# Microbial protein synthesis and milk production in cows offered pasture diets differing in non-structural carbohydrate content

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## ABSTRACT

The effect on ruminal fermentation and milk production of increasing the proportion of non-structural carbohydrate (NSC) in a pasture diet was investigated in early (Trial 1) and late (Trial 2) lactation. Twenty four cows in Trial 1 and 15 cows in Trial 2 were offered pasture (P), 85% P plus 15% protein/NSC mixture (PR), and P+10% (Trial 1) or P+15% (Trial 2) NSC (PE) in a latin square design. All diets were isonitrogenous and P and PR were isoenergetic. PE but not PR increased microbial protein synthesis and decreased rumen ammonia and milk urea levels compared to P in both trials. In Trial 1, neither PE nor PR increased milk yield or milk solids output compared to P. Nitrogen but not dry matter digestibility was reduced on PR and PE compared to P. Ruminal degradation of casein, but not of pasture, was increased on PR and PE compared to P. In Trial 2 the milk yields of cows offered PE and PR were greater than those offered P, and greater on PE than PR. Increasing the ratio of carbohydrate to protein was more effective in improving nitrogen utilisation in the rumen than was increasing the proportion of NSC.

**Keywords:** dairy cows; pasture; microbial protein synthesis; ruminal ammonia; digestibility; ruminal degradation.

## INTRODUCTION

Ryegrass/white clover pastures are often high in crude protein and neutral detergent fibre (CP, 20-30% of dry matter (DM); NDF, 45-50%) and low in non-structural carbohydrate (NSC, 5-25%) compared to overseas recommendations for lactating dairy cows (CP, 16-18%; NDF, 28%; NSC, 35%; NRC, 1989). High ruminal degradability of CP and loss of ammonia ($NH_3$) may limit amino acid supply to the small intestine. Increasing availability of fermentable energy or synchronising energy and CP fermentations are considered important for utilising nitrogen (N) for microbial protein synthesis (MPS) (Stern *et al.*, 1994). The relatively low NSC levels in pasture potentially limit microbial efficiency and milk solids production in New Zealand dairy cows. Increasing the proportion of NSC in the diet may also influence protein digestion rates in the rumen (MacGregor *et al.*, 1983; Siddons and Paradine, 1983). Two trials are described which determined the effect of altering the proportion of NSC in a pasture diet on N utilisation and MPS in early and late lactation. The approach taken was to either replace pasture structural carbohydrate with NSC without increasing total intake, or to provide additional NSC to the diet. Total tract diet digestibilities and ruminal protein degradation rates are also reported for one trial.

## MATERIALS AND METHODS

### Trial 1

Nineteen Friesian and five Jersey cows were allocated to three groups balanced for days in milk, liveweight, and milk yield. Twelve of the Friesians had a fistula in the dorsal rumen. Diets were: pasture at 90% of estimated ME requirement for milk production and liveweight$^{0.75}$ (P); 85% P+15% of ME intake replaced with a NSC/CP mixture (PR); P+10% additional ME as NSC (PE). Diets were isonitrogenous and P and PR were isoenergetic. The replacement protein in PR consisted of urea, lactic casein and formaldehyde-treated casein (20:50:30, N basis). Formaldehyde treatment consisted of soaking 200 kg casein in 1000 l water with 5 l of 40% formaldehyde. The NSC consisted of maize cornflour and dextrose monohydrate (50:50, ME basis). Diets were offered during three 14 day periods with measurements made over days 8-14 in each period. Fifteen cows were housed in individual pens throughout the trial and nine of the fistulated Friesian cows were transferred from pens to metabolism stalls on days 5-14. The remaining 3 fistulated cows, one per diet per period, were used to determine ruminal degradations of pasture and casein.

Ryegrass pasture was fertilised with 90 kg N as urea in a split application (each 45 kg) 28 and 21 days before harvest. Pasture was harvested and offered daily from 0900-1400 and 1600-2200 h. The supplements were drenched in four equal parts at 0900, 1100, 1600 and 1800 h. The amount of N in the PR supplement was adjusted every 4-6 days based on herbage clipped from paddocks to be mown. Cows were drenched twice daily with 10g Mg as $MgCl_2$ mixed with a pluronic-based bloat preventative.

Individual daily pasture DM intakes were determined from feed offered and refused. A bulked sample of pasture offered over days 8-14 was analysed for chemical composition. Milk weights were recorded during days 8-14 and fat, protein and lactose percentages determined on days 10, 12 and 14 for penned cows and daily for metabolism cows.

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Samples of milk obtained by hand were collected at 0700, 1100, 1500 and 1900 h on days 10, 12 and 14 for urea determination. Cows were weighed and their condition assessed (1=very thin to 10=very fat) on two consecutive days at the start and end of each period. Ruminal digesta was sampled from metabolism cows at 0800, 1200, 1600 and 2000 h on days 10, 12 and 14, and pH was determined. Acidified samples for each cow were bulked across days within sample time for $NH_3$ analysis.

During days 8-14 of each period total faecal outputs were collected and daily subsamples were bulked over 7 days and stored at 4°C. Total daily urinary outputs over days 8-14 were collected and aliquot subsamples of each day's urinary output were either acidified with 6 mol/l HCl for subsequent allantoin analysis or diluted 1:4 with distilled water for determination of uric acid. Daily acidified samples were stored at 4°C, bulked over days 8-14 then stored at -18°C prior to analysis. Diluted samples were frozen daily and bulked on thawing. MPS was as described by Chen and Gomes (1992) except that urine was not acidified as voided. The possible loss of allantoin over 24 h in unacidified urine collections was investigated on each of 2 days in Trial 1 by taking a sample of the first urine voided for each cow (at about 1200 h), acidifying half (sample 1) and leaving the remaining half open-topped on the bench beside the collection buckets (sample 2) for acidification at the time of 24 h urine sampling. The correlation coefficients of allantoin concentration in sample 1 versus sample 2 were 0·98 and 0·99 on Days 1 and 2, respectively. Degradation of allantoin appeared minimal under the conditions of urine collection.

On day 10 of each period samples of approximately 30 g of pasture or 10 g of lactic casein (New Zealand Dairy Research Institute, Palmerston North) were weighed into tared nylon bags (mesh opening 44 mm; mesh count 126.6/ $cm^2$; size 10 x 20 cm; Swiss Screens, Australia). The pasture was subsampled from that harvested to feed the cows and was macerated for 10 seconds in a blender to simulate mastication. Eight bags containing pasture and eight containing casein were placed in the ventral rumen of each of the three cows at 0900 h. Two bags containing each substrate were removed at 1, 3, 6, and 12 h after placement, washed under running water until the water squeezed through the bag was clear, and dried for DM and N determination. Two bags each of pasture and casein which were not incubated were also washed to determine the immediately soluble proportions of DM and N.

## Trial 2

Fifteen rumen fistulated Friesian cows were allocated to groups balanced for liveweight and milk yield. The diets were as for Trial 1 except that cows were offered 100% of their estimated ME requirements and 15% additional energy was given in PE. Diets were offered during three 14 day periods but Period 3 was not completed because pastures contained high nitrate levels. All cows spent days 1-4 of each period in pens and days 5-14 in metabolism stalls. Measurements, preparation of pastures and feeding and sampling routines were as for Trial 1, except that cows were not supplemented with Mg or bloat preventative.

## Statistical analysis

Data were analysed as eight 3x3 latin squares (Trial 1) or five incomplete 3x2 latin squares (Trial 2) using SAS Mixed Model procedure (SAS, Version 6.10, SAS Institute Inc., Cary, NC USA) to give means and standard errors of the differences between means (SED). One cow did not complete Period 3 (PR diet, Trial 1).

# RESULTS

## Trial 1

Contents (% of DM intake) of NSC, NDF and CP in P, PR and PE, respectively, were: NSC, 22.5, 28.7 and 28.2%; NDF, 46.0, 40.3 and 42.6%; CP, 23.4, 25.3 and 22.8%. Total DM intake was least ($P<0.001$) for cows when on PR and greatest ($P<0.001$) on PE (Table 1). Neither PE nor PR resulted in more milk than P, although milk yield was higher ($P<0.05$) for cows on PE than on PR. Protein content in milk was similar for all diets ($P>0.05$) but fat content and fat yields decreased ($P<0.01$, $P<0.05$, respectively) on both PR and PE compared to P. The concentration of urea in milk was lower ($P<0.001$) on PE than on P or PR, averaging 8.47, 8.80 and 7.31 mmol/l for P, PR and PE, respectively, (SED 0.13, $P<0.001$). Diet had no effect on changes in liveweight and condition score.

**TABLE 1:** Daily DM intake (DMI), daily production of milk, fat, protein and lactose, change over 14 days in liveweight (LW) and condition score (CS), microbial protein synthesis (MPS) and MPS per kg digestible OM intake in Trial 1, early lactation[a].

| Diet | P | PR | PE | SED |
|---|---|---|---|---|
| Pasture DMI (kg/cow) | 14.1 | 11.9 | 13.9 | 0.14 |
| Total DMI (kg/cow) | 14.1 | 13.6 | 15.0 | 0.14 |
| Milk yield (kg/cow) | 21.9 | 21.6 | 22.4 | 0.32 |
| Fat yield (kg/cow) | 1.00 | 0.92 | 0.94 | 0.028 |
| Protein yield (kg/cow) | 0.72 | 0.70 | 0.72 | 0.017 |
| Lactose yield (kg/cow) | 1.05 | 1.00 | 1.03 | 0.017 |
| LW change (kg) | -6.5 | -4.8 | -2.3 | 4.6 |
| CS change | -0.17 | 0.00 | 0.15 | 0.09 |
| MPS (g N/day) | 271 | 299 | 318 | 28 |
| MPS (g N/kg DOMI) | 24.3 | 27.9 | 26.8 | 2.33 |

[a]DMI and milk production data for 24 cows; MPS data for 9 metabolism cows; see text for DMI of metabolism cows

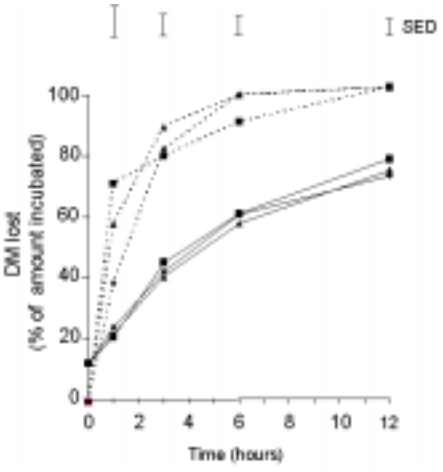
Total daily DMI by cows in metabolism stalls averaged 15.1, 14.3 and 15.8 kg/cow (SED 0.23, $P<0.001$) for P, PR and PE, respectively. N intakes averaged 563, 550 and 546 g/day (SED 9.4) for P, PR and PE, respectively. MPS was similar for cows on PR compared to either P or PE but was higher on PE than on P ($P<0.1$, Table 1). There was no effect of diet on MPS when it was was expressed per kg digestible organic matter intake (DOMI). Ruminal pH values averaged across all sample times were higher on P than on PR and PE (6.08, 5.99 and 6.00 for P, PR and PE, respectively, SED 0.02, $P<0.05$). Ruminal $NH_3$ concen-

trations averaged 22.7, 26.2 and 18.9 mmol/l for P, PR and PE, respectively, (SED 0.9, P<0.001).

DM digestibility did not differ among diets and averaged 80.4, 81.0 and 80.6 (SED 0.36) for P, PR and PE, respectively. Nitrogen digestibility was similar for the PR and PE diets (77.5 and 77.0, respectively), but was 2 percentage units higher for P (79.1, SED 0.80, P<0.05).

The rates and extents of loss of DM and N from pasture incubated in the rumen were not affected by diet. DM losses averaged across diets were 11.6, 21.8, 42.4, 59.1 and 76.0% for 0, 1, 3, 6 and 12 h incubations, respectively, (Figure 1). Corresponding values for N loss were 13.8, 20.4, 45.7, 67.6 and 84.8% (Figure 2). DM and N losses were higher from casein than from pasture on all diets at all times. Rate but not extent of DM and N losses from casein were affected by diet. Within 1 hour of incubation the percentage loss of DM from casein was higher (P<0.01) for cows on PR than on P (71.0 versus 38.2%, SED 10.43, Figure 1) but there was no difference between PE and PR or between PE and P in loss of casein DM. The percentage loss of N from casein after 1 h incubation was similar for PE and PR and higher (P<0.01) on these diets than when cows were on P (81.5, 69.4 and 35.4% for PR, PE and P, respectively, SED 10.86, Figure 2). Losses of casein DM and N were similar for all diets at 3 h. Insufficient casein sample remained after 6 and 12 h for N analysis.

**FIGURE 1:** Percentage of dry matter (DM) lost during incubation of casein (– – –) and pasture (——) in cows on pasture (P,♦), 85% P plus 15% carbohydrate/protein (PR,■) or P plus 10% carbohydrate (PE,▲).




## Trial 2

NSC, NDF and CP contents in P, PR and PE, respectively, were: NSC, 12.4, 20.3 and 21.2%; NDF, 48.9, 43.4 and 44.0%; CP, 18.4, 19.0 and 16.7%. Intake of pasture was lower (P<0.05) on PE than P (Table 2). Total DM intake was least on PR and greatest on PE (P<0.001). Milk yields (Table 2) were higher on PE than on P, on PE than on PR and on PR than on P (P<0.001, P<0.05, P<0.05, respectively). Protein and lactose contents of milk did not differ between diets but fat content was higher on P than on PR or PE (P<0.01). Cows produced more fat (P<0.05), protein (P<0.001) and lactose (P<0.001) on PE than on P,

**FIGURE 2:** Percentage of nitrogen (N) lost during incubation of casein (– – –) and pasture (——) in cows on pasture (P,♦), 85% P plus 15% carbohydrate/protein (PR,■) or P plus 10% carbohydrate (PE,▲).

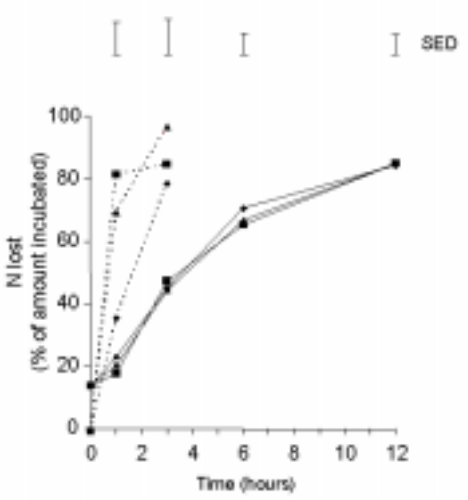



**TABLE 2:** Daily DM intake (DMI), daily production of milk, fat, protein and lactose, change over 14 days in liveweight (LW) and condition score (CS), microbial protein synthesis (MPS) and MPS per kg digestible OM intake in Trial 2, late lactation.

| Diet | P | PR | PE | SED |
|---|---|---|---|---|
| Pasture DMI (kg/cow) | 12.0 | 10.2 | 11.7 | 0.09 |
| Total DMI (kg/cow) | 12.0 | 11.5 | 13.0 | 0.08 |
| Milk yield (kg/cow) | 10.3 | 10.9 | 11.6 | 0.28 |
| Fat yield (kg/cow) | 0.52 | 0.52 | 0.55 | 0.012 |
| Protein yield (kg/cow) | 0.36 | 0.38 | 0.41 | 0.009 |
| Lactose yield (kg/cow) | 0.50 | 0.53 | 0.57 | 0.015 |
| LW change (kg) | -1.03 | -17.8 | 9.0 | 5.6 |
| CS change | -0.15 | -0.25 | -0.25 | 0.19 |
| MPS (g/day) | 139 | 141 | 172 | 11.5 |
| MPS (g N/kg DOMI) | 17.2 | 17.3 | 19.0 | 1.34 |

and more protein (P<0.01) on PR compared to P. Yields of fat, protein and lactose were higher (P<0.05, P<0.01, P<0.05, respectively) on PE than PR. The concentration of urea in milk averaged 7.59, 8.22 and 6.70 mmol/l for P, PR and PE, respectively, (SED 0.24) and was lower (P<0.001) for PE than for both P and PR. Diets did not differ in their effects on change in condition score but liveweight loss was greatest on PR (P<0.01, Table 2).

MPS was higher on PE than on P and PR (P<0.05), but there were no differences among diets when expressed per kg DOMI (Table 2). Ruminal pH values averaged 6.55, 6.46 and 6.44 for P, PR and PE, respectively, (SED 0.05) and were higher on P than on both PR and PE (P<0.1). $NH_3$ concentrations were higher (P<0.001) on PR than on P and PE and averaged 21.1, 24.0 and 18.7 mmol/l for P, PR and PE, respectively, (SED 0.94) across all sample times.

## DISCUSSION

Increasing the proportion of NSC in pasture without increasing energy intake did not increase ruminal micro-

bial protein synthesis or increase milk solids production in early lactation. Adding extra soluble carbohydrate reduced ruminal ammonia and milk urea concentrations and increased microbial protein synthesis but did not affect milk solids yield. Efficiency of microbial synthesis was not altered on the PE diet as DOMI increased. These data suggest that in spring pasture containing 23% NSC the proportion of NSC, *per se*, was not limiting N utilisation but that the total carbohydrate:protein ratio influenced ruminal function. This level of NSC is relatively high for pasture but within the typical range of 5 to 25% (Wilson and Moller 1993; Thom *et al.*, 1989). In late lactation with pasture of 12% NSC, both PR and PE diets increased milk production although only PE increased MPS. Milk protein yield increased by 21 g/cow/day on PR compared to P, without an increase in MPS, and by a further 29 g on PE compared to PR when flow of microbial protein to the intestine increased by about 30 g/day. It appeared that in late lactation the cows were limited by the supplies of both protein to the small intestine and fermen–table carbohydrate.

High ruminal $NH_3$ concentrations indicated substantial degradation of protein in the rumen on all diets. Rate of degradation of N from pasture was not altered by diet but $NH_3$ concentrations were reduced on PE indicating better utilisation of $NH_3$. The increased MPS on PE may have increased protein flow to the small intestine, if supply of ruminally undegraded CP did not change. The lack of production response to PE in Trial 1 may indicate that intestinal supply of protein was not the first limiting nutrient, specific amino acids were limiting, or that the experimental period was too short to detect a production response. The higher ruminal $NH_3$ and milk urea concentrations on PR than P, probably arising from faster degradation of N in casein than in pasture, may have compromised a production response in cows on PR, although this was not apparent in late lactation.

Assuming that 72% of DOMI was digested in the rumen (Ulyatt *et al.*, 1988), microbial efficiency was calculated at 34 gN/kg DOMI ruminally digested in spring pasture. MPS (g N/day) and the calculated efficiency were as high as those reported for cows on total mixed rations (Stern *et al.*, 1994). The calculated efficiency in Trial 2 was lower, at 24 gN/kg ruminally digested OM, suggesting gains in efficiency might be made from manipulating the diet at this time of the year. The lower efficiency may have reflected the low proportion of NSC in the digestible OM, although it did not increase when the proportion of NSC was increased.

The optimal range of ruminal pH for cellulose digestion is 6.4-6.8 (Erdman, 1988) with efficiency of MPS reduced at lower pH values (Strobel and Russell, 1986). Ruminal pH on P in spring was below 6 for much of the day but there was little evidence that it was associated with low microbial efficiency. Parameters defining optimal ruminal function on pasture may differ from those guiding use of feedstuffs in total mixed rations. Low ruminal pH is inconsistent with a diet high in NDF but may be associated with high volatile fatty acid concentrations or low buffering capacity of the forage (Erdman, 1988). The possibilities also exist that the fibre component in spring pasture was not physically effective and therefore did not adequately stimulate salivation (Allen, 1995), or the fermentation rates of the fibre component were higher than might have been assumed for fresh forage.

Proportion of NSC in the diet was associated with an increased loss of N from casein, but not pasture, incubated in the rumen. Siddons and Paradine (1983) and MacGregor *et al.* (1983) also found that *in situ* rates of protein degradation increased with increasing NSC content of the diet. Stokes *et al.* (1991) using ruminally and duodenally fistulated cows showed that the proportion of crude protein digested in the rumen was increased when total diet NSC increased above 24%, but not above 31%. This suggested that the increased rates of digestion in the rumen of CP associated with increased NSC was not an artefact of the incubation technique. Stokes *et al.* (1991) also showed that NDF degradation was not affected by changes in NSC content of the diet. This may explain the observation in the present trial that loss of N from pasture was not affected by NSC content in the diet, as the rate of NDF degradation in the cell walls may be the determinant of the release of protein from pasture.

No difference in DM digestibility was observed between diets but digestibility of N was lower for both PR and PE compared to P. This effect has been observed with starch supplementation in total mixed rations (Cameron *et al.*, 1991). Given that amino acid absorption is incomplete in the intestine (Chalupa, 1984), the decrease in N digestibility for the PE diet may have been due to a greater content of amino acids of microbial origin appearing in faeces. This may not explain the reduction in digestibility on PR, however. The protected casein used in the PR supplement may have had some degree of resistance to proteolysis by abomasal and intestinal proteases. Intake of N in protected casein was about 25 g N/day whereas the difference between diets in faecal N output averaged 7 g N/day (117, 124 and 124 g N/cow/day in faeces for P, PR and PE cows, respectively, SED 4). A third possibility for both the PE and PR diets is that some of the NSC in the supplement by-passed the rumen and stimulated microbial protein synthesis in the caecum and excretion of N in the faeces (Orskov, 1994).

Results indicated that an increased supply of NSC increased ruminal microbial synthesis but production responses were influenced by factors additional to manipulation of the diet. The lack of response to altering the proportion of NSC in spring is consistent with poor responses to supplementation experiments where the grain substitutes for pasture. There may be scope in late lactation for increasing production through manipulating composition of the pasture.

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## REFERENCES


Allen, M.S. 1995. Relationship between ruminally fermented carbohydrate and the requirement for physically effective NDF. *Journal of Dairy Science* **78** (Supplement 1): 265.

Cameron, M.R.; Klusmeyer, T.H.; Lynch, G.C.; Clark, J.H.; Nelson, D.R. 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. *Journal of Dairy Science* **74:** 1321-1336.

Chalupa, W. 1984. Discussion of Protein Symposium. *Journal of Dairy Science* **67:** 1134-1146.

Chen, X.B.; Gomes, M.J. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an overview of the technical details. International Feed Resources Unit, Rowett research Institute, Occasional Publication.

Erdman, R.A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. *Journal of Dairy Research* **71:** 3246-3266.

MacGregor, C.A.; Stokes, M.R.; Hoover, W.H.; Leonard, H.A.; Junkins, L.L.; Sniffen, C.J.; Mailman, R.W. 1983. Effect of dietary concentration of total nonstructural carbohydrate on energy and nitrogen metabolism and milk production of dairy cows. *Journal of Dairy Science* **66:** 39-50.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th revised edition, National Academy of Science, Washington, DC.

Orskov, E.R. 1994. Recent advances in understanding of microbial transformation in ruminants. *Livestock Production Science* **39:** 53-60.

Siddons, R.C.; Paradine, J. 1983. Protein degradation in the rumen of sheep and cattle. *Journal of Science, Food and Agriculture* **34:** 701-708.

Stern, M.D.; Varga, G.A.; Clark, J.H.; Firkins, J.L.; Huber, J.T.; Palmquist, D.L. 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the rumen. *Journal of Dairy Science* **77:** 2762-2786.

Stokes, S.R.; Hoover, W.H.; Miller, T.K; Blauweikel, R. 1991. Ruminal digestion and microbial utilisation of diets varying in type of carbohydrate and protein. *Journal of Dairy Science* **74:** 871-881.

Strobel, H.J.; Russell, J.B. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate limited cultures of mixed rumen bacteria. *Journal of Dairy Science* **69:** 2941-2947

Thom, E.R., Sheath, G.W. and Bryant, A.M. 1989. Seasonal variations in total nonstructural carbohydrate and major element levels in perennial ryegrass and paspalum in a mixed pasture. *New Zealand Journal of Agricultural Research* **32:** 157-165.

Ulyatt, M.J.; Thomson, D.J.; Beever, D.E.; Evans, R.T.; Haines, M.J. 1988. The digestion of perennial ryegrass (*Lolium perenne* cv. melle) and white clover (*Trifolium repens* cv. Blanca) by grazing cattle. *British Journal of Nutrition* **60:** 137-149.

Wilson, G.F. and Moller, S. 1993. Imbalances in New Zealand pastures for dairy cows. Department of Agricultural and Horticultural Systems Management, Massey University, Occasional Publication No. 1, pp 59-70.