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Review

High-sugar grasses

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Abstract

Perennial ryegrass diploids with elevated concentrations of water-soluble carbohydrate (WSC), commonly termed 'high-sugar grasses' (HSGs), have been promoted as a tool for increasing the efficiency of the use of protein (nitrogen (N)) in the rumen and thus offering scope for increasing milk production and animal growth rates, while reducing N losses (in the form of urine) to the environment. Much controversy has arisen about efficacy, largely because benefits have not been seen in all trials, and partly because of variation in the degree to which the sugar trait has been present, or expressed, in several cases reflecting gene×environment or gene×management interactions. Combining the data from multiple trials shows that there is a continuum of response in N-use efficiency (NUE) to WSC:crude protein (CP) ratio, and so 'proof of concept' has been shown. A considerable amount of research is still required to demonstrate a consistently high expression of the trait, and to demonstrate reliably the potential for yield gains and reduced N loss. Associated reductions in nitrous oxide emissions (a potent greenhouse gas (GHG)) are yet to be confirmed. Even more uncertain is the prospect for reducing methane emissions, whether per hectare or per unit energy intake or animal product. Nonetheless, there has been a determined effort to pursue this trait, and to confirm the long-standing hypothesis for NUE. There is no basis as yet for dismissing the prospects of success. The trait remains one of very few low-cost tools being investigated for its potential to mitigate the environmental footprint of livestock production.

Review Methodology: CAB abstracts, Agricola and SciQuest were used as the primary databases for the search. In addition, references were followed through the papers identified by using search engines. Reports on commissioned research were identified by contacting key scientists and have been included.

Introduction

Increasing the readily available metabolizable energy (ME) content of grass forage has been a major goal for plant breeding for livestock production since the 1960s [1–3], so as to meet established feeding standards [4]. Environmental concerns now pose a wider challenge [5]. One commendably determined approach to achieving these combined goals has been the breeding at the Institute for Grassland and Environmental Research (IGER), UK [6, 7] of diploid perennial ryegrass strains with elevated levels of

water-soluble carbohydrates (WSCs) in the harvestable component, i.e. the leaves [8, 9]. It is these cultivars that have been termed 'high-sugar grasses' (HSGs), by IGER, with the aim of increasing WSC content beyond the 4 percentage points (an added 40 g WSC/kg dry matter (DM)), which is the minimum expected to lead to a detectable significant change in animal performance. *Lolium perenne* 'naturally' accumulates high-molecular-weight (HMW) sugars, typically fructans, in the sheath/pseudostem bases, below the grazing height. These provide a buffer to resource regrowth following severe

defoliation [10, 11], and show marked seasonal patterns of accumulation (during summer, autumn and winter) and re-mobilization (during early spring) in cool temperate climates [12]. The capacity to accumulate long-term storage sugar implies that carbon (C) supply from photosynthesis might not be at all times the primary limitation to growth, and so that stimulating sugar accumulation might not be at the expense of total grass yield [13]. The initial focus of breeding was to increase the levels of fructan expressed, now, in the grazeable horizon, in the leaf blades, and so in the diet of grazing ruminants. The HSGs have been shown in many cases to have higher concentrations, also, of low-molecular-weight (LMW) sugars, although major differences in metabolic profiles between HSGs compared with 'control' grasses arise from interactions with temperature, nutrient supply and, notably, endophyte concentration and strain [14–16].

For the livestock industry in the twenty-first century, with its goals of both sustainable food production and reduced environmental impact, the hypothesis for the benefits of a high-WSC diet has rested on how an increased supply of C-skeletons and energy to the rumen microbial population could improve the protein (nitrogen)-use efficiency (NUE) of the rumen, and so increase the supply of protein to the ruminant in the form of microbial protein. An increased capture and utilization of protein in the rumen/ruminant system would be expected to increase performance (milk yield or LWG) per animal, in systems where animal production *per se* is protein-limited, and simultaneously reduce the release of N in labile form (urine) to the environment.

In ruminants, a significant proportion of the N in plant protein in the diet can be lost (predominantly through urine) if the micro-organisms present in the rumen are unable to utilize N released rapidly during the degradation of plant proteins following ingestion [17]. It is proposed that feeding a diet containing a high concentration of readily digestible WSC has the potential to address an imbalance (or asynchrony) in the rate of energy and protein supply for microbial synthesis [17–19]. Improved assimilation of dietary N into rumen microbes results in greater microbial protein flow to the duodenum and therefore an increased supply of protein to the ruminant [20]. It also decreases the production and concentration of ammonia in the rumen, and hence reduces the proportion of N eaten that is returned to the soil in the form of urine [20], thereby avoiding the potential for N losses to the environment – either as nitrate in leaching or nitrous oxide into the atmosphere [21].

Concerns about N in the environment and the potency of nitrous oxide as a greenhouse gas (GHG) with global warming potential (GWP) c. 300 times that of carbon dioxide have increased not only the importance but also the urgency of the research. More recently, interest has focused on how diets of HSG might alter the release of another potent GHG, methane (GWP 25, but of greater total emission).

This summary is based on the text of a comprehensive paper on HSG presented at the Australasian Dairy Science Symposium [22] and a second published by the New Zealand Grassland Association [23]. It incorporates information from a searching review of success in plant breeding programmes [24], and a synthesis of some recently published data on GHG mitigation prospects from HSG [25–28].

Evaluation for Animal Production and Improved NUE

An extensive programme of indoor feeding and field trials was instigated by IGER, using material bulked up into breeding lines, some of which later received cultivar names, and early cultivars [6, 29, 30]. The first cultivar designated widely as being a HSG was AberDove (c. late 1990s and initially 'ba11353'), and the first marketed explicitly as a HSG, was AberDart (c. 2000). These trials compared the selected 'high' population with, in most cases, a 'control' cultivar of 'normal' sugar content. Care must be taken in interpreting experiments in which efficacy is not compared against such a 'control'. Also, in interpreting the results, it is critical to bear in mind to what extent the sugar trait (having higher sugars) was actually expressed at that time. Some trials (outside IGER) simply compared a range of cultivars and retrospectively assessed, which had higher sugar content.

Extensive and thorough field trials, looking for gains in both dairy and liveweight animal sectors, were established in the UK and Netherlands; research was also done in other countries, particularly New Zealand. (For a detailed review of the results and interpretation for each study, see Edwards *et al.* [22, 23] and Table 1.)

For animal performance, early results in the UK looking at milk production in dairy cows [20, 31–33] (see also [34, 35]), were promising, although only one out of the four published studies [20] showed a statistically significant increase in milk yield when cows were fed with an *a priori*-designated HSG (AberDove) compared with a control grass (AberElan). This was the case even though the three other studies used 'time of day' differences in sugar content, or the application of extra N fertilizer to the 'control' grass, to accentuate (intentionally and objectively) differences in sugar and protein content of the forage diet. In the one case where milk yield was significantly greater when cows were fed 'HSG' [20], the authors concluded that milk yield had been increased, notably because of a higher overall digestibility in the HSG cultivar (relative to an uncommonly low digestibility in the control grass in this trial, under the cut-and-carry feeding regime used) and this had led to greater total intake of digestible DM when fed AberDove (HSG), rather than AberElan.

Very substantial efforts were made to explain the apparent lack of confirmation of the hypothesis in a subsequent series of both indoor and outdoor feeding/grazing

Table 1 Summary of the effects of perennial ryegrass cultivars with either high (H) or low (L) water WSC content on herbage intake, liveweight gain, milk yields and utilization of dietary nitrogen (N). Bold pairs in adjacent columns are significantly different from each other, $P<0.05$. From [23]. Reproduced with permission of New Zealand Grassland Association

Source	Country	WSC content (g/kg DM)		Herbage intake (kg DM/day)		Liveweight gain (g/day)		Milk yield (kg/day)		Milk N (% of N intake)		Urine N (% of N intake)	
		H	L	H	L	H	L	H	L	H	L	H	L
Dairy													
[20]	UK	**165**	**126**	11.6	10.7			**15.3**	**12.6**	**0.30**	**0.23**	0.25	0.35
[32][1]	UK	**236**	**166**	17.6	17.0			25.1	26.7	**0.26**	**0.21**		
[31][1]	UK	**234**	**194**	14.6	14.8			21.4	21.9	**0.29**	**0.24**	**0.17**	**0.26**
[33][1]	UK	**243**	**161**	**15.3**	**13.1**			32.7	30.4	0.36	0.37	**0.20**	**0.27**
[37] LS1, 2000	Neth	**192**	**158**	16.2	17.4			26.9	26.3	**0.28**	**0.25**	0.50	0.53
[37] LS1, 2001	Neth	**131**	**93**	14.7	14.9			24.7	23.8	0.23	0.24	0.56	0.53
[37] LS2, 2000	Neth	**195**	**152**	16.1	16.6			26.8	28.2	0.29	0.28	0.47	0.48
[37] LS2, 2001	Neth	113	98	13.7	14.7			22.5	23.9	0.23	0.22	0.55	0.56
[38] 2002	Neth	**144**	**110**	18.0	15.6			**28.8**	**26.0**	0.25	0.25		
[38] 2003	Neth	**131**	**87**	18.4	17.4			25.7	25.2	0.20	0.19		
[43] S, 2004	NZ	**200**	**167**					20.9	20.9				
[43] S, 2005	NZ	**215**	**195**					25.5	25.1				
[43] A, 2006	NZ	170	161					11.3	9.6				
[43] A, 2007	NZ	159	150					**11.7**	**11.0**				
Beef													
[81][2]	UK	90.9	55	**4.3**	**3.6**								
[82]	UK	**243**	**161**	**9.3**	**6.7**								
Lamb													
[44][3], C, I	UK	**143**	**89**	1.0	1.2	**312**	**271**						
[44][3], C, II	UK	**113**	**75**	1.7	1.3	**244**	**194**						
[44][3], C, III	UK	92	84	1.1	1.2	186	175						
[46][4], C	UK	**115**	**100**			47.1	51.5						
[46][4], R	UK	**113**	**100**			98.4	71.7						

Neth, Netherlands; UK, United Kingdom; NZ, New Zealand; LS, Latin square trials 1 or 2; C, continuous grazing; R, rotational grazing; S, Spring, A, Autumn.

Values from [37, 38] are for the highest and lowest WSC cultivars in each year from 2000 to 2004.

[1]These studies used time of day, or N fertilizer, to augment differences in WSC and CP content of the diet.

[2]Perennial ryegrass silage diet.

[3]Data from suckling lambs in consecutive periods (I, II and III).

[4]Data from weaned lambs.
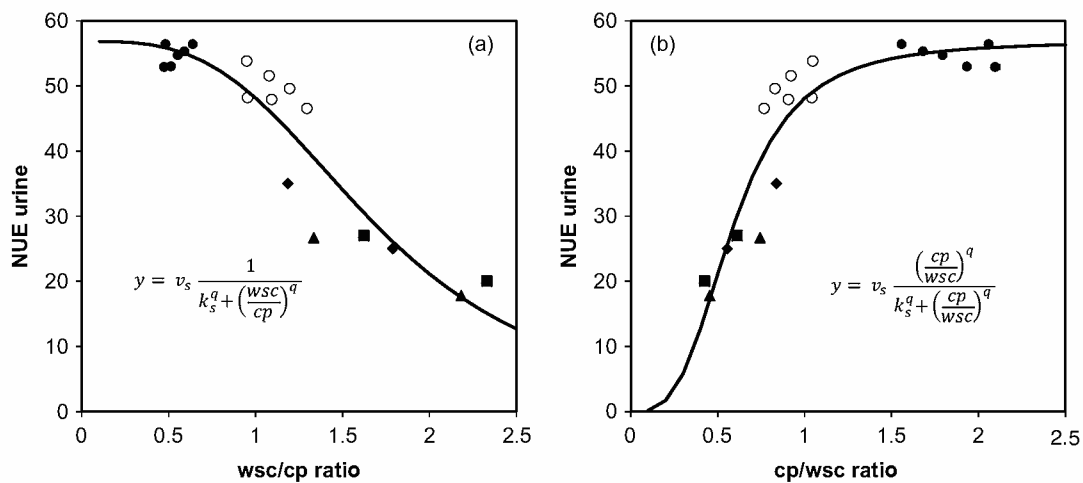
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Figure 1   NUE (N excreted as urine per unit of N eaten) plotted (a) in relation to the WSC:CP ratio and (b) the same data in relation to the inverse, CP:WSC ratio, of the diet. The two plots highlight the problem of fitting lines and interpreting from ratios. The solid lines (equations inset) give the same statistical match regardless of which way the ratio is expressed. Data sources: ●, 2001 data from [36, 37]; ○, 2000 data from [36, 37]; ▲, [31]; ◆, [20, 32]; ■, [33]. Maths courtesy of Jonathan Newman (University of Guelph). From [24], reproduced with permission of Grass and Forage Science, Wiley-Blackwell

studies with dairy cows in the Netherlands [36–41] (and see [42]) using a range of either four or six (in some trials) or eight (in others) cultivars that were shown to have some significant differences in WSC content. Despite determined efforts to test for differences in milk yield between cows fed cultivars chosen for the greatest differences in sugar content, only one trial, in one year of the trial ([38], data for 2002) showed a significant effect of eating grass with its contemporary higher sugar content, in increasing milk yield (see Table 1).

In New Zealand, Cosgrove et al. [43] obtained a marginally significant increase in milk yield between a HSG cultivar AberDart, and a New Zealand standard cultivar 'Impact', on only one occasion ([43], autumn, Table 1), but this was at a time when there was no significant difference in sugar content between the cultivars, ruling out high-sugar content per se as the cause.

In non-lactating animals, significant increases in LWG were seen in two out of three components of a trial using lambs, by Lee et al. [44], in keeping with earlier trials using lamb-grazing cultivars with some differences in WSC content [45]. Trials by Marley et al. [46] showed no significant benefits (see Table 1).

Not only did data collected from within these trials reveal little benefit of the proposed differences in sugar content of the diet on milk yield, there appeared superficially to be no consistent effect on the partitioning of N (NUE). In the UK trials, significant differences in N partitioning were seen (an increased proportion of N eaten was partitioned to milk, and a reduced proportion partitioned to urine) [20, 31–33]. But again, in the Netherlands, there were no significant effects of alleged HSG on the proportion of N eaten partitioned to milk or urine, with the exception of one example in one season ([37],

data from 2000). In the Netherlands, researchers attributed observed changes in total urine N, and proportional N partitioning, to urine, almost entirely to differences in total N intake [47].

Traditionally, in agricultural research, great primacy would be placed on the results derived from farm-based field trials. Research on the topic of the efficacy of HSG diets reveals a sobering thought. Although no clear picture emerged from within any one, or sequence of trials, and the prospects for HSG consequently remained contentious, far greater insights emerged when results were compared between the trials, and so looking for 'proof of concept'.

Synthesis Reveals Strong 'Proof of Concept'

Given the hypothesis concerned, the value of a balance of WSC and protein (N), on N partitioning, a better method to depict the results, to test this premise, was conceived, namely to plot the proportion of N eaten partitioned to milk, and urine, against the ratio of WSC to crude protein (CP), and collate all the results observed.

When all data from the UK and Netherlands trials were taken together and plotted in terms of the WSC:CP ratio of herbage in the diet [22, 23], strong proof of concept was shown. Plotting this way revealed increases in WSC:CP ratio led to a substantial and valuable decrease in the proportion of N eaten that was excreted as urine (Figure 1a) and a relatively small increase in the proportion of N excreted as milk (see [22, 23]). The data reveal a consistent continuum, and hence proof of 'concept' rather than proof of efficacy of any cultivar [24]. What is made clear is that the differences between cultivars (at the time
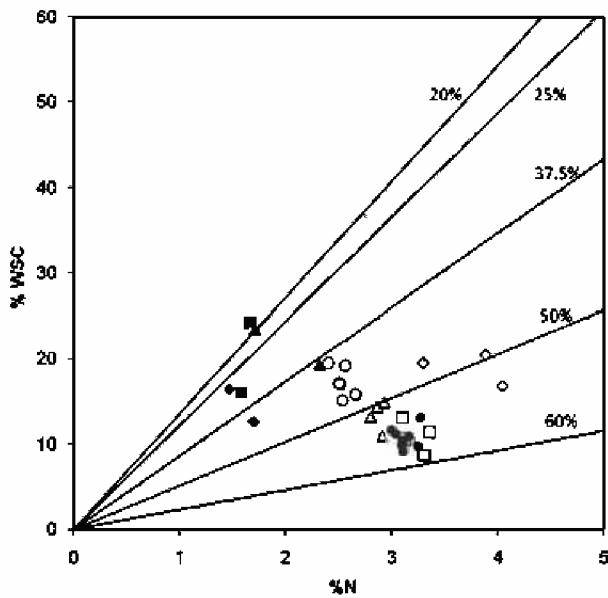
Figure 2 Quantitative targets for breeding high sugar content to reduce N loss in urine. On a 'map' that represents combinations of WSC (on Y-axis) and CP (X-axis, expressed here in terms of herbage N content), we have plotted isolines. Each line represents a single WSC:CP ratio, and so, from Figure 1, a given NUE, shown here as the % of N eaten that is excreted as urine. Superimposed on this 'map' are the combinations of WSC and CP seen in the same trials in UK, Netherlands and New Zealand which were plotted in Figure 1. At low CP (% N) current cultivars achieve low % N loss in urine, but at high CP (e.g. 4% N) sugar content would need to be doubled to achieve the same (c. 25%) N loss in urine. Data sources: ●, 2001 data from [36, 37]; ○, 2000 data from [36, 37]; ▲, [31]; ◆, [20, 32]; ■, [33]; △, □, [38]; ◇, [52]. From [24] reproduced with permission of Grass and Forage Science, Wiley-Blackwell

of those trials) were insufficient to expect significant differences in NUE and (notably more so) in milk yield within any one trial. Moreover, to obtain a desirable low value for the proportion of N eaten excreted as urine can be seen to require a WSC:CP ratio close to 1.5.

This approach can be taken further to develop some much more focused and quantitative 'targets' for plant breeding/manipulation [24]. From the relationships between NUE and WSC:CP ratio seen in Figure 1a, the size of the changes necessary in WSC or CP to create a given size of improvement in NUE, can be calculated. Figure 1a gives an inter-relationship between three dimensions: WSC, CP and the resulting NUE (albeit that two dimensions are collapsed onto one, within the 'x-axis'). A 'graph' (technically a 'map' or 'phase plane') can then be constructed with WSC on one axis, and CP on the other (Figure 2). Any straight line on this map, passing from the origin, represents a single WSC:CP ratio. From the lines fitted to the data, in Figure 1, it is known what that single ratio would mean in terms of the NUE. The graph 'space' can then be filled with a series of iso-lines, each line indicating a single level of NUE to be expected from that

combination of WSC and CP. This enables calculation of how much WSC a forage grass needs to contain to create a given level or change in NUE, and to see how this 'target' changes depending on the level of N in the forage.

Finally, superimposed on this 'map' are the actual combinations of WSC and CP seen in the whole gamut of European Union and New Zealand trials. Current cultivars, and the HSG variants, can already be seen to achieve greater NUE in low nutrient (judged by low forage N) systems, with the elevation of WSC in HSG coming close to delivering the desired WSC:CP ratio of c. 1.5. However, to achieve the same level of (efficient) NUE at higher N requires a far greater increase in WSC content [16, 24]. Whereas to achieve a WSC:CP ratio of 1.5 at 2% N requires a WSC content of 188 g WSC/kg DM, forage at 4% N requires 376 g WSC/kg DM. This is far in excess of the values observed even in HSG in New Zealand (Figure 2). Grasses commonly contain approximately 100–250 g WSC/kg DM. An increase of approximately 40 g WSC/kg DM (as observed in the UK HSG, and in nearly all the trials in the UK and Netherlands) could be effective in grasses with low CP content, but for high-N growing situations (seen more in the Netherlands and New Zealand cases) the calculation suggests that increases of more than 100 g/kg would be required.

Care Needed in Interpretation and Interpolation of Ratios and 'Concentrations'

Biological limits to the scope to repartition the N eaten, between milk, urine, dung (and the small component of N 'retention' in animal tissues) are inevitable and well-recognized [47–50]. The N-partitioning response by the animal is traditionally described in relation to the total daily intake of N (as opposed to a WSC:CP ratio), and there are well-documented analyses of there being upper daily limits to the amount of N that the ruminant can utilize (and so incorporate in milk). The data gathered widely, re-plotted in relation to WSC:CP ratio, and shown with respect to NUE urine in Figure 1a, b, and correspondingly for NUE milk (see [22, 23]), in no way challenge this. The lowest values for the proportion of N eaten being partitioned to milk (NUE milk) in these studies were c. 20%, consistent with the long-standing literature, and occurred at low WSC:CP ratios (c. 0.5), representing a high-protein (N) pasture. In these very same studies, the corresponding NUE urine was c. 55% (Figure 1), and hence about 75% of all N eaten was excreted (urine+milk), the remainder c. 25% voided as dung and a small proportion retained. The highest values of NUE milk were c. 35%, and a corresponding 20% excreted as urine, such that in the highest WSC:CP (cf low protein) diet pastures, only 55% of all N eaten was excreted (milk+urine). It would be ill-advised to extrapolate *outside* the wide range of this data. There is, however, little evidence *within* the range of this data of 'thresholds' to the

scope to re-partition N, usefully, between urine and milk, by altering diet composition *per se*. It is important to note that great care must be taken in fitting (interpolating) and interpreting responses related to ratios, notably where the ratio is close to 1.0 (unity) (for further explanation, see [24]). This is particularly so when some authors prefer to present the inverse (e.g. CP:WSC ratio) as seen in Figure 1b. Inverting the ratio substantially alters the shape of the graph, 'stretching' the graph considerably to the 'right', such that what appears a simple near-linear relationship between NUE milk, for example, and WSC:CP ratio (see [23], Figure 4), for trivial mathematical reasons would become an exponential decline, albeit still a continuum of response. Some authors have proposed to divide this response into two segments, creating the impression of a threshold for the response of NUE milk to diet composition [51, 52]. The collated data from trials, in Figure 1, offers no evidence for any such discontinuity. Concerns must also be expressed where studies reporting the proportion of N eaten partitioned to milk, did not in fact measure intake (of DM or N), but back-calculated these from milk output, as such a calculation requires assuming *a priori* NUE.

There has also been much debate and confusion over the prospects for altering one component of the composition of a feed, e.g. grass WSC, and how this inter-relates with other components, e.g. protein and fibre, given these components are generally presented as (in effect) a 'percentage' (every 10 g/kg is not a quantity but a proportion) and so trivially add to the complement of 1.0 (100%). Increases in WSC are inevitably associated with decreases in all other components, by the simple principle of 'dilution'. To address this, Rasmussen *et al.* [16] proposed a 'sugar-free' analysis of data as an example of how to detect systematic changes in composition, from trivial arithmetic ones. Looking back at Figure 2, the expected trajectory for the effects of a genuine systematic increase in sugar content, on the NUE of a grass forage, and taking account of this trivial 'dilution', would be seen as a diagonal line – an increase in WSC *per se* would lead to an increase in NUE, helped by a simultaneous reduction in N content of the diet. This makes the targets for plant breeding, in terms of the absolute increase in sugars required, a little less demanding.

Prospects for Improving the Expression of the Trait

As changes in one component of forage chemical composition are both trivially, as well as potentially systematically, inter-related, prospects for a more rapid and substantial change in composition depend on greater fundamental knowledge of the control of sugar metabolism [53–55], notably the balance of labile sugars, and their fate either for storage (and the converse – mobilization), or investment more permanently into fibre.

The synthesis of fructans, the major storage sugars in ryegrass, requires the expression of several fructosyl-transferases [55]. Until very recently, only two of three putative genes coding for fructan biosynthesis of the fructan form found in *L. perenne*, had been cloned and functionally characterized, Lp1-SST [56] and Lp6G-FFT [57] (for a brief review, see section on molecular biology of trait expression in Edwards *et al.* [22]). A third gene, '6-FFT/6-SFT', technically required to produce the *L. perenne*-type 'levan' neoseries of fructans, had long remained uncharacterized, but has been reported recently [58]. Similarly, only one of two putative genes for the breakdown of fructan (exohydrolases), had been cloned and functionally characterized '1-FEH' (see [59]), and a '6-FEH' cloned but not characterized [60]. Alternative metabolic transformations have also been proposed [55].

To determine major factors controlling fructan accumulation, Rasmussen *et al.* (unpublished data) and Lasseur *et al.* [11] have looked at the expression patterns of genes putatively coding for fructosyltransferases and exohydrolases, in HSG and control cultivars, under a wide range of environments and metabolic challenges, such as defoliation and regrowth. A particular *isoform* of one of the fructosyltransferases (6G-FFT) has been identified and is believed to be associated with high fructan accumulation in some HSGs [61].

Understanding the factors regulating gene expression offers the best prospect for untangling and removing phenomena that have dogged many attempts at perennial grass forage breeding, namely the prevalence of gene× environment and gene×management (G×E and G×M) interactions (for review, see [24]). Taking just one example: initial trials using AberDove (aka 'ba11353') showed increases in sugar content of 40% and 26%, compared with the UK control 'Fennema' in field-scale studies in the UK [62, 63]. However, a similar comparison in New Zealand, of the same cultivar and source, revealed only small and inconsistent differences in sugar content [64]. A major field assay, at nine sites across Europe [65] showed some 14% increase in sugar per unit biomass in AberDart compared with Fennema, but a reduction in growth in AberDart so that at only one site in nine was the sugar yield per hectare greater in the HSG. Controlled environment studies in New Zealand [64] suggested the expression of the HSG trait might depend on periods of cold pre-treatment, and so a gene× environment interaction (in keeping with Halling [65]). A seed-industry-sponsored field trial in New Zealand [66] also revealed the greater sugar content, and/or the total yield potential, of a number of HSG were not consistent across two major sites (one on the North and one on the South Island) or across years. Locally adapted eco-types have been under development in a number of countries. Moreover, continued determined efforts focusing on breeding for this trait, at IGER (now renamed IBERs), are said to have led to more substantial and consistent expression.

Even when the trait is known to be present, and use is made of plant germplasm from the same populations, but studied under different temperature and management conditions, the complexity of expression of the trait is evident. Rasmussen et al. [16] looked at five cultivars (two a priori designated HSG; AberDart and 'PG1113' – 'Expo'; one EU control 'Fennema', a New Zealand control 'Impact' and a speculative line 'Tuni-7', all while grown at low (10/10°C day/night) or marginally warmer (20/10°C) temperatures, and while regrowing following a common defoliation. This confirmed how low temperatures per se lead to greater sugar content (as is well recognized). Defoliation led to the expected reduction in sugars in all cultivars, at 20/10°C, and a substantial period was necessary before the sugar content of leaves had regained the pre-defoliation levels (again, higher in HSG than in 'controls'). This in itself reveals how sugar content in the diet interacts with management (the duration of regrowth allowed before re-grazing), and so how management alone can be used in some circumstances to modify diet WSC:CP content [67, 68]. It also conveys, conversely, how exploiting HSG may require allowing the sugars (and differences between HSG and controls) to 'rebuild'. But, at the (marginally) lower temperatures (10/10°C), there was no such reduction in sugar content following defoliation in most cultivars, and hence no corresponding period necessary for recovery of sugars or sugar differences. Given the complexities of how animal grazing behaviour leads to different residual vegetation states and emergent regrowth intervals at the bite-patch scale and in both continuous or rotational grazing regimes [69, 70], and the vagaries of season and geographic location, it is clear why field trials alone do not give the most valuable feedback to design and drive forward a programme for systematically altering a plant trait, even one where proof of concept has been shown.

Measurement and Models of Effects of 'HSG' on Methane

Although there are an increasing number of trials investigating the effect of different feed types on methane production by ruminants (e.g. [71, 72]), reports involving the use of HSG are limited. For example, trials feeding diets of differing WSC, protein and fibre content (artificially manipulated using casein, inulin and straw to obtain diets varying in both the absolute levels and ratios of fibre, WSC and protein) to animals in metabolism chambers, reveal little consistent evidence that manipulating the WSC:CP ratio reduced methane emissions per unit feed DM intake (Stefan Muetzel, unpublished data and see [73]). The same conclusions were arrived at following early examples to model ruminant responses to HSG [74]. Current inventory-based procedures that estimate methane emission for ruminants based on either total DM intake, or total energy intake, would clearly predict

greater total methane emission if either total DM intake or total energy intake increased through feeding with HSG grasses. However, because maintenance energy requirement becomes a smaller proportion of total energy requirement if HSG result in increases in individual animal performance, emissions per unit of product may decrease. It is of course critically important not to confuse the effects of an increase in digestible ME intake derived from HSG (energy based on glucose, fructose and fructan), with those where ME has been increased by feeding cereal grains (mainly starch), as the metabolism of starch per se involves major changes in degradation pathway, rumen microbial populations and VFA ratios all beneficial to reducing methane emission [18].

A recent major report of trials testing for methane emissions by animals digesting a range of fresh forages, grown at the field scale in the UK, argues for significant promise in reductions in methane from ruminants grazing HSG diets [28]. However, some concerns still persist. In one trial, the design involved 'zero-grazing', feeding lamb diets from treatments that consisted of HSG growing alone, or with clover, and a control grass growing alone or with clover. While there is no denying that there were significant differences shown across these four treatments in methane emission in total, or per unit feed intake, it is apparent that much of the significance (variance, and the difference) was derived from the presence or absence of clover. Methane production (in total) tended to be substantially lower when clover was included in the diet (though the total methane per animal differences across all four treatments were not significant). Total intake of DM was significantly greater when HSG was fed alone, or with clover. This leads inevitably to the expectation of methane per unit DM intake appearing lower in the two treatments feeding HSG. As with previous studies looking at milk yield in cows [20] the differences between treatments may be more a matter of effects on total intake, than on a fundamental change in metabolism/fate of C and N (and H+) in the rumen. These comments are in no way to be seen as critical of the ongoing substantial efforts being made at IGER/IBERs to explore thoroughly the opportunities for exploiting the HSG trait developed there.

A second trial in the same report [28] involved measurements of methane emission made at intervals over the growing season on lambs grazing outdoors on either HSG or control grass pastures. This puts aside complications that might arise from the presence/absence of clover. Although no detailed experimental results are presented, the report indicates significant reductions in methane emission (approximately 20% lower over the season) for lambs grazing HSG pastures, which together with significantly greater total intake on these pastures, led to substantially lower methane emission per unit DM intake. The interpretation of this study would be greatly enhanced if there were data showing that the pastures designated HSG had been analysed for, and shown to

have, higher sugars. Clearly they had attributes that promoted rapid intake and digestion. The chemical analyses of these pastures would greatly help focus on which attribute reduced methane most. The reasons for this, and the value of untangling the rumen response to multiple changes in feed composition, are apparent in the rumen-process modelling approach described below.

Models

In the description of experimental trials throughout this review, we have drawn attention to how a change in any one component of a total composition, e.g. an increase in the sugar content, of a diet (whether naturally in a fresh forage, or in a 'made-up' feed mix) leads to inevitable trivial dilution of all other components. As a consequence, care must be taken in attributing benefits to, e.g. an increase in sugars per se, when these are associated with reductions in e.g. protein and/or fibre (see [16]).

Mathematical models of rumen function allow prediction of the expected outcome for all types of permutations of feed composition. Recent studies by Ellis et al. [25–27] analysed the effects of diets made up of a wide range of absolute quantities of sugar, protein and fibre, with the 'proportional' concentrations (e.g. g/kg) being an emergent property. In this particularly revealing way, Ellis et al. [25, 26] analysed the effect of HSG on predicted N excretion and milk yield using a dynamic model previously developed by Dijkstra et al. [75] and recently enhanced with components for pH-dependent VFA stoichiometry (after [76]); and a hydrogen (H+) balance sub-model (after [77] and see [78, 79]). The authors analysed the predicted effects of a range of WSC content in the diet from 85 to 248 g/kg DM. CP ranged from 115 to 263 g/kg DM and the neutral detergent fibre (NDF) ranged from 400 to 568 g/kg of DM. With respect to the proposed rumen N-capture benefits of HSG, simulation results showed that NUE increased (a lower proportion of N eaten was excreted in urine) as the WSC content of the diet increased, but this result was affected also by the CP and NDF content of the feed. The greatest N utilization (minimum proportional loss of N in urine) was observed in the simulation when the WSC increased at the expense of CP and least when WSC increased at the expense of NDF. In contrast, simulated milk yield decreased slightly when WSC increased at the expense of CP and increased most when WSC increased at the expense of NDF alone. Results were amplified slightly when conditions of low grass-N fertilization were simulated and in the absence of grain feeding.

The same dynamic modelling approach has also been used to evaluate the effect of HSG on simulated methane emissions [25, 27]. The model indicated an increase in methane emitted (expressed per unit gross energy intake (GEI)) as WSC in feed increased. This is an important metric for expressing methane emissions in current international GHG inventory reporting. 'Intensity' measures of methane production, i.e. methane emitted per unit of animal product, are gaining prevalence in national mitigation strategies. The model predicted an increase in methane emission per unit milk yield, particularly when WSC content increased at the expense of CP, but decreased per unit milk yield when WSC increased at the expense of NDF. This latter result was because of the higher digestibility of WSC (as it replaced NDF), and a higher simulated NDF digestibility, leading to greater total intake and milk yield.

These studies show how field observations will be difficult to interpret without the aid of a theoretical framework for expected responses in ruminant metabolism [26, 27] and without careful attention to effects of HSG diets on total intake, both per head and per ha, emissions (of N or methane) per unit intake of N or gross energy, and whether expressed per unit animal product. The studies also emphasize the risk that mitigation strategies may result in 'pollution-swapping', where a reduction in nitrous oxide emissions, for example, consequent on say HSG leading to a reduced proportion of N eaten being emitted in urine, might be simply offset (or environmental impacts made worse) should the same change in diet lead to an absolute increase per ha, in methane emission. At present, models can help analyse these potential trade-offs, but only more detailed measurements, made at the field scale, involving full analysis of the grazed diet, the emissions of GHG, and fate of N in the rumen, will resolve what are realistic options and outcomes.

Until recently, the value of such an effort would have been limited, as the pasture germplasm available commercially (or for use at a field scale) did not have sufficient differences in diet composition (or composition was not reported) to lead to definitive conclusions. If the current new tranches of HSG cultivars have the more pronounced and consistently expressed sugar contents that are claimed, then measurements may catch up with theory in evaluating just what options for sustained ruminant production, with little or no increase in environmental impact, are available to the livestock industry.

Conclusions

The HSG trait, in diploid perennial ryegrass, has been pursued with admirable determination, notably by the IGER, UK. Considerable effort has gone into evaluating the HSG trait, both for the prospects for increasing ruminant production and for reducing its environmental impact. Proof of concept, at least for reducing the proportion of N eaten by ruminants that is voided as urine has been shown, with evidence also of a smaller increase in the proportion of N partitioned to milk. To achieve this degree of consensus required the interpolation of results across multiple trials. There is far less consensus, as yet, that a HSG diet leads to reductions in methane emission,

and any statements on methane emission responses evidently need to define clearly whether the effect is claimed per animal, per unit intake, per unit product (e.g. milk), or per hectare.

Practical field trials, notably 'on-farm', have long held primacy in the minds of those, understandably, wanting the most convincing pragmatic answers to questions on how to manipulate temperate grassland agriculture to meet society's goals. However, some concern can be raised [24] with regard to how well such pragmatic trials have delivered definitive answers, and how this is an inevitable consequence of the lack of capacity to control experimental treatments or, in some cases, the lack of the appropriate experimental 'control' treatments altogether, to test hypotheses adequately. This has been true even in those cases where a strong hypothesis has been stated a priori and is being pursued. In the case of HSG development, the determination and rigour of the experimentation and theoretical analysis of the prospects and opportunities from this trait, has been exemplary in many quarters.

But one of the greatest problems in establishing a definitive answer as to the efficacy and potential of the HSG trait has been that in the past, there would appear to have been too little difference between the HSG cultivar and the 'control' grass, in any one trial. New cultivars, with new claims for greater gains in WSC, are released continually. This leaves the difficult situation whereby the time the kind of replicated field-scale trial, with appropriate controlled treatments, has been conducted to test the efficacy of that cultivar in modifying N partitioning in ruminants, or reducing GHG emissions, newer cultivars are available. Any lack of evidence of successful effects is all too easily dismissed with statements that the newer cultivars might have given different, more favourable, results. A recent review [24] proposed a catalogue of changes needed in the way new forage cultivars are evaluated, for this reason. We recommend that as soon as plant breeders are content that there is evidence that a new HSG cultivar has the level of difference in WSC from an accepted 'control' that all concerned would respect gives a thorough test of the efficacy of the trait, then a major concerted effort be made to repeat the kind of trials conducted earlier by UK and Netherlands teams, while simultaneously assessing methane output, N balance and performance. The graphs demonstrating 'proof of concept' can readily be used to determine just how much difference in WSC and CP would be needed to create a given expectation of seeing an effect at the field scale, and so justifying the research.

In the meantime, we propose [24] work continues apace on the fundamental mechanisms by which plants accumulate, and their strategy to allocate, carbohydrates to growth or storage, (and hence their levels of WSC), and likewise on what leeway there may be in how much N is required for a given level of DM production (the levels of CP). Whatever the likelihood of success of HSG per se,

there is undeniable value in altering the way grassland agriculture, substantially as a result of the involvement of ruminant animals, uncouples the C and N cycles [80] leading to major inefficiencies in N use. Hence, we might in future come to see 'HSG' as having provided valuable insights into the prospects for lower-N (-requiring) grassland production.

Success to date has depended critically on a culture of research funding that recognized the value of controlled experimentation, theory and field trials, alike. Recent trends to reduce support for agricultural research, and notably to fund instead more on-farm trials, may deeply prejudice progress, at a time when, possibly for the first time, science has the tools to make directed and controlled changes in plant traits and animal metabolism. Pragmatism must be recognized as an outcome of, and not an impediment to, more rigorous science.

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References

1. Cooper JP, Breese EL. Plant breeding: forage grasses and legumes. In: Wareing PF, Cooper JP editors. Potential Crop Production. Heinemann Educational Books, London, UK; 1971. p. 295–318.

2. Smith KF, Reed KFM, Foot JZ. An assessment of the relative importance of specific traits for the genetic improvement of nutritive value in dairy pasture. Grass and Forage Science 1997;52:167–75.

3. Hopkins A, Wilkins RJ. Temperate grassland: key developments in the last century and future perspectives. Journal of Agricultural Science 2006;144:503–23.

4. Blaxter Sir K. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux. Unwin Brothers, Gresham Press, UK; 1980. 351pp.

5. Abberton MT, Marshall AH, Humphreys MW, Macduff JH, Collins RP, Marley CL. Genetic improvement of forage species to reduce the environmental impact of temperate livestock grazing systems. Advances in Agronomy 2008;98:311–55.

6. Humphreys MO. Water-soluble carbohydrates in perennial ryegrass breeding. I. Genetic differences among cultivars and hybrid progeny grown as spaced plants. Grass and Forage Science 1989;44:231–6.

7. Turner LB, Cairns AJ, Armstead P, Ashton J, Skøt K, Whittaker D. Dissecting the regulation of fructan metabolism in perennial ryegrass (Lolium perenne) with quantitative trait locus mapping. New Phytologist 2006;169:45–58.

8. Wilkins PW, Lovatt JA. Progress in improving the ratio of water-soluble carbohydrate to crude protein in perennial ryegrass. Aspects of Applied Biology 2003;70:31–6.

9. Marais JP, Goodenough DCW, de Figueiredo M, Hopkins C. The development of a *Lolium multiflorum* cultivar with a low moisture content and an increased readily digestible energy to protein ratio. Australian Journal of Agricultural Research 2003;54:101–6.

10. Morvan-Bertrand A, Boucaud J, Le Saos J, Prud'homme M-P. Roles of fructans from leaf sheaths and from the elongating leaf bases in the regrowth following defoliation of *Lolium perenne* L. Planta 2001;213:109–20.

11. Lasseur B, Lothier J, Morvan-Bertrand A, Escobar-Guttierez A, Humphreys MO, Prud'homme M. Impact of defoliation frequency on regrowth and carbohydrate metabolism in contrasting varieties of *Lolium perenne*. Functional Plant Biology 2007;34:418–30.

12. Pollock CJ, Jones T. Seasonal patterns of fructan metabolism in forage grasses. New Phytologist 1979;83:9–15.

13. Smith KF, Culvenor RA, Humphreys MO, Simpson RJ. Growth and carbon partitioning in perennial ryegrass (*Lolium perenne*) cultivars selected for high water soluble carbohydrate concentrations. Journal of Agricultural Science 2002;138:375–85.

14. Rasmussen S, Parsons AJ, Bassett S, Christensen MJ, Hume DE, Johnson LJ, et al. High nitrogen supply and carbohydrate content reduce fungal endophyte and alkaloid concentration in *Lolium perenne*. New Phytologist 2007;173:787–97.

15. Rasmussen S, Parsons AJ, Fraser K, Xue H, Newman JA. Metabolic profiles of *Lolium perenne* are differentially affected by nitrogen supply, carbohydrate content and fungal endophyte infection. Plant Physiology 2008;146:1440–53.

16. Rasmussen S, Parsons AJ, Xue H, Newman JA. High sugar grasses – harnessing the benefits of new cultivars through growth management. Proceedings of the New Zealand Grassland Association 2009;71:167–75.

17. Beever DE, Losada HR, Cammell CB, Evans RT, Haines MJ. Effect of forage species and season on nutrient digestion and supply in grazing cattle. British Journal of Nutrition 1986;56:209–55.

18. Beever DE. Rumen function. In: Forbes JM, France J editors. Quantitative Aspects of Ruminant Digestion and Metabolism. CAB International, Wallingford, UK; 1993. p. 187–215.

19. Dove H, Milne JA. Digesta flow and rumen microbial protein production in ewes grazing perennial ryegrass. Australian Journal of Agricultural Research 1994;45:1229–45.

20. Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, et al. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. Grass and Forage Science 2001;56:383–94.

21. Di HJ, Cameron KC. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use and Management 2002;18:395–403.

22. Edwards GR, Parsons AJ, Rasmussen S. High sugar grasses for dairy systems. In: Meeting the Challenges for Pasture-Based Dairying. Proceedings of the Australasian Dairy Science Symposium, 18–20 September 2007, Melbourne, Australia; 2007. p. 307–34.

23. Edwards GR, Parsons AJ, Rasmussen S. High sugar grasses for dairy systems. Proceedings of the New Zealand Grassland Association 2007;69:161–72.

24. Parsons AJ, Edwards GR, Newton PCD, Chapman DF, Caradus JR, Rasmussen S, et al. Past lessons and future prospects: plant breeding for yield and persistence in cool-temperate pastures. Grass and Forage Science 2011;66:153–72.

25. Ellis JL, Bannink A, Dijkstra J, Parsons AJ, Rasmussen S, Edwards GR, et al. A modelling approach to evaluate the feeding of high sugar grasses to cattle: nitrogen and methane. Proceedings of the Animal Science Modelling Group, Guelph, Canada; 2009. p. 532–3.

26. Ellis JL, Dijkstra J, Bannink A, Parsons AJ, Rasmussen S, Edwards GR, et al. The effect of high sugar grass on predicted nitrogen emission and milk yield simulated using a dynamic model. Journal of Dairy Science 2011;94:3105–18.

27. Ellis JL, Dijkstra J, France J, Kebreab E, Parsons AJ, Rasmussen S, et al. Effect of high sugar grass on methane emissions simulated using a dynamic model. Journal of Dairy Science 2012 (January). doi: 10.3168/jds.2011-4385.

28. DEFRA 2010: Ruminant Nutrition Regimes to Reduce Methane and Nitrogen Emissions. Final Report AC0209. 37pp.

29. Humphreys MO. Water-soluble carbohydrates in perennial ryegrass breeding. II. Cultivars and hybrid progeny performance in cut plots. Grass and Forage Science 1989;44:237–44.

30. Humphreys MO. Water-soluble carbohydrates in perennial ryegrass breeding. III. Relationships with herbage production, digestibility and crude protein content. Grass and Forage Science 1989;44:423–9.

31. Miller LA, Theodorou MK, MacRae JC, Evans RT, Humphreys MO, Scollan ND, et al. Efficiency of nitrogen use by dairy cows offered perennial ryegrass with high water soluble carbohydrate concentrations. In Proceedings of the 6th Research Conference of the British Grassland Society, 11–13 September 2000, Aberdeen, UK. British Grassland Society, Reading, UK; 2000. p. 37–8.

32. Miller LA, Baker DH, Theodorou MK, MacRae JC, Humphreys MO, Scollan ND, et al. Efficiency of nitrogen use in dairy cows grazing ryegrass with different water soluble carbohydrate concentrations. In: Gomide JA, Mattos WRS, Carneiro da Silva S, editors. Grassland Ecosystems: An Outlook into the 21st Century. Proceedings of the 19th International Grasslands Congress; 2001. p. 377–8.

33. Moorby JM, Evans RT, Scollan ND, MacRae JC, Theodorou MK. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. Grass and Forage Science 2006;61:52–9.

34. Miller LA, Neville MA, Baker DH, Evans RT, Theodorou MK, MacRae JC, et al. Milk production from dairy cows offered perennial ryegrass selected for high water soluble carbohydrate concentrations compared to a control grass. In Proceedings of the British Society of Animal Science, Annual Meeting, 1999 March, Scarborough, UK; 1999. p. 208.

35. Miller LA, Theodorou MK, MacRae JC, Evans RT, Adesogan AT, Humphreys MO, et al. Milk production and N partitioning responses in dairy cows offered perennial ryegrass selected for high water soluble carbohydrate concentrations. South African Journal of Animal Science 1999;(ISRP-Special issue):281–2.

36. Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S. Effects of perennial ryegrass cultivars on intake,

digestibility and milk yield in dairy cows. Journal of Dairy Science 2005;88:3240–8.

37. Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S. Utilisation of N in perennial ryegrass cultivars by stall-fed lactating dairy cows. Livestock Science 2006;100:159–68.

38. Tas BM, Taweel HZ, Smit HJ, Elgersma A, Dijkstra J, Tamminga S. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilisation in grazing dairy cows. Journal of Dairy Science 2006;89:3494–350.

39. Taweel HZ, Tas BM, Smit HJ, Elgersma A, Dijkstra J, Tamminga S. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrate on intake, rumen function and performance of dairy cows. Animal Feed Science and Technology 2005;121:243–56.

40. Taweel HZ, Tas BM, Smit HJ, Elgersma A, Dijkstra J, Tamminga S. Improving the quality of perennial ryegrass (Lolium perenne L.) for dairy cows by selecting for fast clearing and/or degradable neutral detergent fiber. Livestock Production Science 2005;96:239–48.

41. Taweel HZ, Tas BM, Smit HJ, Elgersma A, Dijkstra J, Tamminga S. Grazing behaviour, intake, rumen function and milk production of dairy cows offered Lolium perenne containing different levels of water-soluble carbohydrates. Livestock Science 2006;102:33–41.

42. Smit HJ, Tas BM, Taweel HZ, Tamminga S, Elgersma A. Effects of perennial ryegrass (Lolium perenne L.) cultivars on herbage production, nutritional quality and herbage intake of grazing dairy cows. Grass and Forage Science 2005;60: 297–309.

43. Cosgrove GP, Pacheco D, Burke JL, Lane GA. Ryegrasses with increased water-soluble carbohydrate: evaluating the potential for grazing dairy cows in New Zealand. Proceedings of the New Zealand Grassland Association Conference 2007;69:179–85.

44. Lee MRF, Jones EL, Moorby JM, Humphreys MO, Theodorou MK, MacRae JC, et al. Production responses from lambs grazed on Lolium perenne selected for an elevated water-soluble carbohydrate concentration. Animal Research 2001;50:441–9.

45. Munro JMM, Davies DA, Evans WB, Scurlock RV. Animal production evaluation of herbage varieties. 1. Comparison of Aurora with Frances, Talbot and Melle perennial ryegrasses when grown alone or with clover. Grass and Forage Science 1992;47:259–73.

46. Marley CL, Fraser MD, Fisher WJ, Forbes AB, Jones R, Moorby JM, et al. Effects of continuous or rotational grazing of two perennial ryegrass varieties on the chemical composition of the herbage and the performance of finishing lambs. Grass and Forage Science 2007;62:255–64.

47. Tas BM. Nitrogen utilisation of perennial ryegrass in dairy cows. In: Elgersma A, Dijkstra J, Tamminga S. editors. Fresh Herbage for Dairy Cattle. Springer, Amsterdam, The Netherlands; 2007. p. 125–40.

48. Hoekstra NJ, Schulte RPO, Struik PC, Lantinga EA. Pathways to improving the N efficiency of grazing bovines. European Journal of Agronomy 2007;26:363–74.

49. Kebreab E, France J, Beever DE, Castillo AR. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. Nutrient Cycling in Agroecosystems 2001;60:275–85.

50. Kebreab E, Dijkstra J, Bannink A, France J. Recent advances in modelling nutrient utilization in ruminants. Journal of Animal Science 2009;87:E111–22.

51. Pacheco D, Lane GA, Burke JL, Cosgrove GP. Water soluble carbohydrates relative to protein in fresh forages: impact on efficiency of nitrogen utilization in lactating dairy cows. Journal of Poultry Science 2007;86:(Suppl. 1): p. 430.

52. Pacheco D, Burke JL, Cosgrove GP. An empirical model to estimate efficiency of nitrogen utilisation from cows grazing fresh forages. In Proceedings of the Australasian Dairy Science Symposium, 18–20 September 2007, Melbourne, Australia; 2007. p. 409–16.

53. Pollock CJ, Cairns AJ. Fructan metabolism in grasses and cereals. Annual Review of Plant Physiology and Plant Molecular Biology 1991;42:77–101.

54. Pavis N, Boucaud J, Prud'homme MP. Fructans and fructan metabolizing enzymes in leaves of Lolium perenne. New Phytologist 2001;150:97–109.

55. Cairns AJ, Turner LB, Gallagher JA. Ryegrass leaf fructan synthesis is oxygen dependent and abolished by endomembrane inhibitors. New Phytologist 2008;80:832–40.

56. Chalmers J, Johnson X, Lidgett A, Spangenberg G. Isolation and characterisation of a sucrose: sucrose 1-fructosyltransferase gene from perennial ryegrass (Lolium perenne). Journal of Plant Physiology 2003;160:1385–91.

57. Lasseur B, Lothier J, Djoumad A, De Coninck B, Smeekens S, Van Laere A, et al. Molecular and functional characterization of a cDNA encoding fructan:fructan 6G-fructosyltransferase (6G-FFT)/fructan:fructan 1-fructosyltransferase (1-FFT) from perennial ryegrass (Lolium perenne L.). Journal of Experimental Botany 2006;57:2719–34.

58. Lasseur B, Lothier J, Wiemken A, Van Laere A, Morvan-Bertrand A, Van den Ende W, et al. Towards a better understanding of the generation of fructan structure diversity in plants: molecular and functional characterization of a sucrose:fructan 6-fructosyltransferase (6-SFT) cDNA from perennial ryegrass (Lolium perenne). Journal of Experimental Botany 2011;62:1871–85.

59. Lothier J, Lasseur B, Le Roy K, Van Laere A, Prud'homme M-P, Barre P, et al. Cloning, gene mapping, and functional analysis of a fructan 1-exohydrolase (1-FEH) from Lolium perenne implicated in fructan synthesis rather than in fructan mobilization. Journal of Experimental Botany 2007;58(8):1969–83.

60. Chalmers J, Lidgett A, Cummings N, Cao Y, Forster J, Spangenberg G. Molecular genetics of fructan metabolism in perennial ryegrass. Plant Biotechnology Journal 2005;2: 459–74.

61. Rasmussen S, Xue H, Newman JA, Parsons AJ. Fructan biosynthesis in grasses – tissue, temperature and cultivar effects on gene expression profiles. In 18th Biennial Meeting of the New Zealand Branch of the International Association of Plant Biotechnology, 16–19 February 2009, Napier, New Zealand; 2009. p. 27.

62. Orr RJ, Cook JE, Atkinson LD, Clements RO, Martyn TM. Evaluation of herbage varieties under continuous stocking. In: Rook AJ, Penning PD editors. Grazing Management. Occasional Symposium No 34 British Grassland Society, Reading, UK; 2000. p. 39–44.

63. Cook JE, Champion RA, Orr RJ, Rook AJ. Intake rate, grazing time and daily intake by sheep grazing perennial

ryegrass varieties under continuous stocking management. In Proceedings of the 6th British Grassland Society Research Conference, 11–13 September 2000, Aberdeen, UK; 2000. p. 67–8.

64. Parsons AJ, Rasmussen S, Xue H, Newman JA, Anderson CB, Cosgrove GP. Some high sugar grasses don't like it hot. Proceedings of the New Zealand Grassland Association 2004;66:265–72.

65. Halling MA, Longland AC, Martens S, Nesheim L, O'Kiely P. Accumulation of water soluble carbohydrates in two perennial ryegrass cultivars at nine European sites. Grassland Science in Europe. Land use systems on grassland dominated regions. In: Luscher A, Jeangros B, Kessler W, Huguenin O, Lobsiger M, Millar N, et al. editors. European Grassland Federation (EGF) 20th General Meeting, 21–24 June 2004, Luzern, Switzerland; 2004. p. 954–6.

66. Hume DE, Hickey MJ, Lyons TB, Baird DB. Agronomic performance and water-soluble carbohydrate expression of selected ryegrasses at two locations in New Zealand. New Zealand Journal of Agricultural Research 2010;53:37–57.

67. Lee JM, Donaghy DJ, Roche JR. Effect of defoliation severity on regrowth and nutritive value of perennial ryegrass dominant swards. Agronomy Journal 2008;100:308–14.

68. Bryant R, Walpot V, Dalley DE, Gibbs SJ, Edwards GR. Manipulating dietary N in perennial ryegrass pastures to reduce N losses in dairy cows in spring. In Proceedings of the 4th Australasian Dairy Science Symposium, 31 August – 2 September 2010, Lincoln, New Zealand; 2010. p. 97–100.

69. Schwinning S, Parsons AJ. The stability of grazing systems revisited: spatial models and the role of heterogeneity. Functional Ecology 1999;13:737–47.

70. Parsons AJ, Dumont B. Spatial heterogeneity and grazing processes. Animal Research 2003;52:161–79.

71. Martin C, Morgavi DP, Doreau M. Methane mitigation in ruminants: from microbe to the farm scale. Animal 2010;4:351–65.

72. Grainger C, Beauchemin KA. Can enteric methane emissions from ruminants be lowered without lowering their production? Animal Feed Science and Technology 2011;166–167:308–20.

73. Hindrichsen IK, Wettstein HR, Machmuller A, Jorg B, Kreuzer M. Effect of the carbohydrate composition of feed concentrates on methane emission from dairy cows and their slurry. Environmental Monitoring and Assessment 2005;107:329–50.

74. Taweel HZ, Smit HJ, Elgersma A. Will feeding high sugar grass reduce methane emission by grazing dairy cows? In: Lloveras J, et al. editors. Sustainable Grassland Productivity. European Grassland Federation (EGF), 21st General Meeting, 3–6 April 2006, Badajoz, Spain; 2006. p. 787–9.

75. Dijkstra J, Neal D, St C, Beever DE, France J. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. Journal of Nutrition 1992;122:2239–56.

76. Bannink A, France J, Lopez S, Gerrits WJJ, Kebreab E, Tamminga S, et al. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Animal Feed Science Technology 2008;143:3–26.

77. Mills JAN, Dijkstra J, Bannink A, Cammell SB, Kebreab E, France J. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: model development, evaluation and application. Journal of Animal Science 2001;79:1584–97.

78. Kebreab E, France J, Mills RA, Dijkstra J. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. Journal of Animal Science 2002;80:248–59.

79. Dijkstra J, Kebreab E, Bannink A, Crompton LA, Lopez S, Abrahamse PA, et al. Comparison of energy evaluation systems and a mechanistic model for milk production by dairy cattle offered fresh grass-based diets. Animal Feed Science and Technology 2008;143:203–19.

80. Parsons AJ, Rowarth JS, Thornley JHM, Newton PCD. Primary production of grasslands, herbage accumulation and use, and impacts of climate change. In: Lemaire G, Hodgson J, Abad Chabbi, editors. Grassland Productivity and Ecosystem Services. CAB International, Wallingford, UK; 2011(in press).

81. Merry RJ, Lee MRF, Davies DR, Dewhurst RJ, Moorby JM, Scollan ND, et al. Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilisation. Journal of Animal Science 2006;84:3049–60.

82. Lee MRF, Harris LJ, Moorby JM, Humphreys MO, Theodorou MK, MacRae JC, et al. Rumen metabolism and nitrogen flow to the small intestine in steers offered Lolium perenne containing different levels of water-soluble carbohydrate. Animal Science 2002;74:587–96.