High sugar ryegrasses for livestock systems in New Zealand

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Abstract

There has been mounting interest over the proposed production and environmental benefits from using perennial ryegrass cultivars bred to have higher water soluble carbohydrate content (high sugar grasses). Here, we objectively review published evidence, from the EU and New Zealand, of the effects of these on milk yield per cow, liveweight gain in sheep, N utilisation and wider trophic interactions. The literature reveals substantial variation in animal responses, though some of the uncertainty in interpretation can be resolved by combining the data from multiple trials, and showing this forms a continuum of response to diet quality. It also reveals variation in the degree to which the sugar trait has been expressed, possibly reflecting a gene x environment interaction. Achieving a more consistent, and probably greater than current, expression of the high sugar trait would be a valuable goal. We suggest 'proof of concept' has been shown, notably for the potential for improving N utilisation in the rumen, and so reducing the proportion of N intake lost in urine. The evidence suggests that this may be a greater challenge, albeit a more valuable goal, because of the relatively high N (crude protein) content forages that predominate in the New Zealand pasture industry.

Keywords: animal performance, high sugar grass, *Lolium perenne*, perennial ryegrass, nitrogen utilisation, trait expression, water soluble carbohydrates

Introduction

Plant breeders have developed perennial ryegrass cultivars with an elevated concentration of water soluble carbohydrates (WSC, also known as high sugar grasses) relative to conventional cultivars (Humphreys 1989a, b, c; Turner *et al.* 2006). This breeding has focussed on increasing the accumulation of high molecular weight storage sugars (i.e. fructans), particularly in leaf blades rather than sheath bases (Pollock & Cairns 1991; Pavis *et al.* 2001a, b). It is proposed that perennial ryegrass with high WSC may improve the balance and synchrony of the nitrogen (N) and carbon (C) supply to the rumen (Miller *et al.* 2001a). This may lead to increased production in ruminants by improving the utilisation of N in the rumen and enhancing the supply of microbial protein to the ruminant (Miller *et al.* 2001a). This also potentially reduces the proportion of N in the diet that is lost in urine, thereby offering environmental benefits through reduced N leaching and emissions of the greenhouse gas nitrous oxide (N_2O) (Di & Cameron 2002). However, considerable debate has arisen over the benefits of high WSC grasses for New Zealand farming systems, including: the degree of improvement in livestock production and N utilisation; concerns over the expression of the high WSC trait under New Zealand conditions; and potential impacts on grazing preference, pasture composition and endophyte. This paper reviews the body of published evidence on the impact of high WSC grasses on these variables. The paper complements a more detailed review (Edwards *et al.* 2007) on the role of high WSC ryegrasses in New Zealand and Australian dairy industries.

Expression of the high WSC trait

Before any benefits of using high WSC ryegrasses can be anticipated across the industry, it is critical to consider just how consistently, and to what degree, an increase in WSC levels has been seen in practice. In UK and European pasture trials there has been a relatively consistent expression of the WSC trait in high WSC cultivars over control cultivars in the order of 10 to 50 g WSC/kg DM (Table 1, and see Cook *et al.* 2000; Orr *et al.* 2000; Halling *et al*. 2004) .

In New Zealand, in the first full year of field-based assessment of WSC concentration under grazing (Aorangi, Manawatu) (Parsons *et al.* 2004), only small and inconsistent differences in WSC content were recorded between the UK high WSC cultivar Aberdart and the UK control cultivar Fennema. Of note, is that these authors made clear their study did not include a New Zealand control cultivar, so conclusions couldn't be drawn about the expression of the high WSC trait in UK cultivars relative to New Zealand cultivars. Further examination of trait expression in controlled environments by Parsons *et al.* (2004), showed low temperatures *per se* increased sugars, notably high molecular weight sugars (fructans), in all cultivars, but a significant difference between the high WSC cultivars (AberDove and AberDart) and Fennema was apparent only after a sustained period of cold and short days (conditions comparable to leaving a UK winter). The major difference between the 24-40% elevation of sugars (between AberDart and Fennema) seen in the UK trials (Cook *et*

Table 1 Summary of the effects of perennial ryegrass cultivars with either high (H) or low (L) water soluble carbohydrate (WSC) content on herbage intake, liveweight gain, milk yields and utilisation of dietary nitrogen (N). Bold pairs in adjacent columns are significantly different from each other, P<0.05.

	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													
Miller *et al.* (2001a)	UK	**165**	**126**	11.6	10.7			**15.3**	**12.6**	**0.30**	**0.23**	**0.25**	**0.35**
Miller *et al.* (2001b)[1]	UK	**236**	**166**	17.6	17.0			25.1	26.7	**0.26**	**0.21**		
Miller *et al.* (2000)[1]	UK	**234**	**194**	14.6	14.8			21.4	21.9	**0.29**	**0.24**	**0.17**	**0.26**
Moorby *et al.* (2006)[1]	UK	**243**	**161**	**15.3**	**13.1**			32.7	30.4	0.36	0.37	**0.20**	**0.27**
Tas *et al.* (2006a) LS1, 2000	Neth	**192**	**158**	16.2	17.4			26.9	26.3	**0.28**	**0.25**	0.50	0.53
Tas *et al.* (2006a) LS1, 2001	Neth	**131**	**93**	14.7	14.9			24.7	23.8	0.23	0.24	0.56	0.53
Tas *et al.* (2006a) LS2, 2000	Neth	**195**	**152**	16.1	16.6			26.8	28.2	0.29	0.28	0.47	0.48
Tas *et al.* (2006a) LS2, 2001	Neth	113	98	13.7	14.7			22.5	23.9	0.23	0.22	0.55	0.56
Tas *et al.* (2006b) 2002	Neth	**144**	**110**	18.0	15.6			**28.8**	**26.0**	0.25	0.25		
Tas *et al.* (2006b) 2003	Neth	**131**	**87**	18.4	17.4			25.7	25.2	0.20	0.19		
Cosgrove *et al.* (2007) S, 2004	NZ	**200**	**167**					20.9	20.9				
Cosgrove *et al.* (2007) S, 2005	NZ	**215**	**195**					25.5	25.1				
Cosgrove *et al.* (2007) A, 2006	NZ	170	161					11.3	9.6				
Cosgrove *et al.* (2007) A, 2007	NZ	159	150					**11.7**	**11.0**				
Beef													
Merry *et al.* (2006)[2]	UK	90.9	55	**4.3**	**3.6**								
Lee *et al.* (2002)	UK	**243**	**161**	**9.3**	**6.7**								
Lamb													
Lee *et al.* (2001)[3], C, I	UK	**143**	**89**	1.0	1.2	**312**	**271**						
Lee *et al.* (2001)[3], C, II	UK	**113**	**75**	1.7	1.3	**244**	**194**						
Lee *et al.* (2001)[3], C, III	UK	92	84	1.1	1.2	186	175						
Marley *et al.* (2007)[4], C	UK	**115**	**100**			47.1	51.5						
Marley *et al.* (2007)[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 Tas *et al* (2006a, b) are for the highest and lowest WSC cultivars in each year from 2000-2004

[1] These studies used time of day, or N fertiliser, 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.

al. 2000; Orr *et al.* 2000) and in NZ at that time, led Parsons *et al.* (2004) to agree with Halling *et al.* (2004) on the possibility of a 'gene x environment interaction', in the trait expression, and on the prospects for developing more locally adapted cultivars. Programmes to develop such cultivars are underway both in NZ (using NZ germplasm) and across Europe.

More recent studies in the Manawatu show the UK high WSC cultivar AberDart had similar WSC to the NZ 'Italian' ryegrass Moata in two successive *spring* seasons, and both were 20 to 40 g WSC/kg DM greater in WSC than the New Zealand control cultivar Impact (Tavendale *et al.* 2006; Cosgrove *et al.* 2007, Table 1). In two successive *autumn* seasons, in the same studies, there were much smaller differences (0-9 g WSC/kg DM, P>0.05) in sugars between the cultivars (Table 1). Some other unpublished trials also show variable, seasonal and geographical, expression of the high WSC trait in New Zealand relative to New Zealand standard cultivars (D.E. Hume, unpublished data).

Effects on dry matter (DM) production

Harvestable DM production is seen as the primary driver of livestock production per ha in New Zealand farming systems. Thus, it is important to confirm that the elevation of WSC concentration does not occur at the expense of DM yield. The pan-European project ('Sweetgrass') in nine northern European (arguably cold) countries showed AberDart had consistently higher (c. 25 g/kg) sugar content than Fennema (Halling *et al*. 2004). However, in these studies total DM yield of Aberdart was significantly depressed, being significantly greater in only one site (in Wales) out of nine. Recent studies in New Zealand, using a range of UK derived high WSC cultivars in a multi site comparison, show these did not consistently differ in yield from NZ controls (Impact and Bronsyn) (D.E. Hume, unpublished data). Therefore, the current crown rust resistant, high sugar cultivars have DM yields at least as great, and on occasions greater, than New Zealand standard diploid cultivars, and are now also available with endophyte. For the industry this would seem therefore a 'one-sided bet'. That is, despite uncertainty over gains, there is no evidence of a detriment to DM production using the high WSC cultivars.

Effect on livestock production

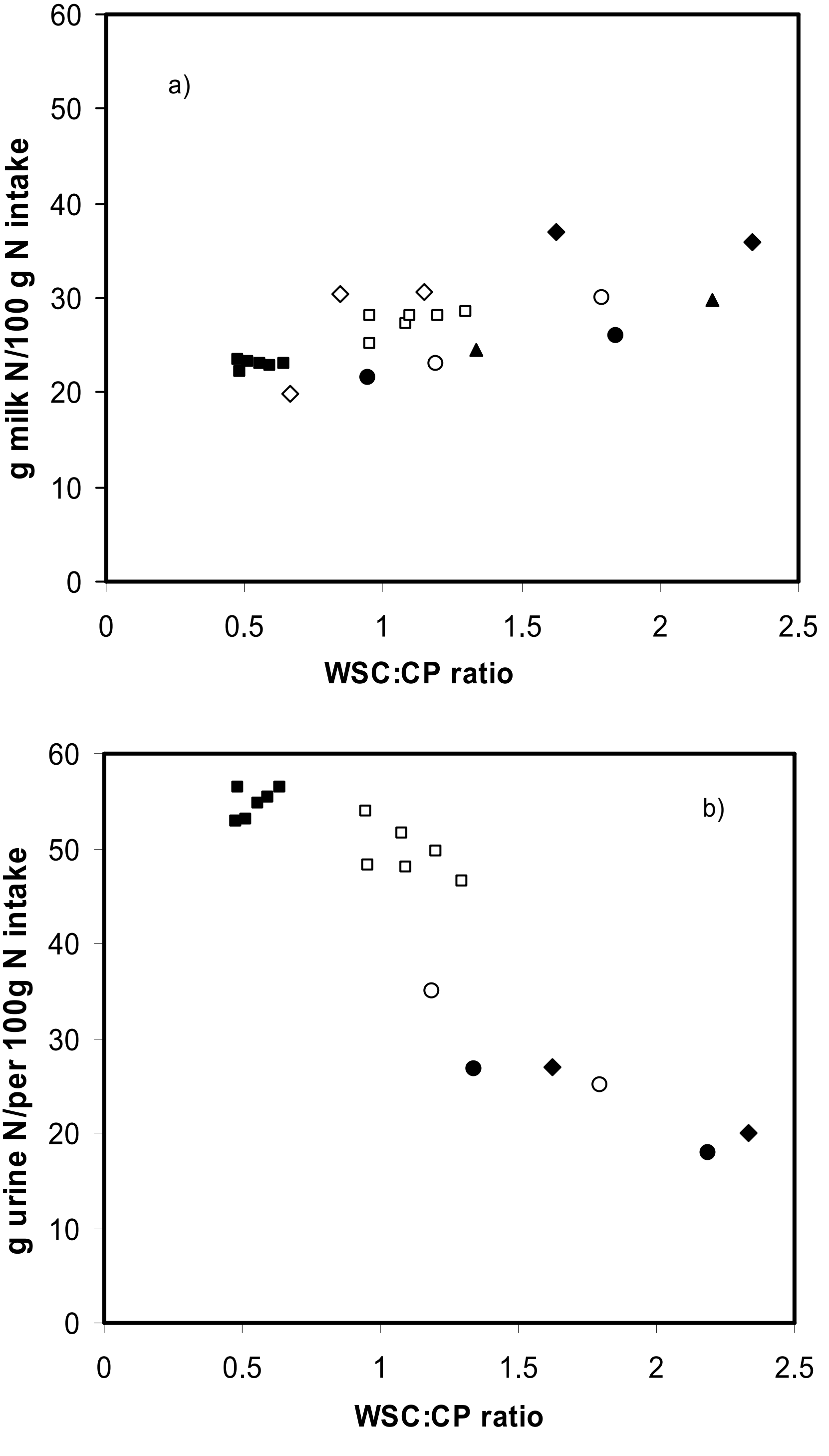
Currently there is limited experimental evidence to indicate that high WSC perennial ryegrass cultivars will increase dairy cow production (Table 1). In one indoor study in the UK (Miller *et al.* 2001a), milk production of late lactation dairy cows was 2.7 kg/day greater (P<0.05) on a high than low WSC cultivar when cows were offered herbage that had been collected after a 6 week regrowth period in mid summer. However, Miller *et al.* (2001a) concluded that the difference was due to higher digestibility in the high WSC cultivar leading to greater intake of digestible DM. Moreover, in two further dairy studies, no differences were detected in milk yields in mid- (Miller *et al.* 2000) and early- (Moorby *et al.* 2006) lactation between a high WSC and control cultivar. This was despite the studies using either 'time of day' (diurnal patterns of sugar accumulation) or the application of N to the control areas, to accentuate differences between diet quality, as a means to test 'proof of concept'. Furthermore, in a further comprehensive set of indoor and outdoor studies in the Netherlands, intake and milk yield were unaffected by perennial ryegrass cultivars regardless of significant differences in WSC concentration (Tas *et al.* 2005, 2006a, b; Table 1).

In the only New Zealand (Manawatu) based study published to date (see Tavendale *et al.* 2006; Cosgrove *et al.* 2007), the yield of milk and milk solids of Friesian dairy cows grazing at pasture in two successive *spring* seasons did not differ among the high WSC cultivar Aberdart, the New Zealand perennial ryegrass control, Impact, and the NZ 'Italian' ryegrass Moata. In the first *autumn* also, milk yield and milk solids did not differ significantly (P>0.05) among cultivars. In the second autumn, milk yield (P=0.05), and milk solids (P=0.006) were significantly greater from the high WSC cultivar (Table 1) even though the difference in WSC concentration was only 0-9 g WSC/kg DM. Cosgrove *et al.* (2007) concluded that while the milk production response in autumn could not be directly attributed to higher WSC, it may be related to other differences between grass types, such as a lower structural fibre of the high WSC grasses compared with the standard.

Working in the UK, and using some of the first germplasm lines produced with the high WSC trait, Lee *et al*. (2001) reported liveweight gain of continuously stocked suckling lambs was greater for a high WSC cultivar in two out of three periods. Earlier work in the UK showed that, over 2 years, total lamb production per ha from grass-only swards of Aurora (noted to be a high WSC cultivar, Humphreys 1989a; Smith *et al*. 2002) was 19% more than from the control cultivar Frances, despite similar herbage productivity (Munro *et al.* 1992). In contrast, Marley *et al.* (2007) did not detect any significant effect (P>0.05) of a high WSC cultivar on liveweight gain of weaned lambs grazing over 10 weeks in summer under either continuous or rotational grazing (Table 1).

In conclusion, there is an inconsistent response of intake, milk yield or liveweight gain to feeding high WSC cultivars (see also Hoeskstra *et al.* 2007). Clearly, there is a need for further science-focussed work to understand why results have been variable (seasonally and geographically) and also to explore how responses might

Figure 1 Combined data from a range of both UK (IGER) and Dutch studies showing a continuum between N utilisation efficiency for milk (a) and urine (b) in dairy cows, in relation to the WSC:CP ratio of the forage component of the diet offered. Data sources: ■ 2001 data from Tas *et al.* (2005, 2006a); □ 2000 data from Tas *et al.* (2005, 2006a); ▲ Miller *et al.* (2000); ○ Miller *et al.* (2001a); ● Miller *et al.* (2001b); ◆ Moorby *et al.* (2006); ◇ Pacheco *et al.* (2007). Note, in all cases except Pacheco *et al.* (2007), the animals also received c. 4 kg/day concentrate.



reflect the changing protein demands of the various livestock classes (Edwards *et al*. 2007). The increased supply of energy to the rumen is proposed to increase *protein* supply to the ruminant, and greater response might be expected in protein limited/demanding systems, than in energy limited ones. Furthermore, testing under grazing (as opposed to the predominantly indoor work in EU), and at the farm scale is needed to gain more evidence of how well the benefits are expressed under NZ farming conditions.

Effect on N utilisation

Initial dairy cow studies in the UK provided strong evidence that feeding dairy cows high WSC cultivars may improve N utilisation in the rumen as indicated by more efficient use of dietary N for milk production and less N excretion in urine (Table 1). Miller *et al.* (2001a) reported a significant reduction in urinary N and an increase in milk N (as a proportion of N eaten) in late lactation dairy cows consuming the high WSC cultivars. A significantly lower proportion of dietary N in urine for the high WSC than control cultivars was also noted in early (Moorby *et al.* 2006) and mid (Miller *et al.* 2000) lactation dairy cows housed indoors. Again the results of Moorby *et al.* (2006) and Miller *et al.* (2000) must be treated with some caution as methods were used to accentuate differences in WSC between cultivars. Moorby *et al.* (2006) cut the low WSC cultivar at 10:00 h and the high WSC cultivar at 14:00 h to emphasise the high WSC cultivar. Miller *et al.* (2000) applied an extra 50 kg N/ha to the control grass in order to increase CP levels relative to WSC. Here, it is uncertain if the high WSC diet *per se* gave the benefits in reduced N emission in urine, or whether application of N to just the control treatment raised N in the control diet and confounded the effects of raised sugar in the high WSC diet.

Further support for the prospects of using higher WSC grasses to improve N efficiency also comes from studies with steers (Table 1). Lee *et al.* (2003) demonstrated, using *in vitro* rumen fermentation, the potential for greater N use efficiency, but were unable to demonstrate this in stall-fed steers (Lee *et al.* 2002). Merry *et al.* (2003, 2006) demonstrated (both *in vitro* and *in vivo*) that silage made from high WSC grasses did improve the incorporation of silage N into microbial N, and saw notable benefits when grass silage was mixed with legume (red clover) silage (see Dewhurst *et al.* 2003a, b). In contrast, a further set of dairy cow studies in the Netherlands consistently reported little benefit of high WSC in improving N use efficiency (Tas *et al.* 2005, 2006a, b; Taweel *et al.* 2006), and attributed observed changes almost entirely to differences in total N intake (Tas 2007). The Dutch studies also drew attention to how the UK work had been conducted using forage diets of rather low crude protein (CP) content.

In conclusion, the literature provides ‘proof of concept’ and promising support that N utilisation can be improved with the use of high WSC grasses, though the evidence suggests this may depend as much on the N environment in which the forage is grown, and so its CP content. Some of the variation (notably between the UK and Dutch studies) may be resolved by combining the data from the multiple trials and showing this forms a continuum of response to diet quality (Fig. 1a, b). The hypothesis for the benefits of high WSC grasses is that high WSC concentrations may correct an imbalance or asynchrony in the supply of energy and protein in the rumen. To test this directly, we need to compare N utilisation, not for a range of WSC contents, or N intakes, but for a range of WSC:CP ratios. Plotting the published data from both UK and the major Dutch trials, in relation to the WSC:CP ratio of the diet, suggests a common relationship between N use efficiency and WSC:CP ratio in dairy cows (see Figs. 1a, b). The lack of response in some trials can be seen to be because there were only small differences in WSC:CP ratio, despite large differences in WSC content.

From an environmental perspective, it is critical to confirm whether the N utilisation benefits will be evident under NZ conditions. These differ from circumstances in the EU in a number of ways: (i) a far greater proportion of the diet in New Zealand is forage based, so that modifications to forage quality will have greater impact, but (ii) diets of fresh forage have higher N content (and more legume) in New Zealand than in the EU (more so as NZ intensifies dairy agriculture). For example, in the studies of Miller *et al.* (2000, 2001a) and Moorby *et al.* (2006), the ryegrass had a CP content ranging from just 92-145 g CP/kg DM. These are low in comparison to the concentrations reported in studies of grazed pasture in New Zealand (e.g. mean 185 g CP/kg DM; range 50-362 g CP/kg DM) (Corson *et al.* 1999). Although CP concentrations in pasture may be inadequate for maximum pasture growth, they generally exceed requirements for milk production (NRC 2001). Under these conditions, where livestock requirements for protein are satisfied, the environmental benefits of high WSC to improve N utilisation and reduce N excretion in urine are more likely to be seen. However this will depend on whether WSC can be elevated sufficiently to restore WSC:CP ratio above *c.* 0.7 (Fig. 1). This we calculate (Edwards *et al.* 2007) may require increases in WSC of 100-200+ g WSC/kg DM, values far greater than has consistently been achieved to date.

Wider Implications of High WSC Grasses

WSC cultivar x environment x management interactions

Changes in WSC concentration, in the form and priorities for metabolic pathways during regrowth, and in response to temperature are well documented (Morvan-Bertrand *et al.* 2001; Lasseur *et al.* 2007). Sugars increase substantially during regrowth following defoliation, and regrowth is generally faster at warm temperatures (defined in the context of each species' adaptive range).

Likewise, low temperatures *per se* lead to a substantially greater total WSC. These responses expose a possible conflict. On the one hand, warm temperatures may stimulate increases in WSC by stimulating recovery of leaf area during regrowth. On the other hand, warm temperatures may reduce sugars relative to colder temperatures. Thus, there is a potential environment (temperature) x management (regrowth duration) interaction. Studies of WSC concentration during contrasting regrowth intervals at different temperatures might only confirm long standing knowledge of the optimal defoliation regime for given local climates. But, if at the same time measures were made of just which genes/pathways were expressed and contributed to sugar level control, then greater opportunities for removing the source of some of the uncertainty in expression in the high WSC trait, may be realised.

WSC cultivar x grazing management interactions

Water soluble carbohydrates are major metabolic and storage components in perennial ryegrass. Thus, if perennial ryegrass is allowed to grow to greater pasture mass, it may be expected to contain higher WSC. Further, grazing systems that allow greater accumulation of pasture mass (e.g. rotational grazing) might be expected to have higher WSC concentrations than those that are maintained at low pasture mass (e.g. continuous grazing). In the UK, Marley *et al.* (2007) tested this idea, but found no difference in the differential of WSC concentration between the high WSC cultivar Aberdart and the control cultivar Fennema between rotational and continuous sheep grazing. But note also in the Marley *et al*. (2007) study that there was little difference in WSC content between cultivars.

WSC cultivar x nutrient cycling interactions

A decrease in urine N, and a greater relative release of organic N in dung, may have immediate environmental benefits in reducing N leaching and emissions of greenhouse gases (Di & Cameron 2002). However, it is unclear how possible changes in root exudation of sugars or sustained changes in the C:N ratio of decaying plant biomass, would affect the function of major microbial systems in the soil. Of note is that changes in leaf WSC concentration potentially parallel, and may even exceed, the proposed impacts of elevated atmospheric CO_2 seen as a part of climate change (Edwards & Newton 2007; Newton & Edwards 2007). Research is underway to evaluate if major changes in plant quality could mitigate, or exacerbate, impacts due to 'climate change'.

WSC cultivar x endophyte interactions

In New Zealand, the endophyte (*Neotyphodium lolii*) status of perennial ryegrass is of crucial importance in determining the grasses agronomic value and impact on livestock (Easton & Fletcher 2007). Any changes in endophyte concentration and in the secondary metabolites they produce may thus have important implications for New Zealand pasture systems. Rasmussen *et al.* (2007) studied interactions between high WSC cultivars, fungal endophyte strains and N supply in a laboratory study. They showed that the endophyte concentration – determined by quantitative PCR – was reduced by 40% under high N supply and by 50% in the high WSC cultivar (Fig. 2). Production of the alkaloids peramine, ergovaline, janthitrems and lolitrem B were also reduced under both high N supply and in the high WSC cultivar, and in both cases the effects of N supply and high WSC were additive.

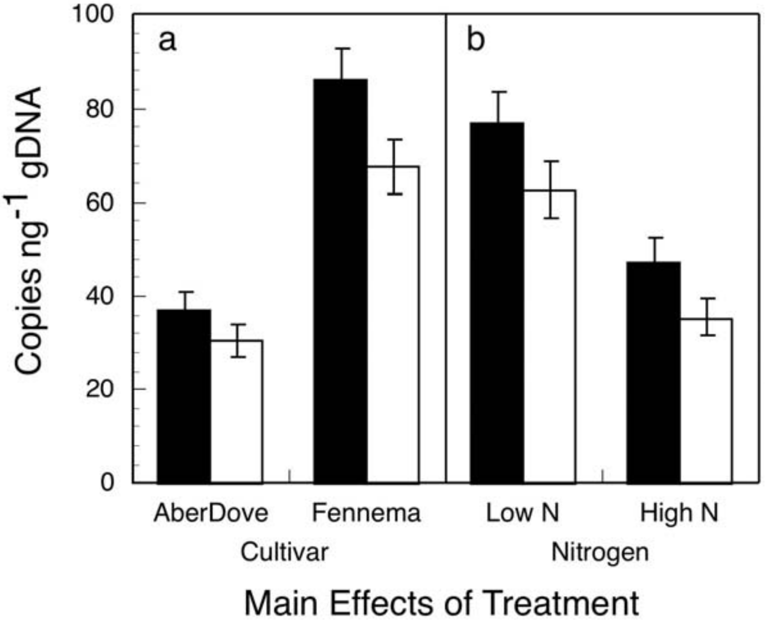
In conclusion, the changes in response to WSC content and N supply may have important implications for New Zealand pasture systems by affecting the balance of risk (e.g. toxicity) and benefit (e.g. insect deterrence) of having endophyte in the pasture. What remains a concern is whether the concentration of peramine – the alkaloid responsible for deterrence of Argentine stem weevil – is at times reduced below critical levels for protection from this insect, thus jeopardising plant productivity. However, this concern is based on one laboratory study and field based studies of interactions between endophyte, high WSC cultivars, N fertiliser and insect pest attack are currently underway to determine the generality of this response.

WSC cultivar x diet preference x pasture composition interactions

If the high WSC trait were to alter grazing preference there may be important implications for the persistence of pastures, pasture quality and pasture composition (Cosgrove & Edwards 2007). Mayland *et al.* (2000) noted cattle preference for tall fescue cultivars was positively correlated with total non structural carbohydrate, while Smit (2005) found dairy cows consistently preferred perennial ryegrass cultivars with a high WSC concentration.

The potential impacts of this preference may be positive or negative. On the one hand, high preference may promote overgrazing and reduced persistence. On the other hand, high preference may overcome the reluctance of dairy cows to graze to the low pasture residuals (e.g. 1300 to 1500 kg DM/ha) that are proposed in New Zealand (Hoogendoorn *et al.* 1988; van Bysterveldt

Figure 2 Main effects of (a) cultivar and (b) N availability on fungal endophyte concentrations expressed as the number of copies of two 'single copy' fungal genes NRPS-1 and chitinase per unit total (plant plus fungal) genomic DNA. Black bars denote NRPS-1, white bars denote chitinase. Aberdove = high WSC cultivar; Fennema = control WSC cultivar. Bars show the untransformed means and standard errors. There were no significant interactions. (From Rasmussen *et al.* 2006, with permission from New Phytologist).



2005) to enhance pasture quality (e.g. by better white clover growth and by reducing seed head development and build up of dead material). Further, the proportion of white clover in the pasture may also be increased if the use of high WSC cultivars reduces the partial preference (60-70%) observed for white clover (Parsons *et al.* 1994; Cosgrove & Edwards 2007). The one study set up to test this (Francis *et al.* 2006) was hampered by the lack of significant difference in WSC between high and control cultivars, and no difference in partial preference for white clover was observed.

Consequently, the use of high WSC grasses may potentially have important impacts on pasture composition. However, there are limited data available to confirm this. To date, studies of high WSC perennial ryegrasses have generally used pure perennial ryegrass swards so that measurements of pasture chemical composition and animal response are not confounded by the presence of clover. Ultimately, the value of grasses – whether high WSC or conventional cultivars – should be assessed in the presence of clover as they are used on-farm (Harris 1990). There is considerable scope in which to investigate this. For example, Munro *et al*. (1992) showed that the increase in lamb output between Aurora (high WSC cultivar, Humphreys 1989a; Smith *et al*. 2002) and control ryegrasses was more pronounced when the ryegrass was grown with clover than as a pure sward.

Future prospects for meeting WSC:CP goals

Progress to date in increasing the sugar concentration of perennial ryegrass by traditional breeding has seen annual gains of "approximately 4 g WSC/kg DM" from 1992-2000, accompanied by a 1g WSC/kg DM per annum reduction in CP (Wilkins & Lovatt 2003; and see Marais *et al.* 2003). In keeping with this, our review points towards differences in the field between high WSC cultivars and controls which are rarely greater than 40 g WSC/kg DM. Earlier, we argued that a greater and more consistent expression of the trait in plants may be necessary. Molecular techniques may offer the greatest prospects for going beyond the existing, potentially conservative, natural range of expression. Detailed studies of gene expression and associated enzyme activity could provide benefits from better understanding *per se*, but molecular techniques also offer prospects for non transgenic (cisgenic) if not fully transgenic, up-regulation of the trait (see Edwards *et al.* 2007 for discussion of molecular biology approaches to improving WSC trait expression).

Conclusion

- The development of perennial ryegrass cultivars with high WSC represents a substantial effort to progress industry goals of greater production with an eye to environmental concerns. This work displays an exemplary level of commitment, not just to developing forage plants, but to testing 'proof of concept' and to ensuring the proposed benefits can be exhibited through animals. The proposed benefits of the trait have been exposed to a level of scrutiny that has, to be fair, been far greater, and more critical, possibly than for any other grass cultivar development (see e.g. Crush *et al.* 2006).

- Available evidence reveals substantial variation in livestock production responses but the effect has never been negative. A more consistent response of improved N utilisation was noted across studies, indicating valuable potential prospects for reducing environmental impacts of farming if the WSC:CP ratio can be elevated in the high CP forages grown in New Zealand.
- There is a serious need for further field based, year round, confirmation, of how well the trait is expressed and how great the animal responses are under New Zealand conditions. Here, research should move beyond the current short term studies to include farm scale evaluations so the full ramifications of the trait under different farm managements can be explored. But it is important these studies have an appropriate control treatment, an adjacent area resown with a control cultivar to ensure the benefits of resowing with a high WSC grass are due to the grass cultivar *per se* and not the benefits of resowing *per se* (eg N mineralisation).
- But field/farm based studies *alone* may not suffice. These can serve to establish whether current forage germplasm works at present, but run the risk that further evidence of uncertain performance benefits will dismiss future prospects completely. Science-focussed research is also needed (it was, after all, the origin of the opportunity) to explain the variation seen to date; to elevate WSC sufficiently to restore WSC:CP ratio in high N forage, and so to secure future opportunities.
- Areas in which additional research is required are the molecular basis of the control of sugar accumulation, effects of high WSC cultivars on pasture composition and pasture persistence (pests/weeds), and interactions with endophyte and mycorrhizae, under a range of NZ grazing management combinations.

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