# The chemical composition of high-sugar and control ryegrasses in grazed pastures at different latitudes throughout New Zealand

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## Abstract

Extrapolating from single-site animal studies of the effects of water soluble carbohydrate (WSC) on methane and nitrogen emissions requires knowledge of geographical and temporal variation in plant chemical constituents. To provide this, samples of grazed pasture were collected from experiments at four different latitudes over one year. At each site, one high-sugar perennial ryegrass (HSG) and two control cultivars of perennial ryegrass, were sampled at each grazing during a 12-month period and analysed for concentrations of WSC, crude protein (CP) and fibre (NDF). Compared with the controls the HSG was higher in WSC (overall mean 299 vs 260 g WSC/kg DM; P<0.01 at each site), lower in NDF (426 vs 460 g NDF/kg DM; P<0.01 at each site) but did not differ in CP. There were significant differences among sites (P<0.001) with highest concentrations of WSC in Canterbury followed by Southland, Waikato and Manawatu (295, 278, 266 and 254 g WSC/kg DM, respectively). Concentrations of WSC were highest in spring and approximately double those in autumn. Animal responses to cultivars with a higher concentration of WSC will be tempered by these large natural variations, which must be accounted for when extrapolating or scaling-up to regional or national outcomes.

**Keywords:** water soluble carbohydrate, perennial ryegrass, protein, fibre, latitude, variation

## Introduction

High-sugar perennial ryegrasses (*Lolium perenne*; HSGs), selected for elevated concentrations of water soluble carbohydrate (WSC; sugar) can increase animal liveweight gain (Lee *et al*. 2001), milk production (Cosgrove *et al*. 2007; Miller *et al*. 2001b) and improve the utilisation of dietary protein, thereby reducing nitrogen excretion (Pacheco *et al*. 2007, 2009; Miller *et al*. 2001a), under some circumstances. Whether these benefits occur depends on many factors including dry matter (DM) yield, DM intake and sward structural characteristics (Wims *et al*. 2013).

Recent data from the United Kingdom (DEFRA 2010) and New Zealand (Jonker *et al*. 2014) suggests that the high-sugar trait may also reduce methane emissions. In a review, Janssen (2010) proposed mechanisms by which higher sugar may reduce emissions. These included a reduction in rumen pH, high propionate fermentation, and an increase in the rate of passage of digesta from the rumen, conditions which arguably would occur in the rumen of animals consuming an HSG diet.

However, plant constituents other than just WSC may also affect methane and nitrogen emissions. The direct effects of WSC may be influenced by the absolute concentrations of WSC, not just the increment provided by the high-sugar trait, and by the associated reductions in concentrations of protein and/or fibre that result when WSC increases. Using a modelling approach to account for the combined effects of multiple plant constituents Ellis *et al*. (2011, 2012) indicated methane emissions could increase or decrease depending on whether higher WSC is offset by reduced protein or by reduced fibre.

To extrapolate *in vivo* data on methane emissions (e.g. for national inventory purposes) requires data on the variation in plant constituents that is likely to occur, and the extent to which the high-sugar trait in HSG ryegrasses is expressed under differing conditions. To provide these data the chemical composition of one high-sugar cultivar and two cultivars of perennial ryegrass which had not been selected for elevated WSC were measured in grazed pastures at four sites, representing a range of latitudes in New Zealand. Latitude was selected as the experimental factor to provide environmental variation because it embodies differences in temperature and solar radiation, known to influence WSC accumulation and storage in ryegrasses (Parsons *et al*. 2004). This paper reports the seasonal profile in the concentration of WSC in HSG and control perennial ryegrass cultivars growing at different latitudes.

## Materials and Methods

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 New Zealand Plant Breeding and Research Association.

### Sites

The lowland trial sites (<50 m above mean sea level) 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 × two levels of white clover × eight cultivars of perennial ryegrass × 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 × no clover main plots and samples were taken from three of the five replications (nine plots). These cultivars, selected on the basis of similar heading dates, were the high-sugar diploid perennial ryegrass cultivar 'Abermagic' (flowering date +15 days relative to 'Nui') with AR1 endophyte (89% endophyte infection based on a grow-out test) and control diploid perennial ryegrass cultivars 'Alto' (+14 days) and 'Prospect' (+12 days) each with AR37 endophyte (85% and 75% endophyte infection, respectively). The low N treatment received 50 kg N/year applied as two applications of urea at 55 kg each, in autumn and spring. The Canterbury site received 100 kg N/year, reflecting the higher rates typically used under irrigation in this region. Main plots were individually fenced but cultivars within plots were grazed in common. 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 dry matter (DM)/ha, 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 and 1500 h from an area of approximately 100 mm × 80 mm at 10 sites randomly within each plot using hand or electric shears to 40 mm above ground level and composited into a single sample. These samples, comprising about 100 g fresh

**Table 1** The annual mean maximum and minimum air temperatures, mean daily total solar radiation and annual total rainfall for 2013 compared with the 30-year means (1984-2013) and standard deviations (SD), at each of four sites.

| | | Mean max °C | Mean min °C | Solar rad MJ/m² | Rainfall mm |
|---|---|---|---|---|---|
| Waikato | 2013 | 20.2 | 8.9 | 14.7 | 975 |
| | 30-yr mean | 19.0 | 8.8 | 14.2 | 1145 |
| | SD | 0.58 | 0.46 | 0.45 | 136.7 |
| Manawatu | 2013 | 18.9 | 9.0 | 14.2 | 842 |
| | 30-yr mean | 17.7 | 8.7 | 13.7 | 996 |
| | SD | 0.63 | 0.43 | 0.61 | 143.8 |
| Canterbury | 2013 | 17.5 | 7.7 | 13.9 | 762 |
| | 30-yr mean | 16.9 | 6.9 | 13.6 | 608 |
| | SD | 0.55 | 0.45 | 0.55 | 127.3 |
| Southland | 2013 | 15.6 | 6.5 | 12.4 | 1081 |
| | 30-yr mean | 14.6 | 5.8 | 11.5 | 1078 |
| | SD | 0.50 | 0.40 | 0.62 | 127.7 |

weight for each plot, were immediately frozen in liquid nitrogen, stored frozen and subsequently freeze-dried and ground to pass a 1 mm 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 (calibrated using the anthrone method (Thomas 1977) to determine the concentrations of high- and low-molecular weight sugars), crude protein (CP) and neutral detergent fibre (NDF).

### Data analysis

Within site, the ryegrass cultivar effect on annual mean concentrations of WSC, CP and NDF (2 d.f.) was determined by ANOVA (SAS), using the replicate × cultivar interaction (4 d.f.) as error term. Site effects (3 d.f.) and the site × cultivar interaction (6 d.f.) were tested using the replicate within site interaction. Seasonal differences in WSC were not statistically compared, but an average least significant difference (LSD; P=0.05) for each site was derived from a repeated measurements analysis of cultivar effects over the successive sampling dates. This LSD can help identify the occurrences of cultivar differences through the year.

## Results

### Climatic conditions

The 2013 annual mean maximum and minimum air temperatures, solar radiation and rainfall and the 30-year means are shown in Table 1. Across all sites the 2013 mean maximum temperature was warmer than the long-term means (based on variations from the mean of greater than one standard deviation), as was the mean minimum in Canterbury and Southland, and solar radiation was higher than the mean in Waikato and Southland. Mean temperatures and solar radiation decreased as latitude increased. For Waikato and Manawatu, 2013 was drier than the long-term mean, whereas for Canterbury it was wetter.

### Cultivar effects

'Abermagic' had a higher annual mean concentration of WSC than 'Alto' or 'Prospect', consistently highly significant (P<0.01) at all sites (Table 2). The additional sugar in the HSG ranged from 34.5 g/kg DM in Waikato to 47 g/kg DM in Southland. In contrast to WSC, the concentration of NDF was lower in 'Abermagic' than in either of the control cultivars, again consistently highly significant (P<0.01) at all sites. Differences among cultivars in CP were much smaller in comparison and not significant (P>0.05).

### Site effects

There were significant differences among sites (P<0.001) in the mean concentrations of WSC (Table 2), in the order of Canterbury (295 g WSC/kg DM), Southland (278 g/kg DM), Waikato (266 g/kg DM) and Manawatu (254 g/kg DM). For CP, concentrations in Southland were significantly higher, and in Canterbury significantly lower, than Waikato or Manawatu (P<0.001). For NDF, concentrations in Canterbury were significantly higher, and in Southland significantly lower, than in Waikato or Manawatu (P<0.001). For WSC, CP and NDF the ranking of cultivars was similar at all sites (site × cultivar interaction not significant, P>0.05).

### Seasonal effects

The seasonal profiles in the concentrations of WSC at

**Table 2** The annual mean concentrations (g/kg DM) of water soluble carbohydrate (WSC), crude protein (CP) and neutral detergent fibre (NDF) in three cultivars of perennial ryegrass in grazed pastures at each of four sites.

| Site | Cultivar | WSC | CP | NDF |
|---|---|---|---|---|
| Waikato | 'Abermagic' | 289a[1] | 149 | 429a |
| | 'Alto' | 255b | 156 | 456b |
| | 'Prospect' | 254b | 153 | 461b |
| | Mean | 266y[2] | 153x | 449x |
| | P | 0.005 | 0.233 | <0.001 |
| | $LSD_{0.05}$ | 15.5 | 8.8 | 8.5 |
| Manawatu | 'Abermagic' | 280a | 152 | 418a |
| | 'Alto' | 248b | 156 | 454b |
| | 'Prospect' | 235b | 157 | 461b |
| | Mean | 254z | 155x | 444x |
| | P | <0.001 | 0.074 | <0.001 |
| | $LSD_{0.05}$ | 9.4 | 4.2 | 8.2 |
| Canterbury | 'Abermagic' | 319a | 138 | 447a |
| | 'Alto' | 285b | 141 | 481b |
| | 'Prospect' | 279b | 136 | 488b |
| | Mean | 295w | 139y | 472w |
| | P | 0.005 | 0.305 | 0.002 |
| | $LSD_{0.05}$ | 16.5 | 7.9 | 13.3 |
| Southland | 'Abermagic' | 309a | 180 | 409a |
| | 'Alto' | 263b | 189 | 437b |
| | 'Prospect' | 261b | 189 | 442b |
| | Mean | 278x | 186w | 429y |
| | P | 0.002 | 0.229 | 0.004 |
| | $LSD_{0.05}$ | 17.1 | 14.0 | 12.4 |
| | P (site means) | <0.001 | <0.001 | <0.001 |
| | $LSD_{0.05}$ (site means) | 11.1 | 5.7 | 7.3 |

[1] Cultivar means within site with different letters (a or b) differ significantly P<0.05

[2] Site means with different letters (w, x, y or z) differ significantly P<0.05

each site are shown in Fig 1. While there were occasions at each site where ‘Abermagic’ was not significantly higher than the other cultivars, there was only a single occasion, in early spring in Waikato, where it was significantly lower ($P<0.05$) than one of the control cultivars (‘Alto’). Concentrations in all cultivars showed a similar seasonal profile, reaching their lowest concentrations during autumn (approximately 200 g WSC/kg DM) and their highest concentrations during the spring (approximately 350 g WSC/kg DM). The timing of these annual troughs and peaks tended to be later as latitude increased.

## Discussion

It is notable that the mean concentration of WSC across the year for all cultivars at all sites was relatively high at 273 g WSC/kg DM. Moller *et al*. (1996) and Stevenson *et al*. (2003) reported overall mean concentrations of approximately 120 g WSC/kg DM and 235 g WSC/kg DM, respectively for dairy pastures, and Easton *et al*. (2009) and Hume *et al*. (2010) recorded overall means of 231 and 216 g WSC/kg DM, respectively, from swards intermittently defoliated by cutting or sheep grazing. Parsons *et al*. (2004) reported an annual profile of concentrations that ranged from 60 to 180 g WSC/kg DM, with an annual mean of approximately 120 g WSC/kg DM, in continuously stocked sheep pastures in Manawatu. The high concentrations in the current study may be attributable to the weather conditions prevailing during the year of measurement, particularly the higher solar radiation and lower rainfall (Roche *et al*. 2009), and possibly also the comparatively low application rate of nitrogen fertiliser.

The overall annual mean concentration of WSC in the HSG, ‘Abermagic’ (299 g WSC/kg DM) was higher than in either of the control cultivars (mean 260 g WSC/kg DM), an overall mean increase of 39 g WSC/kg DM. This greater accumulation of WSC in the HSG is at the upper end of the 20–40 g/kg DM additional sugar that has been recorded in other trials in New Zealand (Cosgrove *et al*. 2007; Easton *et al*. 2009; Hume *et al*. 2010) and elsewhere (Halling *et al*. 2005; Taweel *et al*. 2005). The seasonal profile indicates that the level of expression of high WSC in the HSG varied at the different sampling time-points, and at times was not significant. There was no apparent consistency in this across seasons or sites, and differences among cultivars in developmental stage when grazed (Bryant *et al*. 2012; Rasmussen *et al*. 2009), or in their response to environmental conditions (Parsons *et al*. 2004), may contribute to the observed differences in expression of the high-sugar trait.

This enhanced expression of WSC accumulation was greatest in Southland (+47 g WSC/kg DM) and least in Waikato (+34.5 g WSC/kg DM). While the

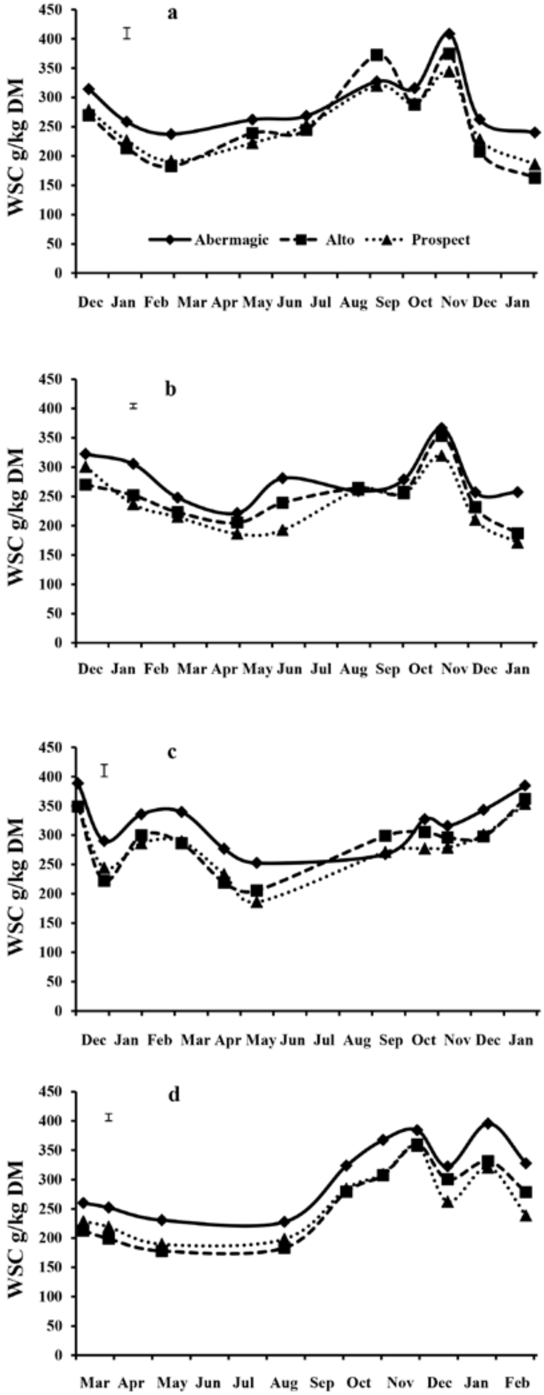


**Figure 1** Seasonal profiles in the concentration of water soluble carbohydrate in three cultivars of perennial ryegrass in grazed pasture at four sites: a) Waikato, b) Manawatu, c) Canterbury and d) Southland (note different X-axis range for Southland reflecting the spring sowing). The average $LSD_{0.05}$ shown for each site is Waikato 19.1, Manawatu 8.2, Lincoln 20.7 and Southland 12.5.

regional differences in expression of additional WSC were modest in absolute amounts, the proportionately greater expression at the higher latitude South Island sites is consistent with expected responses to cooler temperatures, lower solar radiation and greater seasonal contrasts in day length, as demonstrated under controlled-environment conditions (Parsons *et al*. 2004).

The annual mean concentrations of WSC were 266, 254, 295 and 278 g/kg DM in Waikato, Manawatu, Canterbury and Southland, respectively. There are few published studies comparing concentrations measured throughout a year across multiple sites. Easton *et al*. (2009) recorded higher mean concentrations of WSC of 253 g/kg DM in Canterbury compared with 208 g/kg DM in Manawatu. Hume *et al*. (2010) also recorded significantly higher (+32 g WSC/kg DM) concentrations in Southland than in Manawatu in spring, but not in autumn.

As anticipated, the seasonal variation in WSC was large and consistent across sites. Concentrations in spring (up to 350 g WSC/kg DM) were nearly double those in autumn (200 g WSC/kg DM). This difference between spring and autumn is consistent with some previous field studies (Moller *et al*. 1966; Parsons *et al*. 2004; Cosgrove *et al*. 2009; Easton *et al*. 2009), but differs from the very low seasonal variation recorded by Stevenson *et al*. (2003).

Higher concentrations of WSC are, by definition, offset by lower concentrations of other primary constituents, often fibre and/or crude protein. In this study, higher WSC due to cultivar effects was offset mainly by reductions in the concentration of NDF and the effects on CP were small by comparison. In contrast, the effects on NDF and CP associated with regional differences in concentration of WSC were less consistent. In Canterbury the high WSC was associated with high NDF but low CP. In previous analyses of the changes in composition associated with higher WSC, reductions in CP of 0.5 units for each unit increase in WSC were greater than for NDF (0.25 units for each unit increase in WSC) and greater than simple dilution alone would explain under field conditions (Cosgrove *et al*. 2009), but under controlled-environment conditions greater for NDF than for CP, and largely explained by simple dilution (Rasmussen *et al*. 2009). These associated changes in other constituents have important nutritional consequences (e.g. DM intake increases as NDF reduces; Lee *et al*. 2001) and environmental consequences (e.g. N excretion reduces as CP reduces; Miller *et al*. 2001a; Pacheco *et al*. 2007, 2009), and may affect methane emissions (Ellis *et al*. 2011, 2012).

The overall mean concentration of CP was low (158 g CP/kg DM), probably a consequence of the high concentrations of WSC. Only in Southland was the annual mean concentration of CP (186 g CP/kg DM) above the accepted minimum for lactating dairy cows (NRC 2001). The concentrations at the other sites were 153, 155 and 139 g CP/kg DM in Waikato, Manawatu and Canterbury, respectively. Given the seasonal pattern in the concentration of crude protein that was the inverse of the pattern for WSC, with lowest concentrations during spring and highest concentrations in autumn (data not presented), it suggests that these pastures were deficient in protein especially during spring. They were sown as ryegrass monocultures, without the high-CP white clover companion species that would normally help elevate overall dietary protein concentrations, providing that the proportion is sufficiently high. However, they were fertilised with N, albeit at a low rate.

The focus in this comparison of ryegrass cultivars growing at different latitudes has been on chemical composition, particularly WSC, as an indicator of nutritive value and to inform the interpretation and extrapolation of animal response data on, for example, methane and nitrogen emissions. The extent to which a cultivar that has been selected for a particular trait contributes to environmental or production goals ultimately depends on the expression of that trait and on many other factors such as DM production, DM intake and grazing management.

## Conclusions

To extrapolate animal responses (e.g., methane, nitrogen emissions) to particular traits such as high-sugar from single-site studies to wider regional- or national-scale effects, it is necessary to quantify the extent of variation in chemical composition at similar scales. Seasonal effects were the greatest source of variation in the concentration of WSC, followed by regional and then cultivar differences. The effects of one factor, e.g., cultivar traits, must be considered with reference to other factors such as season and region. With an increasing capacity to modify plant composition there is, correspondingly, an increasing need for animal response studies to determine the optimum concentrations of primary constituents, and identify targets for future improvement to rectify excesses and deficits.

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