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Clover-rich diets and production, behaviour and nutrient use by cows in late lactation

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ABSTRACT

The nutritional value of legumes for high milk production is seldom captured because grazed dairy pastures in NZ typically contain insufficient legume to satisfy the cows' requirements. This paper reports interim results from a project investigating novel methods of forage presentation to cows, to enhance the contribution of legume to their diet. Four groups of spring-calving cows (n=5) in late-lactation were allocated to four treatments in a balanced, cross-over design. Two treatments consisted of spatial and temporal arrangements of grass and white clover allocated to cows as follows: i) continuous free access to grass and white clover growing separately as side-by-side monocultures (G/C) and ii) grass-only at 'night' between the afternoon and morning milking and clover-only during the 'day' between morning and afternoon milking (GnCd). These were compared against controls of iii) a typical mixed ryegrass-white clover pasture (Mix; an industry standard) and iv) a ryegrass-only pasture (Grass; a forage standard). Cows grazed each treatment for 8 days before moving to the next treatment in the pre-determined sequence. Measurements included milk production and composition, the time spent grazing during the day and the night (separately for grass and white clover in G/C) and the concentrations in the milk of skatole and indole, as metabolic indicators of nutrient acquisition and utilisation in the contrasting forage treatments. Cows offered G/C or GnCd produced 19.4 and 17.9 kg milk/cow/day, respectively, compared with 15.0 and 14.6 kg/cow/day when offered Mix or Grass, respectively (P=0.01, SEM=0.74). The diurnal distribution of grazing was influenced by pasture treatment (treatment x time interaction P <0.001). Cows on G/C grazed proportionately more at 'night' and cows on GnCd proportionately less at 'night' compared with Grass or Mix. GnCd constrained the cows to spend less time grazing clover than those offered G/C (245 vs. 408 mins grazing clover during 24 hrs) and more time grazing grass (253 vs. 105 mins in 24 hrs). The apparent 'restriction' in GnCd to grazing clover-only during the day did not significantly reduce milk yield compared with G/C. The spatial and temporal allocations of grass and clover affected the concentrations of indolic compounds in PM and AM milk, suggesting there is scope to improve the efficiency of protein utilisation in the rumen.

Keywords: grass; white clover; grazing behaviour; skatole, indole.

INTRODUCTION

The proportion of clover in mixed-species pasture (e.g. 10-20%) is generally too low for the nutritional benefits of white clover for milksolids (MS) production to be fully realised by commercial farmers. Cows eating clover produce more milksolids (MS) than those eating ryegrass (Rogers *et al*., 1982). While this superiority is often attributed to its typically higher concentration of protein (nitrogen), even at the same concentration of N, clover is superior to grass (Ulyatt 1981). The proportion of clover required to maximise milk production is 55-65% (Harris *et al*., 1997), and this is very close to the proportion of clover that cows (and sheep) prefer to eat when given free choice (Newman *et al*., 1992; Parsons *et al*., 1994). However, both research and practice clearly show that it is seldom possible to create mixed pasture with the high proportion of clover that cows prefer, let alone sustain that high proportion.

There are alternative ways of growing and presenting grasses and legumes that might allow these benefits to be more readily captured. Cows in mid-lactation given continuous free access to pasture comprised of ryegrass and white clover growing separately, side-by-side, produced more milksolids than those given conventional pasture comprised of grass and clover growing as a mixture (Nuthall *et al*., 2000; Marotti *et al*., 2001). The basis for the production responses in these studies was the high proportion of clover in the cows' diet, and its high nutritive value compared with ryegrass, rather than a higher intake of dry matter. Short-term studies indicated that animals given this novel pasture arrangement could benefit from both higher dry matter intake and higher nutritive value of clover (Cosgrove *et al*., 1999; Marotti *et al*., 2002; Champion *et al*., 2004), and recently Rutter *et al*. (2003) reported that cows fed in this way grazed more, ate more and produced more milk.

The objective of this study was to determine if the behavioural responses to alternative methods of pasture allocation seen to date would translate to higher milk yield and milksolids production by cows in the later stages of lactation during autumn. Data was collected on grazing behaviour, milk yield and composition, and in addition the concentrations in the milkfat of skatole and indole were monitored as indicators of protein utilisation in the rumen (Lane *et al.*, 2002).

MATERIALS AND METHODS

This study was conducted during April 2005, on the Massey University No 1 Dairy Unit, located on Manawatu sandy loam soils adjacent to the Manawatu river.

Treatments

Four treatments consisted of different spatial and temporal allocations of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) as follows: Grass-only, 24 hrs a day (Grass); Mixed ryegrass-white clover pasture, 24 hrs a day (Mix); Continuous, free access to ryegrass and white clover, growing separately side-by-side as monocultures in the area ratio of 0.75 clover and 0.25 grass (G/C); Grass-only at night and clover-only during the day (GnCd). The mixed pasture contained 9 ± 5 % white clover and 91 ± 5 % grass (mean ± SD).

Experimental design

A balanced, cross-over design was used. Cows were allocated to treatments for 8 days. Following the final milk sampling on the morning of day 8 the cows moved directly to the next assigned treatment in the sequence. The experimental unit was the group mean.

Pasture establishment and maintenance

Approximately 15 hectares of existing mixed-species pastures (10 paddocks) at the site were used to create the composition required for each treatment. The areas for Grass-only and Clover-only were created by spraying the existing pasture in October 2003 with selective herbicides to remove legumes and grasses, respectively. Unsprayed, resident pasture was used for the Mix treatment. The spatial and temporal configurations of grass and clover required for G/C and GnCd treatments were achieved using temporary electric fences. Paddocks were allocated to treatments such that each treatment comprised equal areas of old grass pasture where the ryegrass component contained wild-type endophyte and more recently sown ryegrass pasture containing AR1 endophyte. Urea was applied to the grass-only and mixed pasture areas at 35 kg N/ha, 6 times annually (at approximately 2-monthly intervals).

Animals

Twenty spring-calving cows (527 ± 40 kg liveweight, 3.8 ± 0.4 condition score; mean ± SD) were selected from the main herd and allocated to 4 groups of 5 cows balanced for parity, Production Worth and days-in-milk (average 232 days in milk as at March 30; range 201-254). All cows were dosed twice daily at milking for prevention of bloat (Bloateze, containing 700g/l alcohol ethoxylate and 70 g/l ethylene/propylene co-polymer; Farmers Industries Ltd. Mount Maunganui). In addition, cows were dosed with a Rumensin® antibloat rumen capsule (releasing 320 mg monensin sodium/day, Elanco Animal Health, Manukau) 5 days prior to the start of the experiment and water troughs were treated daily with Bloateze for additional protection against bloat.

Pasture allocation and chemical composition

For each group, the total daily feed allowance (54 kg DM/cow/day) was offered as fresh breaks after each milking in the ratio of 40% during the 'day' and 60% during the 'night'. Pasture mass was assessed using an electronic rising-plate meter (Farmworks Precision Farming Systems, Feilding), and allocation protocols included a minimum pre-grazing and post grazing mass of 2400 kg DM/ha and 1600 kg DM/ha, respectively. For G/C, the masses of grass and clover were measured independently and the total area to be allocated (0.75 clover:0.25 grass) was calculated using the lesser mass. Samples of grass, clover and mixed grass-clover pasture were taken at 8:00 AM and 5:00 PM of the last 24 hours grazing for each of the 4 periods in the cross-over. These samples were handplucked to represent herbage being consumed by the cows in each treatment. Separate samples were taken in the morning (AM) and afternoon (PM) to describe diurnal changes in chemical composition and to relate milk composition in the PM and following AM, respectively, to the herbage ingested during the preceding interval. Samples were stored frozen and subsequently freeze-dried, ground to pass a 1 mm sieve, and analysed using near-infra red reflectance spetroscopy (FeedTech, AgResearch Grasslands, Palmerston North).

Milk yield measurements

Milk yield was recorded at each milking using Metatron™ (Westfalia, Germany) meters and proportional in-line samplers. Subsamples for each cow were retained for analysis of milkfat and protein (FT 6000 Fourier Transform infrared analyser, Foss Electric, Hillerød, Denmark). Daily yield was obtained by summing the PM and following AM yields. Milk yield data presented in this paper is the treatment mean of PM and AM yields for the last 3 days of each period (i.e. allowing 5 days adjustment to treatment) and milk composition is the mean of samples taken at the PM and AM milking of the last day of each period. Yield of milksolids was calculated from the estimates of milk yield and composition at the final PM and AM sampling.

Milkfat metabolites

For analysis of metabolites, milk samples taken from each cow within a treatment at the final PM and AM milkings were pooled proportional to milk yield. Milkfat was extracted from these pooled PM and AM samples and stored at -20 ºC for subsequent analysis for skatole and indole by HPLC with fluorescence detection (Lane *et al.*, 2002).

Behaviour measurements

Cows were observed continuously for the final two, consecutive 24 hour periods, of each 8-day measurement period when cows were accustomed to the diet. One observer recorded activity for two groups of 5 cows, noting at 1-minute intervals whether they were grazing or not, and for G/C whether they were grazing grass or clover. The observations of grazing were summed to give the total duration of grazing for the 'day' and for the 'night'. These were defined as the periods between the morning and afternoon milking, and the period between the afternoon and morning milking, respectively. The 'night' included approximately 2 hours of daylight following the afternoon milking. Cows were off pasture for approximately 2 hours at each milking, and observations resumed when the cows returned to the paddocks. The total time available for grazing during the 'day' was 450 mins and during the 'night' 810 mins.

Statistical analysis

Treatment means were compared by ANOVA after removing group and period effects. For milk yield and milksolids, separate analyses were conducted for daily totals and for PM and AM data. Time was included in the analysis of variance as a stripped-plot factor for grazing duration (day, night) and milkfat metabolites (PM, AM).

RESULTS

Pasture species composition

The chemical composition of the pasture offered to cows in each treatment in the morning and in the afternoon is shown in Table 1. The composition of G/C is shown for grass and clover separately, and as a weighted mean for the treatment calculated from the dietary proportions of each using the time spent grazing on each. The PM pastures for Grass, Mix and G/C treatments had slightly higher soluble carbohydrate, metabolisable energy, organic matter digestibility and lower protein than the AM pasture. For GnCd the contrast between AM and PM pasture composition was greater because of the switch from clover to grass following the afternoon milking. The PM pasture (grass) contained less protein and ME and was less digestible than was AM pasture (clover).

TABLE 1: The concentrations (mean ± SD, g/kg DM) of neutral detergent fibre (NDF), water soluble carbohydrate (WSC), protein (PROT), organic matter digestibility (OMD) and metabolisable energy (ME, MJ/kg DM) of pastures offered to cows in the morning (AM) and in the afternoon (PM) as four treatments consisting of Grass, mixed grass-white clover (Mix), continuous free access to grass and white clover (G/C) and grass at night and clover during the day (GnCd).

Time	Treatment	Species	NDF	WSC	PROT	OMD	ME
AM	Grass	grass	536±70	148±21	224±27	750±38	11.3±0.5
	Mix	grass+clover	546±6	143±17	225±36	739±18	11.2±0.3
	G/C	grass	543±55	132±23	251±24	792±29	11.7±0.2
		clover	482±43	160±17	249±15	810±27	12.1±0.2
		weighted mean[1]	494	155	249	806	12.1
	GnCd	clover	468±42	159±17	263±20	823±25	12.3±0.1
PM	Grass	grass	577±19	172±24	210±22	802±44	11.8±0.3
	Mix	grass+clover	547±53	196±12	218±30	810±40	11.9±0.3
	G/C	grass	557±38	170±40	231±18	823±42	12.1±0.3
		clover	467±21	190±21	234±12	831±57	12.6±0.5
		weighted mean[1]	485	186	233	829	12.5
	GnCd	grass	581±41	172±19	202±37	777±68	11.6±0.8

[1] mean of grass and clover weighted according to the proportion of time grazing each component

Milk yield and composition

Daily milk yield for the clover-rich treatments G/C and GnCd (19.4 and 17.9 kg/cow/day, respectively) was significantly (P=0.01, SEM=0.33) higher than for Grass and Mix (14.6 and 15.0 kg/cow/day, respectively) (Figure 1a). Similar treatment effects were apparent in the daily yield of milksolids (G/C, 1.58; GnCd, 1.50; Mix, 1.37; Grass, 1.29; P<0.05, SEM=0.1; Figure 1b), although the magnitude of the difference was partially reduced by the lower milkfat concentration (P < 0.05) for the clover-rich treatments (Figure 1c) and lower milk protein (P < 0.01) for the GnCd treatment (Figure 1d), in the morning milk.

FIGURE 1: The yield of a) milk (kg/cow/d), b) milksolids (kg/cow/d), c) milk fat (%) and d) milk protein (%) at the morning (AM) and afternoon (PM) milking of cows offered Grass, mixed grass-white clover (Mix), continuous free access to grass and white clover (G/C) and grass at night and clover during the day (GnCd) (LSD $_{0.05}$ for comparing treatment means of AM or PM milk yield 1.5; milksolids 0.13; milkfat 0.7; milk protein 0.1).

Figure 1a

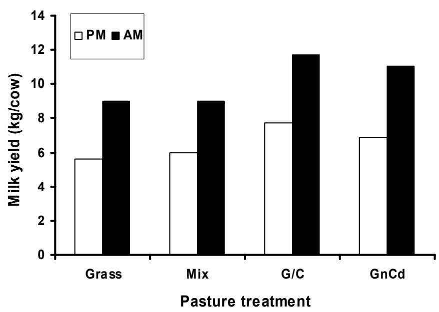


Figure 1b

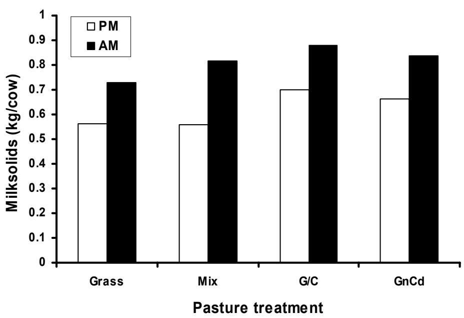


Figure 1c

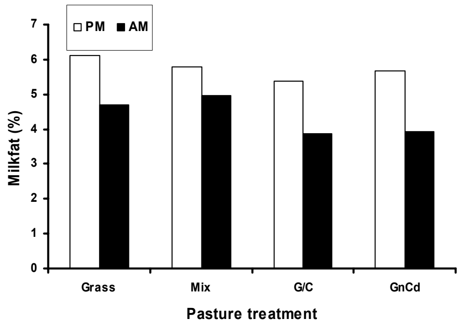
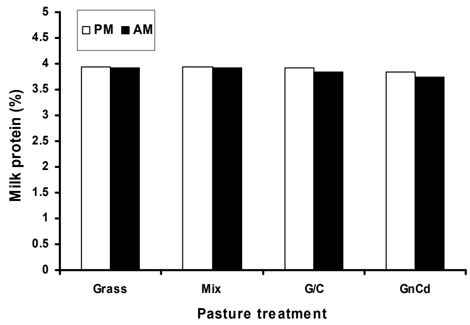


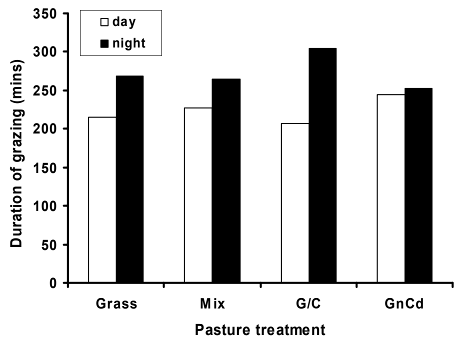
Figure 1d



Duration of grazing

The duration of grazing during the day (225 mins) and during the night (275 mins) did not differ significantly among the treatments. However, there was a pasture treatment x period of day interaction (P < 0.001). The G/C and GnCd treatments were different from the Grass and Mix (Figure 2). The G/C cows grazed more at night and less during the day compared with cows grazing Grass and Mix. In contrast, the GnCd cows distributed their grazing evenly between day and night. For G/C, the proportions of time grazing grass and clover were 0.14 and 0.86, respectively, during the day and 0.19 and 0.81, respectively, during the night.

FIGURE 2: The duration of grazing by cows during the night and during the day by cows offered Grass, mixed grass-white clover (Mix), continuous free access to grass and white clover (G/C) and grass at night and clover during the day (GnCd) (LSD $_{0.05}$ for comparisons among treatment means for different periods of the day 52).



Skatole and indole concentrations in milkfat

The concentrations of skatole (P < 0.05) and indole (P < 0.01) in milkfat were higher in the afternoon milk than in the morning milk for each pasture treatment (Figure 3a, b). The clover-rich treatments resulted in higher concentrations of both compounds compared with the grass dominant treatments (P < 0.01). However, there was a pasture treatment x milking time interaction for both constituents ($P < 0.001$). Milkfat from G/C cows was higher in skatole and indole in both the PM and AM milk compared with the Grass and Mix treatments. In contrast, while the concentrations of skatole and indole in the PM milkfat of the GnCd cows were higher than for the G/C cows, concentrations in the AM milkfat was low and similar to the Grass and Mix treatments.

FIGURE 3: The concentration of skatole (a) and indole (b) in the milkfat at the afternoon (PM) and morning (AM) milking of cows offered Grass, mixed grass-white clover (Mix), continuous free access to grass and white clover (G/C) and grass at night and clover during the day (GnCd) ($LSD_{0.05}$ for comparisons among treatment means for different times of milking for skatole 170; indole 99).

Figure 3a

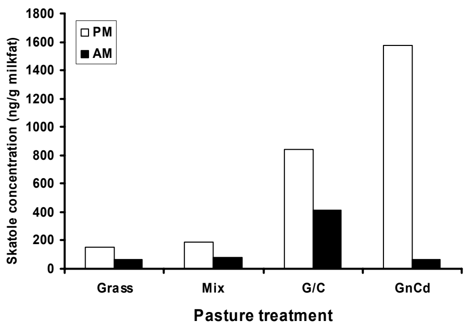
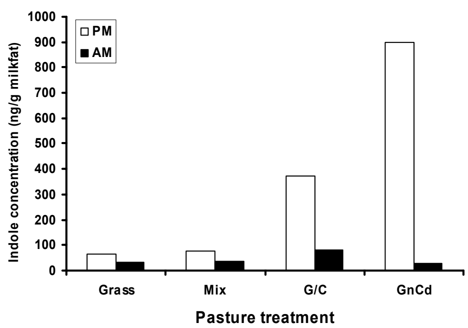


Figure 3b



DISCUSSION

Many studies have demonstrated the high nutritive value of white clover for animal production. However, this potential is seldom realised in practice. The proportion of clover in mixed-species pasture cannot be sustained at the high level required for clover to contribute effectively to the diet and hence production of animals at pasture. Growing grass and white clover separately instead of as a mixture, as proposed by Chapman *et al*. (1996), allows greater control over the proportion of grass and clover grown and offered to animals. In this study, sustaining a high proportion of clover in the diet substantially increased milk production by cows in the latter stages of lactation during autumn. This increase in milk yield for G/C compared with the Grass or Mix treatments (32% higher yield of milk and 20% higher yield of milksolids) is consistent with results reported by Nuthall *et al*. (2000) and Marotti *et al*. (2001) for cows in mid-lactation. The greater milk yield of G/C and GnCd compared with Grass and Mix appears to result from the greater opportunity to eat clover, as, in contrast to results recorded by Rutter *et al*. (2003) for cows in early lactation, there was no increase in total time spent grazing compared with Grass or Mix.

The treatment GnCd was tested because allocating forages in this way may be simpler to implement in practical terms (Rutter *et al*., 2001). In the course of 24 hours cows still get a clover-rich diet, and providing clover following the morning milking and grass following the afternoon milking aligns approximately with diurnal patterns of preference in ruminants noted by Parsons *et al*. (1994). However, compared with G/C, where animals ingest both grass and clover in most bouts of grazing, GnCd alters the temporal synchrony of the intake of grass and clover. This may affect nutrient digestion and metabolism, and constrain the opportunity for cows to fully satisfy the nutritional or behavioural purposes of diurnal patterns in preference (see Newman *et al*., 1995). One of the benefits of G/C demonstrated by Champion *et al*. (2004) was that it offered opportunity for a substantial increase in total intake (often seen as extended grazing time) compared with animals eating grass-only or clover-only. Eating grass and clover in the desired proportions and in close temporal synchrony may be important elements of that 'boost'. In this study there was no difference between G/C and GnCd in the total time spent grazing over 24 hours, and the main difference between the two methods of allocation was in the proportions of grass:clover in the diet of 0.5:0.5 for GnCd and 0.18:0.82 for G/C (estimated from the proportional time spent eating each species). This lower proportion of clover in the diet of the GnCd cows did not significantly reduce milk production compared with G/C. However, as discussed below, aspects of nutrient digestion and utilisation were affected by the way in which grass and clover was presented to the cows.

Skatole and indole were measured in the milkfat as indicators of nutrient acquisition and metabolism in the different treatments, and showed large differences between the treatments. Skatole and indole arise in the rumen from the microbial degradation of the amino acid tryptophan from dietary proteins (Deslandes *et al*., 2001 and references cited therein). The levels in milkfat of pasture-fed cows are much higher than in cows fed a concentrate diet, and may serve as an indicator of dietary protein utilisation in the rumen (Lane *et al.,* 2002). Sheep fed white clover had higher concentrations of skatole and indole in the rumen and in the blood (Schreurs *et al*., 2003, 2006a, 2006b) than sheep fed ryegrass. This was attributed to both the higher protein content of white clover compared to ryegrass, and its rapid degradability. While the concentrations of skatole and indole in milkfat for the cows on the Grass and Mix treatments (Figure 3) were comparable with previously reported levels for dairy cows fed a mixed ryegrass-clover pasture in late spring (Lane *et al*., 2002), those for the G/C and GnCd diets greatly exceed the highest concentrations in early spring reported in that study. This is the first report to link the clover content of forage diets to concentrations of skatole and indole in milkfat. It is likely that the high levels of milkfat skatole and indole relates to the higher intake and protein content of the G/C and GnCd diets, as the amount of protein degraded in the rumen increases with intake and with the protein content of the diet (Kebreab *et al*., 2002). Monensin, the active ingredient of Rumensin, can suppress the conversion of tryptophan to skatole and indole (Mohammed *et al*., 2003), and even higher concentrations in milkfat may have been recorded if cows had not been treated with Rumensin. The suppression may have differed between grass and clover-rich diets, mediated through the differences in protein degradability and the resulting levels of free tryptophan in the rumen.

The concentrations of indoles in the milk is affected by their metabolism in the cow as well as by rumen processes. The indoles are absorbed from the rumen through the rumen epithelium into the blood stream where the majority is removed by the liver (Roy *et al*., 2004) and the residue exchanges passively into the mammary gland (Roy *et al*., 2002). The recent observation of higher concentrations of indole and skatole in milkfat from afternoon milking than from morning milking for cows grazing ryegrass (Tavendale *et al*., 2005), was suggested to relate largely to the different spacing of feed intake and milk collection for the two milking intervals and the time for clearance of the indoles from the cow. This pattern was observed here for the Grass and Mix treatments. The large difference between concentrations at the afternoon and morning milkings for the GnCd diets demonstrates little if any carry-over between the two milkings. The low concentration in the morning reflected the grass-only diet during the night, and was as low as the Grass treatment where cows ate grass day and night.

Continuous, free access to grass and clover (G/C) provided the greatest opportunity for cows to attain their preferred diet. These cows could freely choose during both the day and the night. Relative to this treatment, the other treatments constrained the diet choice of cows in some way, either temporally (e.g. GnCd), or by the absence of clover (e.g. Grass) or its low proportion (e.g. Mix). The G/C cows showed the greatest duration (and proportion of their 24-hr total) of grazing at night, consistent with the observation by Penning *et al.* (1991) of more bouts of grazing at night on a clover diet. The GnCd cows, whose access to clover was confined to the day only, showed the greatest duration of grazing during the day. This marked contrast in diurnal distribution of grazing activity indicates a behavioural adjustment by the cows to the challenge of the restricted availability of clover.

The GnCd treatment also provides insight into the way physical and metabolic factors may regulate intake at short-term time scales. During the day the GnCd cows grazed clover for 245 minutes compared with 177 minutes grazing clover by the G/C cows (85% of the 207 minutes of grazing during the day). This greater duration of eating clover would impose a considerably higher physical and metabolic load to deal with during the day than for the G/C cows. While this greater duration of eating (and probably higher intake) during the day may have been offset by the lower apparent consumption at night, clover is considered to impose a short-term (i.e. meal-scale) constraint to intake, as shown by the shorter, but more frequent grazing bouts on clover compared with those on grass (Penning *et al*., 1991). It appears that the cows have been able to overcome a meal-scale constraint (i.e. rank that constraint lower) in response to the challenges provided by this treatment i.e. they seek to minimise the constraint to total daily nutrient intake (imposed by being confined to grass-only at night) by increasing intake during the day.

Animals are also capable of varying their instantaneous rate of intake over a considerable range (Newman *et al*., 1994). It is possible that the GnCd cows increased their rate of intake of clover during the day compared with the G/C cows, to compensate for the restricted duration of access to white clover. The high levels of skatole/indole in the PM milk for the GnCd cows compared to the G/C cows, and the comparatively small (and non-significant) reduction in milk yield resulting from the restricted access both indicate that intake of clover during the day may have been higher than indicated simply by the time spent grazing. Further research is required to test this suggestion.

In this study we report the production effects per cow. Further steps towards implementation require an evaluation of the responses per hectare, taking account of the effects of these different spatial arrangements of grass and clover on dry matter production, as has been done for sheep farm systems (Cosgrove *et al*., 2003). While this study was based on white clover, there are alternative legumes (lucerne, red clover for example) with greater annual dry matter production than white clover that may be equally suitable.

CONCLUSION

Novel approaches to growing and allocating forages to grazing animals, such as tested here, offer considerable scope to improve the productivity of pasture-based grazing systems. Aligning the species composition of pasture with the animals own goals in nutrient intake can result in the sustained provision of high legume diets, and better captures the high nutritive value of legumes as increased animal product. The high levels of indoles in the milkfat of the cows on G/C and GnCd suggest there is scope to improve the efficiency of protein utilisation in the rumen and further enhance the efficiency and precision of nutrient allocation to grazing animals and their production from clover-rich diets.

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