Ryegrass to lucerne - effects of dietary change on intake, milk yield and rumen microflora bacteria of dairy cows

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ABSTRACT

The value of legumes for milk production has been attributed to higher intakes by cows and a higher nutritive value compared to ryegrass. However, abrupt changes in diet can adversely affect production, even when the change is to feed with a higher feeding value. A 13-day indoor experiment compared the responses of 12 cows that were changed from ryegrass to lucerne (*Medicago sativa* L.) (L) on Day 4, with 12 cows remaining on ryegrass (R). Immediately after the change to lucerne there was a 24% decrease in milk yield; this was temporary, as from four days after the change these cows averaged 20% more milk than those fed ryegrass (L = 15.7, R = 13.1 kg milk/cow/d; P <0.001). The temporary decrease in milk yield was not due to decreased intake as lucerne-fed cows had higher intakes (L = 16.5, R = 14.8 kg dry matter/cow/d; P <0.001). Only small differences in the rumen microbial populations were apparent on the day diets changed. The temporary decrease in milk yield was more likely associated with a change in metabolism of the resident bacteria. Improved understanding of the microbial consequences of an abrupt dietary change will enable better management decisions to be made on-farm.

Key words: dairying; diet change; dry matter intake; lucerne; milk yield; rumen microflora.

INTRODUCTION

For many years the success of the New Zealand dairy industry was determined by the amount of pasture dry matter (DM) grown and utilised for milk production, but the drive for increased production has required more feed and increased use of supplements such as maize or pasture silages. Higher levels of production have introduced many factors that affect the success of a farm system and it is likely that the industry's forage base will diversify to meet these challenges. Previous experiments have demonstrated increased milksolids (MS) production from legume-dominant diets based on white clover (Rogers & Robinson, 1984; Harris *et al*., 1997; Harris *et al*., 1998a) or birdsfoot trefoil (lotus) (*Lotus corniculatus* L.) (Harris *et al*., 1998b; Woodward *et al*., 1999) due to high nutritional value and intake (DMI) of these legumes.

Recent dairy farmlet trials have evaluated productivity and profitability by comparing a farmlet based on ryegrass-white clover with a forage mixed ration (FMR) farmlet that also included annual ryegrass, lotus, red and white clovers and lucerne (*Medicago sativa* L.) (Woodward *et al.*, 2008). MS production per cow was 9.5% higher on the FMR farmlet in 2006/2007 and 5.5% higher in 2007/2008, with most of this advantage in late summer-autumn when the FMR cows were producing 20% more milk. However, during the trial it was observed that regular changes from ryegrass pasture to lucerne caused short-term reductions in MS yield. It was not clear if this reduction was due to depressed intakes or some other factor. The objective of this indoor feeding experiment was to measure how changing cows from pasture to a lucerne diet affected DMI, MS yield and rumen microflora.

MATERIALS AND METHODS

Experimental design and management

A 13-day indoor feeding experiment was conducted in March 2008 in the Calan Gate facility at DairyNZ's Lye Farm, Hamilton, New Zealand, using 24 multiparous Holstein-Friesian dairy cows in late lactation (206 ± 15 (standard deviation) days in milk (DIM)). The cows had been grazed together on lucerne dominant pastures during the day and perennial ryegrass dominant pastures during the night for four days before the indoor feeding commenced so all were accustomed to lucerne, and switching to ryegrass pastures for a further ten days. Measurements of daily milk yield and, milkfat and milk protein concentration, during this period, together with age, breeding worth (BW) and production worth (PW) indices, live weight (515 ± 28 kg) and DIM were used to allocate cows to the two treatments. Each group included four cows with permanent rumen fistulae.

The first three days of the experiment served as an adaptation period during which all cows were fed individually in the Calan Gates on a ryegrass-based diet. On Day 4, 12 of the cows were changed to a lucerne diet (L) with the remainder on a ryegrass-based diet (R). Data presented here were based on

measurements made during the 13 days of indoor feeding.

Intake and feed measurements

Cows were fed freshly cut lucerne or ryegrass at 08:00 h and 16:00 h daily to ensure daily refusals were approximately 15% of feed offered. Individual DMI of the lucerne and ryegrass offered and refused was calculated from the fresh weights and DM content estimated after drying sub-samples for 48 hours at 95°C. The botanical compositions of both feeds were determined daily. Chemical composition and energy content of bulked samples of feed offered during the measurement period after they had been oven-dried at 65°C, were measured on alternate days using near infrared spectrophotometry (NIRS systems 6500; FeedTECH, Palmerston North, New Zealand).

Milk measurements

Milk yield (kg/cow/d) was measured daily as an afternoon plus the following morning's production and samples taken to determine fat % and protein %, using Fourier-transform infrared spectroscopy (FT120 analyser, Foss Electric, Hillerød, Denmark).

DNA extraction from freeze-dried and ground rumen contents

Rumen samples collected from the fistulated cows at 16:00 h on Day 2, when all cows were fed ryegrass, on Day 4 as the first day of the diet change and on Days 6 and 13, were freeze-dried and ground for bacterial DNA extraction. Freeze-dried and ground rumen contents (100 mg) were bead-beaten using a FastPrep bead beater (Thermo Savant, Holbrook, New York, USA) with 1 g of zirconium bead (0.5mm) in 1 mL of saline EDTA buffer (0.15 M NaCl, 0.1 M EDTA, pH 8) containing 1% (wt/vol) sodium dodecylsulphate (SDS) and 500 µL of buffer-saturated phenol (Sigma Aldrich, St Louis, Missouri, USA). Extracted DNA was treated with phenol:chloroform:isoamyl alcohol (25:24:1, v/v) to remove proteins, ethanol (70% EtOH) precipitated, and the DNA pellet redissolved in TE buffer (10 mM Tris. HCl, 1 mM EDTA, pH 8).

Primers and PCR amplifications

The PCR primers used for amplifying the variable region 3 (V3) of the bacterial 16S ribosomal RNA gene and for Denaturing Gradient Gel Electrophoresis (DGGE) analyses were 337f (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG; GC-clamp underlined) and 514r (TTA CCG CGG CTG CTG GCA C) and were synthesised by Integrated DNA Technologies (San Diego, California, USA). PCR amplifications contained 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 2.5 mM $MgCl_2$; 0.2 mM (each) dATP, dCTP, dGTP, and dTTP; 0.5 µM (each) primer; and 0.5 U of Platinum Taq DNA Polymerase High Fidelity (Invitrogen, Auckland, New Zealand). Amplifications were carried out in 0.2 mL microtubes in a model PTC 200 thermal cycler (BioRad, Sydney, NSW, Australia) using a touch-down programme of 94°C for 2 minutes, start annealing 61°C for 30 seconds, elongation at 68°C for 30 seconds, followed by melting at 94°C for 30 seconds, decreasing by 0.5°C/cycle for 10 cycles, 22 cycles of annealing at 56°C for 30 seconds, elongation at 68°C for 30 seconds, and melting at 94°C for 30 seconds and a final elongation at 68°C for three minutes.

Denaturing Gradient Gel Electrophoresis (DGGE)

Aliquots of PCR products (3 to 5 µL) were separated by agarose gel electrophoresis in 1.5% (wt/vol) agarose gels to confirm product sizes. DGGE gel loading buffer (10 µL, 0.05% bromophenol blue, 0.05% xylene cyanol, 70% glycerol w/v in H_2O) was added to stop the reaction. The PCR products were separated in 6.5% polyacrylamide gels as described by Yu & Morrison (2004) except that a 40 to 65% linear gradient of denaturant was used and run at 60°C for 16 hours at 50V in a 2401 TTGE/DGGE system (CBS Scientific Company Inc, Del Mar, California, USA). DGGE Markers 1 and 5 (Nippon Gene, Toyama, Japan) were used and gels were stained with SYBR GOLD (10,000 × concentration in DMSO; 3 µL stain diluted in 15 mL water) for 10 minutes and destained in fresh water for 5 minutes. Gels were photographed using a Gel Logic 200 imaging system (GL 200, Eastman Kodak Company, Rochester, New York, USA). DGGE bands were detected and analysed using the band-searching algorithm of BioNumerics software (BioSystematica, Tavistock, Devon, UK).

Statistics

Each variable was initially analysed as repeated measures data with Cow as the subject and Treatment, Day and their interaction as fixed effects using AREPMEASURES procedure in GenStat 11 (VSN International, Hemel Hempstead, Hertfordshire, UK). There was a significant interaction of Treatment with Day for each variable, so the data for each day for each variable were analysed separately using analysis of variance with treatment as a fixed effect and Cow as the blocking variable. Cow means for each variable from Days 2 and 3 of the Calan Gate feeding, when all cows were fed ryegrass, were included in the analysis as a covariate.

TABLE 1: Dry matter (DM) content, chemical composition and metabolisable energy content of the lucerne and ryegrass offered to cows during Days 4 to 13.

Component	Diets	
	Lucerne	Ryegrass
Dry matter (%)	24.1	17.3
Crude protein (g/100 g DM)	22.2	18.9
Lipid (g/100 g DM)	1.8	3.4
Acid detergent fibre (g/100 g DM)	30.7	28.5
Neutral detergent fibre (g/100 g DM)	40.9	49.3
Ash (g/100 g DM)	10.2	10.4
Metabolisable energy (MJ/kg DM)	10.6	10.6

RESULTS AND DISCUSSION

Feed characteristics

The lucerne pasture was almost entirely lucerne (95%) with the remainder weeds and dead matter. The ryegrass pasture was 60% perennial ryegrass, 7% white clover, 15% other grasses (mainly summer grass), 12% weeds (mainly broad-leaved plantain and oxalis) and 6% dead matter. The quality of both feeds provided sufficient energy and protein for cows in late lactation (Holmes *et al.*, 2002) (Table 1).

DMI and milk production

The change in diet to lucerne on Day 4 resulted in an immediate increase in DMI (L = 16.5, R = 14.8 kg DM/cow/d; P <0.001) and a 24% decrease in milk yield (L = 10.8, R = 14.2 kg milk/cow/d; P <0.001), which was similar to observations in farmlet trials (Woodward *et al.*, 2008). The lucerne-fed cows always had a higher DMI than those fed ryegrass (Table 2), ranging from 12% higher on Day 4 to 23% on Day 10 (Figure 1). A multitude of factors have been associated with voluntary intake, including palatability and post-ingestion factors (Grovum & Chapman, 1988) that can be influenced by physical plant cues such as fibre content, plant and canopy structure, and chemical cues such as aroma, flavour, toxins, carbohydrate content, organic acid content. Regardless of cause, the higher DMI of the lucerne-fed cows was not a consequence of availability and was consistent with previous experiments where cows were fed other legumes such as white clover and lotus (Harris *et al*., 1997; Harris *et al*., 1998a; Harris *et al*., 1998b; Woodward *et al*., 1999).

Milk yield after the dietary change was only 7% below that of cows fed ryegrass on Day 5, the second day of lucerne feeding (L = 12.9, R = 13.9 kg milk/cow/d; P <0.01). By Day 6 the cows fed lucerne were producing 6% more milk than those fed ryegrass (L = 14.5, R = 13.6 kg milk/cow/d; P <0.01) and from Day 7 onward the cows fed lucerne averaged 20% more milk than those fed ryegrass (Table 2).

MS yield after the dietary change followed a similar pattern to milk production, with an initial 24% decrease, followed by increases so from Day 7, lucerne-fed cows averaged 21% more MS (P <0.001) than those fed ryegrass (Figure 1, Table 2). Analyses of the daily milk composition data showed the change to lucerne reduced milkfat and increased milk protein concentrations over three days, so from Day 7, lucerne-fed cows had 8% lower milkfat (P <0.05) and 13% higher milk protein concentrations (P <0.001) than ryegrass-fed cows (Table 2). The milk yields and composition stabilised from Day 7.

Previous experiments where ryegrass was substituted with legumes have also increased MS production and the effects of feeding lucerne on milk composition mimicked changes reported when white clover, lotus and sulla were fed (Harris *et al*., 1997; Harris *et al*., 1998a; Harris *et al*., 1998b; Woodward *et al*., 1999; Woodward *et al*., 2002). In the current experiment, the reduction in milkfat was countered by an increase in milk protein, and so MS concentration was similar for both diets. Once milk production had stabilised by Day 7, feed conversion efficiency (FCE) (g MS/MJ Metabolisable energy (ME)) was higher (P <0.01) for the lucerne-fed cows than the ryegrass-fed cows (Table 2).

TABLE 2: Dry matter intake, milk production, milk composition and feed conversion efficiency data from cows changed from ryegrass to lucerne on Day 4 or fed ryegrass throughout the experiment. The data here are the mean values for the two treatments from Days 7 to 13 once milk production had stabilised. SED = Standard error of difference; DM = Dry matter; MS = Milksolids; ME = Metabolisable energy.

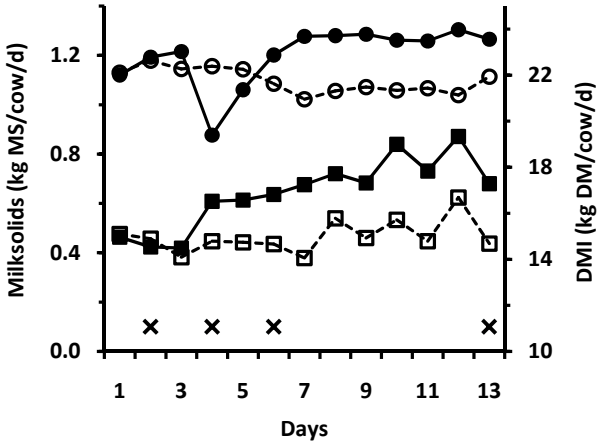
Production measurement	Diet		SED	Significance
	Lucerne	Ryegrass		
Dry matter intake (kg DM/cow/d)	18.0	15.2	0.5	***
Milk yield (kg/cow/d)	15.7	13.1	0.6	***
Milkfat (%)	4.32	4.69	0.19	*
Milk protein (%)	3.81	3.39	0.05	***
Milksolids yield (kg/cow/d)	1.28	1.06	0.05	***
Feed conversion efficiency (g MS/MJ ME)	6.71	6.59	0.04	**

Bacterial population analysis

Introduction of a new diet can decrease DMI and reduce production (Grovum & Chapman, 1988), especially if animals are not familiar with the feed (Fukasawa *et*

al., 1999), but in this experiment DMI increased while milk production declined. The immediate decrease in MS production was probably due to post-ingestive factors that influenced the efficiency of nutrient utilisation from the lucerne. Reduced efficiency may be a consequence of adaptation by the rumen microflora, a change in products of digestion for absorption or a need for adaptation to absorbed nutrients affecting metabolic efficiency.

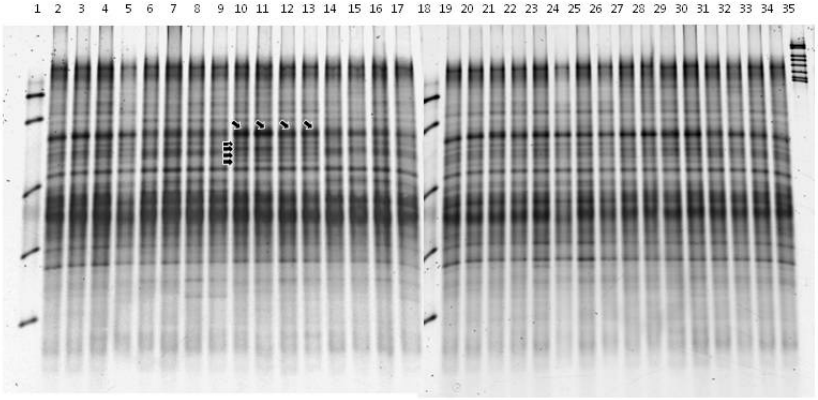
FIGURE 1: Daily milksolids yield of cows changed from ryegrass to lucerne (●) on Day 4 and cows fed ryegrass (○) throughout the experiment. Daily dry matter intakes (DMI) of cows changed from ryegrass to lucerne (■) on Day 4 and cows fed ryegrass (□) throughout the experiment are also shown. DGGE analyses of rumen microflora were done on the days marked (**X**).



In general, the DGGE fingerprint patterns of rumen bacterial populations (Figure 2) were remarkably similar between the cows fed lucerne and ryegrass. Variation in DGGE patterns between individual animals was also minor in most cases. An analysis of banding similarity for each of the samples showed no significant correlation between banding patterns and treatment groups. However, some small differences in banding pattern were observed in samples collected on Day 4, the day the diet changed. A prominent band near the top of the gel, indicated by an arrow in Figure 2, showed an increase in intensity while several bands immediately below it were present in all the ryegrass-fed animals but absent or substantially reduced in the lucerne-fed group.

The general similarity of banding patterns between treatments groups indicates that the composition of the bacterial microflora populations in the rumen was not significantly disturbed by the change in diet from ryegrass to lucerne, although some slight alteration in abundance of some of the bacterial inhabitants may have occurred in the first day of diet change. The resolution of the DGGE technique is such that it is likely that only major shifts in bacterial populations will be seen. Smaller adjustments in large populations or changes to small groups of bacteria may not be apparent on DGGE gels. Minor effects could be expected because differences in the chemical composition of the ryegrass and lucerne diets were not large. Diet is thought to be one of the main determinants of species diversity in the rumen (Hobson, 1997). Large changes in diet or antibiotic supplementation have been associated with changes in microbial bacterial (Edwards *et al.*, 2005; Karnati *et al.*, 2007) or protozoal (Regensbogenova *et al.*, 2004) DGGE patterns on a number of occasions. However, not all studies have shown shifts in microbial populations. Addition of monensin to the rumen of dairy cows showed that archaeal DGGE profiles of methanogen populations were not affected over six months (Hook *et al.*, 2009). The primer set used in the current study circumscribes only the bacterial component of rumen microflora so it is possible that there were undetected changes in archaeal, protozoal or fungal

FIGURE 2: PCR-DGGE profiles of V3 regions of 16S rRNA genes amplified from DNA extracted from rumen contents of lucerne- or ryegrass-fed cows. Samples are identified by lane numbers across the top of the profile: Lanes 2-5: Lucerne cows on Day 2, two days before diet change; Lanes 6-9: Ryegrass cows on Day 2; Lanes 10-13: Lucerne cows on Day 4, day of diet change; Lanes 14-17: Ryegrass cows on Day 4; Lanes 19-22: Lucerne cows on Day 6; Lanes 23-26: Ryegrass cows on Day 6; Lanes 27-30: Lucerne cows on Day 13; Lanes 31-34: Ryegrass cows on Day 13; Lanes 1 and 18: DGGE marker 1; Lane 35: DGGE marker 5. A prominent band near the top of the gel, indicated by arrow, showed an increase in intensity while several bands immediately below it were present in all the ryegrass-fed cows but absent or substantially reduced in the lucerne-fed cows.



populations. However, in the present study, it is more likely that the metabolism and products of metabolism of the resident bacteria, rather than their populations, changed in response to the different diet. Metabolic changes within bacteria can occur quickly, which may also explain why the diet change did not manifest itself as a perturbation of bacterial populations.

CONCLUSION

This experiment showed a change from ryegrass pasture to lucerne caused a significant drop in production, despite increased DMI, that does not appear to be explained on the basis of bacterial populations. Four days after the change in diet, MS production exceeded that of cows fed ryegrass by 21%, and milk protein production was increased by 34% suggesting an increase in amino acids available for milk synthesis. This experiment did not explain the decrease in production, or the lower efficiency of nutrient capture in the three days after a change in diet, but it highlighted the extent of the reduction in feed conversion efficiency in response to a sudden change in feeding. Previous trials when ryegrass was substituted with white and red clovers or lotus (Harris *et al*., 1998a, b) did not suggest adverse effects of a sudden change, so the responses reported here may be specific to lucerne.

If lucerne is to be fed in conjunction with ryegrass, an appropriate grazing management would need to be developed to minimise short-term reductions in MS yield. Climatic requirements for lucerne growth, and the lower DM yield compared to ryegrass in the Waikato of 16 versus 22 t DM/annum (Woodward *et al.*, 2008), suggests it could be fed throughout lactation but comprise only 15 to 30% of the diet and be fed in conjunction with ryegrass pasture. This, and previous experiments, highlight the need for specialist feeding of supplements, especially when they comprise a high proportion of a diet. It also highlights the need to measure impacts of changing diets to optimise management. This may include changes in feed availability and intake.

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