The effect of clover-rich diets on cows in mid lactation: production, behaviour and nutrient use

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Abstract
Mixed species pastures typically have insufficient clover to capture its high nutritional value as extra milk production. This paper reports interim results of a study investigating novel methods of presenting white-clover-rich diets to cows. Cows were offered continuous, free access to grass and clover growing separately side-by-side in the same paddock (G/C), or grass-only at night between the afternoon and morning milking and clover-only during the day between the morning and afternoon milking (GnCd). These treatments were compared against controls of grass-only pasture (Grass) and mixed grass-clover pasture (Mix; 92% grass, 8% clover). Cows offered G/C or GnCd produced 2.0 and 1.9 kg MS/cow/day, respectively, compared with 1.5 kg MS/cow/day for cows grazing Grass and Mix. The AM and PM milkfat of cows grazing G/C contained high levels of skatole, an indicator of protein digestion and metabolism, compared with Grass and Mix. For GnCd, levels were high only in the PM milk and low in the AM milk following the grass-only diet at night. The GnCd cows grazed proportionately more during the 'day' and less at 'night' than the G/C cows. Manipulating the spatial and temporal allocation of grass and white clover to align nutrient allocation with the nutritional and behavioural needs of the cow boosts MS production, but there is scope to improve the efficiency of protein utilisation in the rumen.
Keywords: grass, white clover, milk production, grazing behaviour, skatole

Introduction
The nutritional value of legumes for high milk production is well-established (Rogers *et al*. 1982; Beever *et al*. 1986; Harris *et al*. 1997). Cows produce more milk on legume than on grass diets because the nutritive value of grass is lower (Ulyatt 1981). Use of nitrogen (N) fertiliser on grass only partially remedies this, as even at the same level of nitrogen intake from N-fertilised grass or clover, animal performance is 15% greater on a clover diet (Ulyatt 1981; Thomson 1984). However, the potential of legumes is seldom realised in practice because grazed dairy pastures in NZ typically contain insufficient legume.

Previous studies indicate gains in production, above that achieved from typical mixed grass-clover pastures, when cows are given continuous free access to ryegrass and white clover growing separately side-by-side in the same paddock (Nuthall *et al*. 2000; Marotti *et al*. 2001; Rutter *et al*. 2003). However, despite the productive benefits, and the opportunities that spatial separation may provide for sustaining a pasture composition that more closely matches preference (Newman *et al*. 1992; Parsons *et al*. 1994) and nutrient requirements of lactating cows, growing these species separately within the same paddock may present practical challenges. Rutter *et a*l. (2001) proposed, instead, a temporal separation in the grazing of these two species, meaning they could be grown in separate paddocks. Although over the course of 24 hours animals still get a clover-rich diet, such a method of allocation precludes them from eating both species during each bout of grazing during the day, and this asynchrony may affect production and nutrient utilisation compared with free access to adjacent monocultures.

This paper reports results from a project investigating novel methods of forage presentation to cows, to enhance the proportion of legume in their diet. The hypothesis we tested was that temporal separation in feeding grass (at night) and white clover (during the day) to cows in mid-lactation would be as good for milk production as giving them continuous free access to both grass and clover. The responses to these contrasting methods of pasture allocation were evaluated in terms of milk production, grazing behaviour and nutrient metabolism.

Materials and Methods
This study was conducted between November 8 and December 15, 2004, on the Massey University No 1 Dairy Unit, located on Manawatu sandy loam soils adjacent to the Manawatu River.

Treatments and experimental design
Four treatments were compared. 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) clover-only during the 'day' between morning and afternoon milking and grass-only at 'night' between the afternoon and morning milking (GnCd). These were compared against

controls of iii) a typical mixed grass-white clover pasture (Mix; an industry standard) and iv) a grass-only pasture (Grass; a forage standard). The mixed pasture contained 8 ± 5% clover and 92 ± 5% grass (proportion of green DM above ground level).

Four groups of spring-calving cows (n=8) in mid-lactation were allocated to each of four treatments in a balanced, cross-over design with four periods. Cows grazed each treatment for 8 days. They then returned to a single group and grazed a grass paddock for a 2 day wash-out period before moving to the next treatment in the pre-determined sequence. The experimental unit was the group mean.

Animals

Thirty-two spring-calving Friesian cows (482 ± 72 kg; mean ± SD) in their 3rd to 7th lactation were separated from the main herd and allocated to four groups balanced for age (4.9 ± 1.1 years), days in milk (91 ± 19 days as at November 8) and Production Worth index (117/76). Cows were dosed with a Rumensin® antibloat capsule prior to starting the experiment and dosed twice daily at milking with Bloateze (700 g/L alcohol ethoxylate and 70 g/L ethylene/propylene co-polymer; FIL, Mount Manganui). Water troughs were treated daily with Bloateze for additional protection.

Pastures

The trial used nine paddocks of resident mixed-age grass-clover pastures. In four recently sown paddocks, ryegrass predominated and five contained a mix of grass species. Four paddocks consisting of adjacent grass and clover monocultures in the area ratio of 0.25:0.75 were created by using selective herbicides to remove grass (creating the white clover monoculture) or white clover (creating the grass monoculture). The grass-only treatment used two paddocks of grass monoculture created by selectively removing clover, and the Mix treatment used two paddocks of the resident pasture. Paddocks were blocked according to endophyte strain in the ryegrass, which consisted of wild-type endophyte in older paddocks and AR1 in more recently sown paddocks. Within each 8 day measurement period, cows grazed paddocks containing ryegrass with wild-type endophyte for 4 days and AR1 endophyte for 4 days, to ensure treatment and period were not confounded by endophyte status. Urea was applied to the grass-only and mixed pasture areas at 35 kg N/ha, 6 times annually.

Pasture allocation

The total daily feed allowance for each group of 54 kg DM/cow/day was offered as fresh breaks twice daily in the proportions of 40% following the morning milking and 60% following the afternoon milking. Pasture mass was assessed using a rising-plate meter (Farmworks Precision Farming Systems, Feilding), calibrated separately for grass and clover. Allocation protocols included target minimums for pre-grazing and post-grazing mass of 2400 and 1600 kg DM/ha, respectively. For G/C, the total area to be allocated (0.75 clover:0.25 grass) was calculated using the lesser of the mass of grass or clover.

Measurements

Pasture composition: Samples of grass, clover or mixed pasture were taken by hand plucking to represent the diet being consumed by the cows. These samples were collected on five occasions during the trial at 8:00 AM and 5:00 PM to characterise the diet contributing to the milk harvested at the afternoon and following morning milking, respectively. Samples were stored frozen, and subsequently freeze-dried, ground to pass a 1 mm sieve and analysed for concentrations of protein, water soluble carbohydrate and fibre, organic matter digestibility and metabolisable energy density by near-infrared reflectance spectroscopy (FeedTech, AgResearch Grasslands, Palmerston North).

Milk production: Milk yield was recorded at each milking using Metatron™ (Westfalia, Germany) meters and proportional in-line samplers. Milk samples were collected at the PM and following AM milking on days 2, 5 and 8 during each 8-day period, and analysed for fat, protein and lactose content (FT 6000 Fourier Transform infrared analyser, Foss Electric, Hillerød, Denmark). Milk yield data are presented as the treatment mean of the PM plus AM yield for the last 3 days of each measurement period. Milk composition is the treatment mean of samples collected at the final samplings of each period, and, together with yield for that 24-h period, was used to estimate milksolids production for each treatment.

Grazing behaviour: Cows were observed continuously for two consecutive, 24-h days at the end of each period to determine the time spent grazing during the 'day' and during the 'night'. Each of two observers recorded activity for two groups of eight cows, noting at 1 minute intervals whether or not they were grazing and for G/C whether they were grazing grass or clover. Day and night refer to the intervals between successive milkings. Cows were removed from pasture at 6:30 AM and 3:00 PM and were off pasture for approximately 2 h at each milking. The 'night' included approximately 3-4 h of daylight following afternoon milking until dusk (on November 8 sunrise was at 06:00 hours and sunset at 20:10 hours). The total time available for grazing during the 'day' was 450 minutes and during the 'night' 810 minutes.

Milkfat skatole: The concentration of skatole in milkfat was measured as an indicator of protein digestion and metabolism in the contrasting forage treatments. Milk samples at the final PM and AM milking of each 8-day period were subsampled and individual cow samples within each treatment group and milking time pooled. Milkfat was extracted from these pooled subsamples, stored at -20 °C for subsequent analysis for skatole concentration (ng/g milkfat) by HPLC with fluorescence detection (Lane *et al*. 2002).

Statistical analysis

Treatment means and interactions with time ('day' vs. 'night' for grazing behaviour and AM vs. PM for milkfat metabolites) were compared by ANOVA after partitioning group and period effects. In the ANOVA, time was included as a strip-plot factor (sub-plot in strips; Cochran & Cox 1957). For milk yield and composition, separate analyses were conducted for AM, PM and daily totals.

Results

Pasture chemical composition

The chemical composition of pastures offered to cows following milking in the morning (AM sample) and in the afternoon (PM sample) is shown in Table 1. For Grass, Mix and G/C the AM pasture differed from PM pasture mainly in the lower concentration of WSC. For GnCd the diet was characterised by the diurnal switch from clover (AM) to grass (PM), and this accentuated the diurnal difference in WSC (140 and 214 g WSC/kg DM for AM and PM, respectively), and included a difference in protein concentration (282 and 186 g protein/kg DM for AM and PM, respectively).

Milk yield and composition

Cows offered G/C or GnCd produced 27.1 and 25.1 kg milk/cow/day, respectively, compared with 20.6 and 20.5 kg/cow/day when offered Grass or Mix, respectively ($P<0.01$, SEM 0.3) (Fig. 1). Milksolids yields were 2.0 and 1.9 kg MS/cow/day for G/C and GnCd, respectively, and 1.5 kg MS/cow/day for Mix and Grass ($P<0.05$, SEM 0.03).

Grazing behaviour

The distribution of grazing between day and night differed among treatments (treatment x time of day interaction $P<0.001$, SEM 17.5). The G/C cows grazed more at night (261 min) and less during the day (194 min) (Fig. 2) compared with GnCd cows which grazed more during the day than the night (249 vs. 214 min). GnCd constrained the cows to spend less time eating clover than those offered G/C (250 vs. 365 min eating clover during 24 h) and more time grazing grass (215 vs. 90 min in 24 h).

Milkfat skatole

The contrasting temporal and spatial allocations of grass and clover had a large effect on skatole concentrations in AM and PM milk (Fig. 3). Concentrations were higher in PM milk than AM milk and were higher for G/C than for Grass or Mix. However, there was a pasture treatment x time of milking interaction ($P<0.001$, SEM 177). The GnCd resulted in the highest concentration of any treatment in the PM milkfat (after an all-day diet of clover), but the concentration in the AM milkfat (following an all-night diet of grass) was as low as Grass and Mix.

Discussion

Growing grass and clover separately instead of as a mixture, as proposed by Chapman *et al*. (1996; and see

Table 1 The concentrations (mean ± SD, g/kg DM) of protein (PROT), water soluble carbohydrate (WSC), neutral detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy (ME, MJ/kg DM) of grass (gr) and clover (cl) pastures offered to cows in the morning (AM) and in the afternoon (PM) as four treatments consisting of Grass, mixed grass-white clover (Mix; 8 ± 5% clover and 92 ± 5% grass), continuous free access to grass and white clover (G/C) and grass at night and clover during the day (GnCd).

Time	Treatment	Species	PROT	WSC	NDF	OMD	ME
AM	Grass	gr	188±35	165±8	537±27	751±45	11.6±0.4
	Mix	gr+cl	200±19	156±18	508±35	738±42	11.4±0.6
	G/C	gr	186±40	159±4	534±43	736±23	11.4±0.3
		cl	270±12	149±6	447±13	838±12	12.6±0.2
		mean[1]	253	151	464	817	12.4
	GnCd	cl	282±5	140±13	435±11	845±19	12.7±0.2
PM	Grass	gr	185±24	201±5	505±38	784±40	12.2±0.4
	Mix	gr+cl	191±29	190±7	503±37	775±43	12.0±0.5
	G/C	gr	190±28	197±6	511±28	776±46	12.1±0.6
		cl	246±10	183±9	463±22	868±22	13.2±0.3
		mean[1]	235	186	473	849	13.0
	GnCd	gr	186±29	214±20	468±45	808±35	12.5±0.3

[1] Mean of grass and clover in G/C weighted according to the proportion of time grazing each component

Figure 1 The yield of milksolids (kg/cow/d) 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 comparison among treatment means of AM or PM milksolids 0.034).

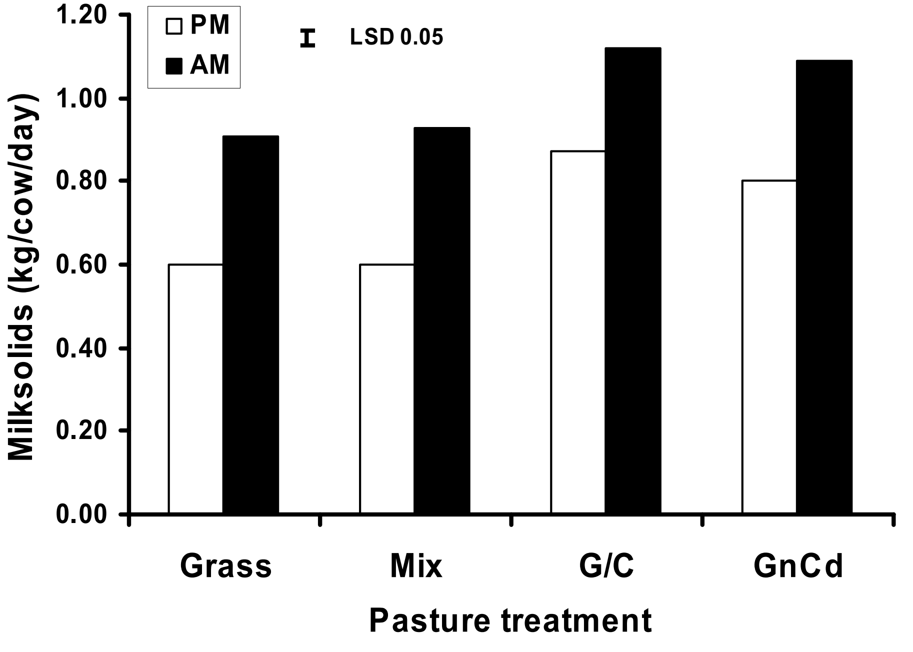


Figure 2 The duration of grazing (min) during the night and during the day by cows offered Grass, mixed grass-white clover (Mix), continuous free access to grass and white clover (G/C) and grass at night and clover during the day (GnCd) (LSD $_{0.05}$ for comparisons among treatment means for different periods of the day 28).

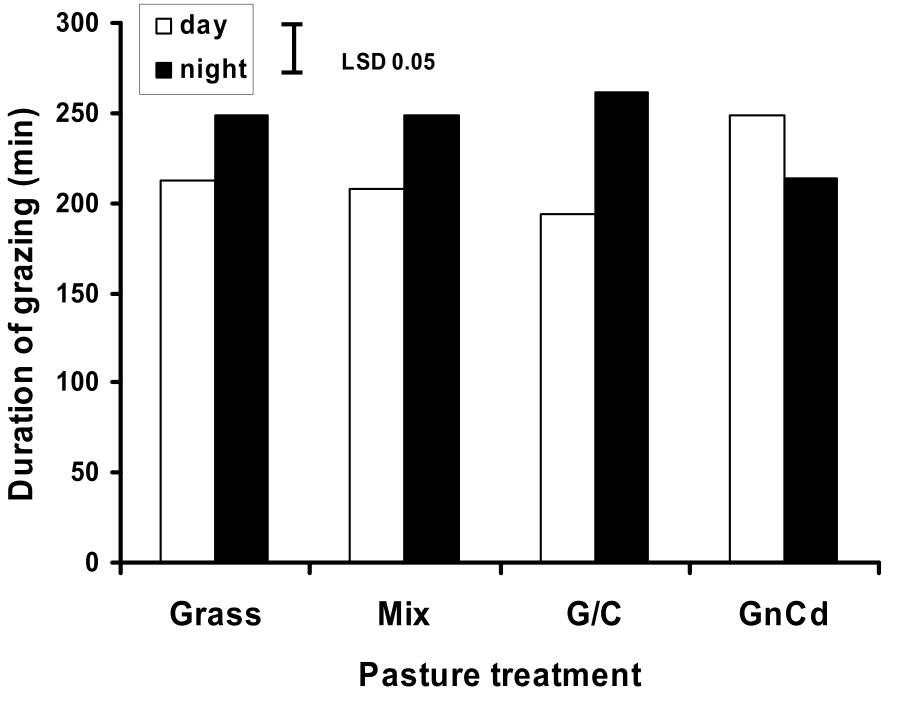
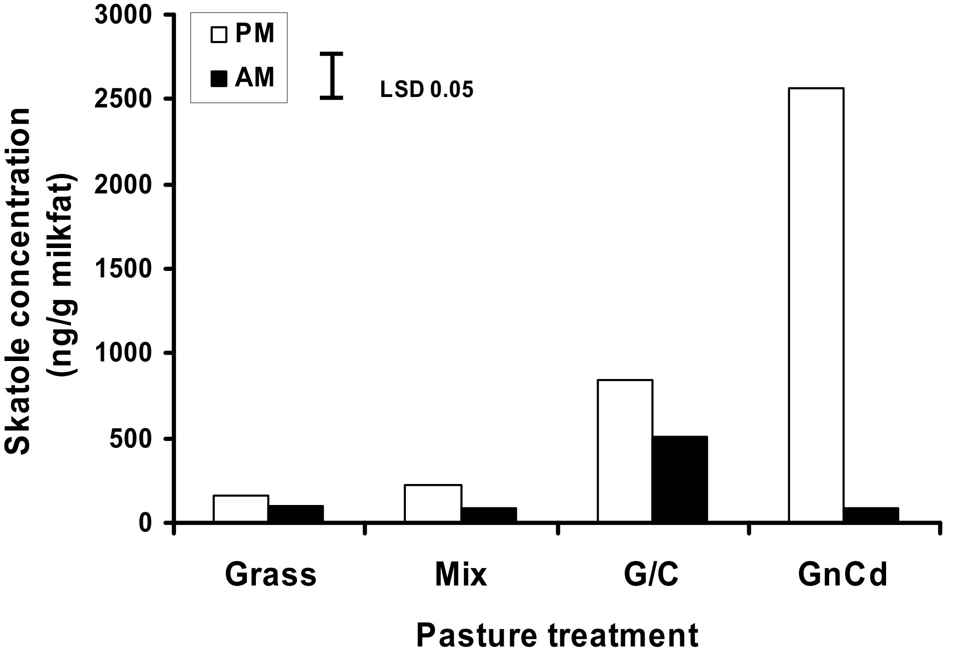


Figure 3 The concentration of skatole in the milkfat (ng/g) at the afternoon (PM) and morning (AM) milking of cows offered Grass, mixed grass-white clover (Mix), continuous free access to grass and white clover (G/C) and grass at night and clover during the day (GnCd) (LSD $_{0.05}$ for comparisons among treatment means for AM or PM skatole 253).



reviews by Chapman *et al*. 2006; Parsons *et al*. 2006), allows greater control over the proportions of grass and clover grown and offered to animals. In this study, using novel pasture arrangements, and so sustaining a higher proportion of clover in the diet, MS production per cow increased by 25% (GnCd) to 30% (G/C) compared with Grass or a mixed pasture containing 8% clover (Mix). The increase for G/C compared with Grass is consistent with results reported by Nuthall *et al*. (2000) and Marotti *et al*. (2001), but greater than that recorded for cows in late lactation (Cosgrove *et al*. 2006). It is not clear if this difference between trials relates to stage of lactation *per se*, or seasonal differences in the chemical composition of the grass and/or clover. There were only minor differences between November/December and April in the concentrations of protein and carbohydrate of the grass and clover, although NDF in grass (grass comprised approximately 20% of the G/C diet and 50% of the GnCd diet) tended to be lower in this study than it was in autumn.

The greater MS production from G/C and GnCd appears to result from the greater opportunity to eat clover, as there was no increase in the total time spent grazing on these treatments compared with Grass or Mix, in contrast to results reported by Rutter *et al*. (2003). One of the benefits of offering animals continuous free access to grass and clover growing separately (G/C), demonstrated by Champion *et al*. (2004), was that it offered opportunity for a substantial increase in intake (often seen as extended grazing time) compared with Grass or a Mix. The opportunity to eat both grass and clover in close temporal synchrony in G/C may be a significant element of that 'boost'. However, in previous comparable studies (Marotti *et al*. 2002), there was no 'boost' in the total time spent grazing. This suggests that temporal separation in allocating grass and clover (i.e. GnCd) may be a feasible alternative, and it would be simpler to implement than G/C. In GnCd, although the proportion of total grazing time spent eating clover over the course of 24 h was 50% compared with 80% in G/C, there was no significant reduction in milk production.

The novel spatial and temporal arrangements of grass and clover may affect nutrient digestion and metabolism. Skatole arises in the rumen from the microbial breakdown of tryptophan from dietary proteins (Deslandes *et al*. 2001) and is influenced by the concentration and intake of dietary proteins (Kebreab *et al*. 2003). The concentrations of skatole in milkfat for the cows on Grass and Mix were comparable with the levels reported for cows grazing mixed ryegrass-white clover pasture in spring (Lane *et al*. 2002). However, the much higher concentrations of skatole for G/C cows in the morning and afternoon and in the afternoon milkfat for GnCd, appears to be related to the high clover content of these diets. Recent studies showed higher concentrations of skatole in the rumen, relative to protein intake, for sheep fed white clover compared with those fed ryegrass (Schreurs *et al*. 2003).

The diurnal difference in skatole concentration for the Grass, Mix and G/C treatments, was accentuated in GnCd by eating clover-only during the day. The very high concentration in the PM milk, but the low concentration in the AM milk following overnight feeding on grass, indicates no carry-over from one milking to the next. The higher concentration of skatole in PM compared with AM milk has previously been attributed primarily to the different intervals between successive milkings and the resulting difference in the time for indolic compounds to clear from the cow (Tavendale *et al*. 2005), although the diurnal difference in WSC concentration of the forage may also be a factor. The high concentration of skatole in the PM milk from GnCd may also reflect a behavioural response by the cows grazing this treatment. Of all the treatments, G/C provided the greatest freedom for cows to eat their preferred diet and distribute their grazing as they wished, whereas the GnCd cows were constrained temporally. On the basis of time spent grazing, the intake of clover during the day was estimated to be approximately 60% higher for the GnCd cows compared with the G/C cows. This high intake of clover in the short interval between AM and PM milking (approximately 8 h), and the rapid degradation of this large quantity of protein in the rumen may have contributed to the high concentration of skatole in PM milkfat.

Novel approaches to growing and allocating forages, such as tested here, can align the species composition of pasture with the animals' own goals in nutrient intake, sustain the provision of high legume diets, and better capture the high nutritive value of legumes as increased milksolids. The high levels of indolic compounds 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 *precision* of nutrient allocation to grazing animals and their production from clover-rich diets. Greater precision in nutrient allocation may in future contribute to meeting more demanding targets in product composition (e.g. flavour, fatty acid profiles) and in environmental standards (e.g. nitrates, methane). These are influenced by diet composition, and successful delivery to these goals by modification of plant traits will depend critically on precise, targeted allocation of pastures.

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