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## Effect of water soluble carbohydrate concentration of ryegrass on the partial preference of sheep for clover

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### ABSTRACT

A trial was carried out to determine whether grass cultivar or water soluble carbohydrate (WSC) concentration would influence the partial preference of sheep for clover and ryegrass. Six mixed age Coopworth ewes were housed in individual pens and once a day offered swardlet monocultures of white clover adjacent to one of three perennial ryegrass cultivars. One swardlet of each grass cultivar was shaded and the other left un-shaded in order to create differences in WSC concentration. WSC concentration did not differ among ryegrass cultivars but was lower in shaded (210 g/kg dry matter (DM)) than non-shaded (230 g/kg DM) ryegrasses. Neutral detergent fibre was significantly higher in the un-shaded (439 g/kg DM) than non-shaded (422 g/kg DM) ryegrass ( $P < 0.01$ ). The total number of bites from clover swardlets fed adjacent to shaded grass swardlets was 21% greater ( $P = 0.026$ ) than when fed adjacent to non-shaded grass swardlets. Sheep took a significantly lower proportion of bites from clover when paired with un-shaded (44%) than shaded (55%) ryegrass ( $P = 0.03$ ). There were no significant effects of ryegrass cultivar on any aspect of the preference tests. The results of this work support the hypothesis that partial preference for clover can be manipulated by altering the WSC concentration of companion ryegrass by shading but not, in this case, by ryegrass cultivar.

**Keywords:** diet selection; diet preference; *Lolium perenne*; *Trifolium repens*; water soluble carbohydrates.

### INTRODUCTION

Grazing sheep and cattle exhibit preferences for plant species and plant components (Rutter *et al.*, 2004) and are able to discriminate and select a diet that differs in proportion to what would be obtained if they grazed at random. Studies show that when offered ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) as adjacent monocultures, sheep and cattle most often select a diet of approximately 70% clover and 30% ryegrass (see Rutter *et al.*, 2004 for a review). This partial preference for clover may produce an immediate nutritional benefit for the animal but depletes the preferred species from pastures and therefore may reduce diet quality in the long term. A lower partial preference for clover may be a strategy to increase clover content in the pasture and diet in the longer term (Edwards *et al.*, 2008).

Dietary preference for grasses has been shown to be positively correlated with water soluble carbohydrate (WSC) content (Tava *et al.*, 1995) suggesting that grasses bred to express a high sugar content may reduce the partial preference for clover. Francis *et al.* (2006) tested this hypothesis but lack of trait expression meant no conclusions could be drawn. The present study was conducted to determine if WSC content, as influenced by cultivar or shading, influenced the partial preference of sheep for clover and ryegrass.

### MATERIALS AND METHODS

An indoor trial using six mixed-age Coopworth ewes with a liveweight range of 56.6 to 69.0 kg was

carried out at Lincoln University starting 3 April 2008. The experimental design was a latin square with six comparisons each offered to one of six sheep each day for six days and repeated during the next six days. The comparisons were a swardlet of 'Demand' white clover paired with one of three ryegrass cultivars which had either been shaded or non-shaded. The perennial ryegrass cultivars were 'Aberdart' (marketed as a high sugar diploid), 'Bealey' (tetraploid) and 'Bronsyn' (diploid). A basal diet of 500 g/d fresh weight of meadow hay and 1 kg/d of APR pellets (Archers, Ltd, Rangiora) was offered before and after the preference test. A common diet was fed to all sheep to ensure that preferences were not affected by previous diet. Water was available *ad libitum* at all times in the pens.

Swardlets were sourced from monoculture plots grown at the Field Service Centre, Lincoln University. Each day at 8:00 h two swardlets of each of the three grass cultivars and clover ( $n = 6$ ) including soil were dug to fit within a 22 x 42 x 10 cm deep plastic tray. Grass swardlets to be shaded were moved indoors to an area shaded with hessian sacks. The other grass and all clover swardlets remained outdoors. WSC concentration is lowest at sunrise, accumulates throughout the day on exposure to light reaching a peak in early afternoon (Orr *et al.*, 1997). The shading was designed to maintain WSC at a lower concentration.

Before the preference test, 10 height measurements were taken from each swardlet with a ruler and each sward weighed to the nearest 1 g. Pre-grazing pasture samples were also sourced from

**TABLE 1:** Pre-grazing height (cm) and the decline in height (cm) due to grazing, of three ryegrass cultivars either shaded or non-shaded and their respective clover swardlets. SEM = Standard error of the mean.

Treatment	Grass pre-grazing (cm)	Clover pre-grazing (cm)	Decline in grass (cm)	Decline in clover (cm)
<b>Cultivar</b>				
Bealey	18.0	12.8	3.44	4.04
Aberdart	17.0	12.7	3.62	3.40
Bronsyn	17.8	12.0	3.87	2.96
SEM	0.039	0.027	0.462	0.363
P value	0.235	0.036	0.805	0.119
<b>Shade treatment</b>				
Non-shaded	17.9	12.6	4.10	3.56
Shade	17.2	12.3	3.19	3.38
SEM	0.031	0.022	0.377	0.296
P value	0.191	0.400	0.097	0.673

mini swardlets harvested at the same time as the main ones and cut to ground level before each test was made. All pasture samples were frozen, freeze-dried, ground and nutritional parameters estimated by near infra-red spectrophotometry by the Lincoln University Analytical Service.

Swardlets were then strapped into pairs and offered to the sheep. Preference tests were conducted between 12:30 h and 13:00 h on each day. The number of bites taken from each swardlet was recorded for one minute after which the swardlets were removed. A bite was classified as the prehending bite in which the sheep pulled herbage from the sward and was associated with the sound of herbage ripping and the pulling motion of the sheep's head. Post-grazing, the swardlets were reweighed, 10 post-grazing height measurements taken and the pasture cut to ground level and bagged.

**TABLE 2:** Main effects of perennial ryegrass cultivar and shading on mean nutritive parameters and pre- and post-grazing height of three cultivars of perennial ryegrass and clover. DM = Dry matter, SEM = Standard error of the mean.

Treatment	WSC (g/kgDM)	NDF (g/kgDM)	ADF (g/kgDM)	DOMD (g/kgDM)	Crude protein (g/kgDM)
<b>Cultivar/species</b>					
Bealey	229	421	235	734	147
Aberdart	224	418	225	740	162
Bronsyn	207	453	246	724	154
Clover <sup>a</sup>	117	182	161	723	294
SEM	8.88	4.74	2.69	4.28	4.05
P value	0.23	<0.001	<0.001	0.04	0.05
<b>Shade treatment</b>					
Non-shaded	230	422	232	734	153
Shade	210	439	239	732	15.6
SEM	7.25	3.87	2.20	3.49	3.31
P value	0.06	<0.01	0.05	0.68	0.58

<sup>a</sup>Clover was not included in the separation of means.

Bite mass and dry matter (DM) apparently consumed were calculated from the change in weight of swardlets before and after grazing. The percentage of bites from clover was calculated as a measure of dietary preference.

The experimental protocol was approved by the Lincoln University Animal Ethics Committee.

Data was analysed as an ANOVA (Payne *et al.*, 2007) of two replicates of a latin square with six sheep, three cultivars and two shading treatments as fixed effects. Where treatment effects were significant, means were separated by a least significant difference test (P <0.05).

## RESULTS

There was no statistical difference in the mean pre-grazing height of the three grass cultivars or between shaded and non-shaded swardlets however clover swardlets paired with Bronsyn swardlets were 0.8 cm and 0.7 cm shorter on average (P = 0.036) than the clover swardlets paired with Bealey or Aberdart respectively (Table 1). Neither cultivar nor shading treatment had a significant effect on the decline in grass or clover height with grazing.

There was no significant effect of grass cultivar on WSC concentration (Table 2). WSC concentration was 9% lower (P = 0.06) in shaded than non-shaded grasses. There was a highly significant (P <0.001) effect of cultivar on acid detergent fibre (ADF) and non detergent fibre content (NDF). Bronsyn had the highest concentrations of NDF and ADF followed by Bealey then Aberdart (Table 2). Shading significantly increased NDF concentration and ADF concentration (Table 2). Organic matter digestibility and protein concentration were higher in Aberdart than Bronsyn and Bealey (Table 2). Shading did not affect digestibility or protein concentration (Table 2).

Although there was no significant effect of cultivar on the mass of grass or clover grazed, there were trends suggesting sheep preferred Aberdart over the other grass cultivars. Table 3 shows sheep removed 5.78 g DM from Aberdart swardlets which was 25% and 23% more DM than that removed from Bealey and Bronsyn swardlets respectively. Similarly, there was no significant effect of shading on the mass of clover or grass removed but a trend for sheep grazing non-shaded grass to remove more grass (+12%) and less clover (Table 3).

Sheep took significantly fewer (P = 0.026) bites from clover swardlets paired with non-shaded grasses (Table 3). Sheep took a

**TABLE 3:** Main effects of cultivar and shading treatment on bite mass, total number of clover bites and the proportion of clover bites. DM = Dry matter, SEM = Standard error of the mean.

Treatment	Mass grazed (g DM)		Bite mass (mg DM)		Total no. of clover bites	% of bites from clover	Mass of clover grazed (% of total)
	Grass	Clover	Grass	Clover			
<b>Cultivar</b>							
Bealey	4.64	4.52	271	197	22.5	49.9	48.5
Aberdart	5.78	4.33	260	276	19.3	48.1	45.9
Bronsyn	4.70	4.02	261	178	21.2	50.9	44.4
SEM	0.490	0.406	109	78	1.83	4.47	3.77
P value	0.194	0.682	0.975	0.031	0.475	0.903	0.744
<b>Shade treatment</b>							
Non-shaded	5.32	4.19	0.298	0.241	18.6	44.0	43.1
Shade	4.76	4.39	0.231	0.194	23.4	55.3	49.4
SEM	.400	0.332	0.031	0.022	1.50	3.65	3.08
P value	0.330	0.684	0.139	0.139	0.026	0.033	0.150

significantly lower proportion of bites from clover when paired with non-shaded shaded (44%) than shaded (55%) ryegrass.

## DISCUSSION

The results of this work support the hypothesis that partial preference for clover can be manipulated by altering the WSC concentration of companion ryegrass by shading but not, in this case, by grass cultivar.

Shading decreased mean WSC concentration by 10% and increased NDF by 4% indicating a shift from non-structural to structural carbohydrates in shaded grass. These effects are similar to those of Hight *et al.* (1968) who showed that shading perennial ryegrass caused a reduction in the proportion of soluble carbohydrate from 6.3 to 2.4% and an increase in the proportion of cellulose and lignin from 16.8 to 19.6% and 5.6 to 6.5% respectively. Smith *et al.* (1998) also found that high sugar grasses with a higher WSC concentration had a lower NDF concentration. Ciavarella *et al.* (2000) used shading to reduce the WSC concentration of *Phalaris aquatica* L. to 62 mg/g DM compared with non-shaded strips at 126 mg/g DM WSC, although 24 hour shading was used.

Shading increased the partial preference for clover as indicated by the significantly higher number of clover bites and greater proportion of bites taken from clover. This suggests that sheep were responding to the shift in the proportion of non-structural to structural carbohydrates due to shading by increasing the proportion of clover in their diet. It is not clear of course, whether this change in diet selection is primarily a response to the decrease in WSC or an increase in NDF concentration in the grass or the balance between the two. Crude protein concentration has also been shown to influence feed preferences (Kyriazakis, 2003). However, there was no significant difference

in the protein concentration of shaded and non-shaded grasses and therefore this cannot explain the effect of shading on partial preference

This result is consistent with other studies of preference of forages differing in WSC concentration. Ciavarella *et al.* (2000) found Merino wethers selected 2.6 times more non-shaded grass than shaded grass when offered as a choice. Burritt *et al.* (2005) found lambs preferred hay cut in the afternoon over hay cut in the morning, presumably because of the higher WSC content of the afternoon hay.

In our study, partial preference was not altered by offering different ryegrass cultivars. However, Aberdart, a cultivar bred in the United Kingdom to have higher WSC concentration in lamina than standard ryegrass cultivars did not express this trait under the conditions of this trial. Other studies with high sugar grasses have also noted a lack of trait expression in Australia (Francis *et al.*, 2006) and New Zealand (Parsons *et al.*, 2004), which may be attributed to the environment in which it is grown (Parsons *et al.*, 2004). There were significant differences in NDF and ADF concentrations between cultivars but this did not affect the partial preference for clover although significant differences in preference between pair of ryegrass cultivars has been shown previously (Bryant *et al.*, 2008).

An unusual observation in this work was that the typical preference ratio for clover and grass of 70:30 observed by other workers (Rutter *et al.*, 2004) was not seen in this experiment where less than 50% of total intake was from clover. Using a similar swardlet technique, Concha and Nicol (2000) showed that as the pre-grazing height of clover decreased relative to that of grass, the partial preference for clover decreased. These authors also showed that the bite mass of clover was greater than that of grass at the same pre-grazing height but that clover bite size decreased with pre-grazing clover height. Although in the present study, the pre-grazing height of clover and grass were similar, bite

mass of clover and grass was also similar. The low bite mass of clover may have contributed to the relatively low proportion of clover in diet selected in this study.

Reducing the WSC concentration of grass swardlets by shading caused a significant increase in the partial preference shown for clover. This suggests that not only are sheep able to make nutritional decisions based on a small number of bites, but that the balance of WSC and NDF concentration may play an important role in determining the partial preference of grass and clover. The results of shading indicated that an increase in WSC concentration may be a method for manipulating partial preference for clover. However, unless differences in WSC concentration of greater than 2% units exists between grass cultivars, it is unlikely that differences in partial preference for clover will be influenced by grass cultivar. Expression of the high sugar trait in New Zealand pastures needs to be investigated further to ensure expression is more reliable if the impacts of high sugar grasses on clover preference and other factors such as nitrogen utilisation in livestock are to be demonstrated.

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