospect	236	172	467	27	81
				0.10	0.99	0.06	0.25	0.37
		Nitrogen	High N	232	186	447	27	84
			Low N	250	158	472	27	77
				0.21	0.11	0.11	0.95	0.11

Table 17 The effect of ryegrass cultivar and level of nitrogen (N) fertiliser applied on the annual mean concentrations of water soluble carbohydrates (WSC), crude protein (CP), neutral detergent fibre (NDF), lipid and ash in three cultivars of perennial ryegrass for two years in Canterbury (Year 1 = 2013, and Year 2 = 2014).

Region	Year	Effect		WSC	CP	NDF	Lipid	Ash
Canterbury	1	Cultivar	Abermagic	295	158	445	19	71
			Alto	259	162	472	17	75
			Prospect	251	160	482	17	76
				<0.0001	0.622	0.0001	0.009	0.002
		Nitrogen	High N	243	177	464	19	80
			Low N	294	143	468	17	68
				0.027	0.01	0.562	0.077	0.006
	2	Cultivar	Abermagic	294	162	450	22	74
			Alto	259	168	483	20	78
			Prospect	253	169	486	20	79
				<0.0001	0.048	<0.0001	0.0004	0.0003
		Nitrogen	High N	249	188	457	22	83
			Low N	288	145	488	19	71
				0.021	0.022	0.001	0.025	0.013

Table 18 The effect of ryegrass cultivar and level of nitrogen (N) fertiliser applied on the annual mean concentrations of water soluble carbohydrates (WSC), crude protein (CP), neutral detergent fibre (NDF), lipid and ash in three cultivars of perennial ryegrass for two years in Southland (Year 1 = 2013, and Year 2 = 2014).

Region	Year	Effect		WSC	CP	NDF	Lipid	Ash
Southland	1	Cultivar	Abermagic	299	185	411	17	77
			Alto	257	194	436	15	84
			Prospect	250	197	442	16	86
				<0.0001	0.015	<0.0001	0.026	0.0001
		Nitrogen	High N	260	198	430	17	84
			Low N	278	186	429	15	80
				0.011	0.021	0.658	0.001	0.062
	2	Cultivar	Abermagic	286	165	452	26	75
			Alto	249	175	476	25	81
			Prospect	249	172	481	25	81
				<0.0001	0.031	<0.0001	0.281	0.0003
		Nitrogen	High N	254	183	456	28	82
			Low N	268	158	484	23	76
				0.026	0.014	0.012	0.011	0.024

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