



Effects on Performance of Sulla and/or Maize Silages Supplements for Grazing Dairy Cows

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ABSTRACT : The objective of this study was to investigate the effects of either maize or sulla silage supplementation to grazing dairy cows in summer. Forage mixtures used in the four week trial were based on previous experimental results but inclusion of rumen fistulated cows in five treatments enabled rumen sampling and use of *in sacco* incubations to determine the diet effects on digestion kinetics. Sulla and maize silages were used to supplement pasture and to meet minimum requirements for dietary protein concentration. Five groups of ten cows were grazed on a restricted daily allowance of 18 kg dry matter (DM) pasture/cow to simulate a summer pasture deficit, and four of these five groups received an additional 6 kg DM cow⁻¹ d⁻¹ of silage (sulla, maize, or sulla and maize silages). A sixth group was given a relatively unrestricted (38 kg DM cow⁻¹ d⁻¹) pasture allowance. The silage mixtures and pasture were incubated *in sacco* during the final week of the trial. The pasture was of high nutritive value and not typical of usual summer conditions, which favoured a response to quantity rather than quality of silage supplements. There was no difference in cow performance with the four silage supplements and the low milk solids (MS) production (about 1.0 kg MS d⁻¹) relative to full pasture (1.3 kg MS d⁻¹) showed the principal limitation to performance was dry matter intake. Milk composition was not affected by silage type and the low level of pasture substitution (0.29) suggested metabolizable energy (ME) was the principal limitation to performance. Samples of rumen liquor and *in sacco* data demonstrated significant effects of supplement; DM degradation rates (k) was highest (0.084 h⁻¹) when cows were fed 6 kg sulla silage whereas diets with a high proportion of maize silage were slowly degraded (p<0.01). (**Key Words :** *In sacco*, Milk, Pasture, Silage Mixtures)

INTRODUCTION

A previous study examined responses to maize, pasture, lotus (*Lotus corniculatus* L.) and sulla (*Hedysarum coronarium* L.) silage supplementation and showed clear benefits of lotus silage for milk production when pasture supply was restricted (Woodward et al., 2002). Although maize silage did maintain milk production over the four week experimental period the cows did not gain weight in contrast to other silages and the dietary CP was less than cow requirements (NRC, 2001). The potential of sulla silage was not expressed because the long stalks and sub-optimal quality resulted in substantial refusals. These

silages provided a foundation for further evaluation, with more focus on achieving adequate dietary CP using fewer silage types.

The poor cow response to maize silage, in contrast to lotus silage (14.97 vs. 17.23 kg milk, respectively; Woodward et al., 2002) emphasised the importance of meeting cow protein requirements especially as maize silage has a very low protein concentration (6.9% DM) and is not suitable for feeding with low quality summer pasture. However when maize silage is fed in a balanced diet, it provides a source of non-structural carbohydrates (NSC; mainly starch) which may complement pasture for much of the year (Kolver et al., 2001). Sulla is of interest because it is a high yielding forage legume in temperate climate countries (Waghorn et al., 1998) containing condensed tannins (CT) and high concentration of NSC which offers good potential for high quality silage production (Niezen et al., 1998). Balancing dietary protein deficiency by feeding sulla and improving readily fermentable carbohydrate intake with maize silage may optimise milk solid production from cows grazing poor to medium quality in summer in

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Received December 2, 2005; Accepted March 31, 2006

New Zealand.

In their study, Niezen et al. (1998) also experimented a dry summer with restricted pasture availability, typical of the Waikato (main dairy region in New Zealand) dairy environment. Maize and sulla silages were fed alone or as mixtures to account for about 40% of total DMI. The use of maize silage as a sole supplement enabled data from their trial to be compared with literature reports (Stockdale, 1995; Phillips, 1988; Kolver et al., 2001; Woodward et al., 2002).

The objective of this study was to examine the effects of supplementation of grazing dairy cows with either maize or sulla silage or a combination of both during the summer in New Zealand. Data obtained from this trial, with kinetic information, were used as inputs to the Cornell Net Carbohydrate and Protein System (CNCPS) model to determine the first limiting nutrient and provide information concerning rumen digestion parameters.

MATERIAL AND METHODS

Sixty Friesian dairy cows (15 primiparous and 45 multiparous including 10 with rumen fistulated; 483 kg liveweight (LW); 14.3 kg milk d^{-1} ; 156 days in milk) were allocated to six treatments and balanced for milksolid (MS) yield and LW. The overall design comprised a uniformity (covariance) period of one week, when all cows were grazed on ryegrass pasture (80% *Lolium perenne* L. and 20% *Trifolium repens* L.) enabling their subsequent allocation to six groups to be fed the experimental diets for three weeks.

Treatments

The six treatments enabled the effects of supplementing a pasture diet with either maize and/or sulla silages to be compared with unsupplemented pasture:

- FP: full pasture allowance (38 kg DM $cow^{-1} d^{-1}$)
- RP: restricted pasture allowance (18 kg DM $cow^{-1} d^{-1}$)
- PMS: restricted pasture
 - + 4 kg DM of maize silage $cow^{-1} d^{-1}$
 - + 2 kg DM of sulla silage $cow^{-1} d^{-1}$
- PSM: restricted pasture
 - + 4 kg DM of sulla silage $cow^{-1} d^{-1}$
 - + 2 kg DM of maize silage $cow^{-1} d^{-1}$
- PS: restricted pasture+6 kg DM of sulla silage $cow^{-1} d^{-1}$
- PM: restricted pasture+6 kg of maize silage $cow^{-1} d^{-1}$

Use of full and restricted pasture allowance allowed substitution rates to be calculated as well as impacts of pasture availability.

Feeding and cow management

Each treatment group was given a new paddock of

pasture on a daily basis with a back-fence using electric fence. Daily pasture allowances for each treatment group were estimated by pre-grazing herbage biomass (Bonham, 1989) and allocation of paddocks size accordingly. Silages were fed to cows on a group basis from portable feed troughs (one trough per five cows) once cows returned to the paddock after morning milking. Silage DM was determined by quick drying (microwave) confirmed by drying for 24 h at 100°C (Woodward et al., 2002), and sufficient placed in troughs to provide six kg supplement DM $cow^{-1} d^{-1}$. Troughs were collected from paddocks when cows were at afternoon milking and any refusal were weighed and sampled for DM determination. Water was available *ad libitum*.

Cows were rotational grazed throughout the treatment periods in treatment groups of 10 animals but each group was divided into two groups for measurement on Tuesday, Wednesday and Thursday of each week. On these three days, each group of 10 cows were split into two groups of five (same cows in each group each week) when milk and pasture measurements and samples were collected in order to replicate the treatments. On the remaining four days each week (Friday, Saturday, Sunday, Monday) the replicate groups were combined into treatment groups (six groups of 10 cows) for ease of management. Cows in each treatment group were grazing similar pastures (same area) in adjacent plots.

The full allowance (38 kg DM $cow^{-1} d^{-1}$) of pasture was intended to provide unrestricted feed, while the restricted pasture allowance of 18 kg DM $cow^{-1} d^{-1}$ was intended to mimic summer conditions with feed shortages.

Measurements

Pasture intakes : pasture intakes of each treatment group were estimated by using a rising plate meter to measure pre- and post-grazing herbage mass (50 measures per 24 h for each group). This was done three times per week during the measurement period for each group of 10 cows to coincide with milk sampling days. Twelve quadrants (1 m²) of pasture were cut one day of each week pre- and post-grazing (on representative pasture) to calibrate the rising plate meter (Hodgson et al., 1999).

Silage intakes : silage intakes of each group were measured by weighing silage pre- and post-feeding. Pre-feeding weighing was done every day, but post-feeding weighing of refusals was done only during the measurement period to coincide with days on which pasture intakes were estimated.

Pasture quality : two types of samples were collected using an electric clipper, for analyses of pasture quality:

i) Pre-grazing pasture samples were cut to ground level from each of 12 pasture paddocks on each day during the measurement period. Samples from each pasture paddock

were bulked within weeks to provide one sample for each group of five cows per week to indicate the quality of pasture on offer.

ii) A second pre-grazing pasture sample was cut to estimated grazing height at about five cm above ground level from each pasture paddock on each day during the measurement period. Samples were bulked to provide one sample for each group of five cows per week to indicate quality of pasture consumed. Both pasture types were sampled to determine DM content (100°C; 24 h) and sub-samples dried at 60°C were used for NIRS analyses (Corson et al., 1999).

Silage quality : samples of the silages offered were taken on measurement days and bulked to provide one sample per week. Samples were taken of silage refusals over the same period and bulked for analyses. Silages and refusals were sampled for dry matter measurement (100°C; 24 h) and NIRS analysis. Composition of silage offered and refused enabled calculations of the dietary composition (nutrient×intake) for each constituent (DM, CP, lipid, ADF, NDF, and ME):

Dietary composition

$$= ((\text{kg offered} \times \text{concentration constituent offered}) - (\text{kg refused} \times \text{concentration constituent refused})) / \text{kg intake}$$

Liveweight was measured before milking on three mornings per week during weeks one, three, and four of the trial. Milk yield was measured for each cow on three days per week, and samples taken to measure fat, protein, and lactose concentration as described in Woodward et al. (2002).

***In sacco* incubation and digestion kinetics**

Two rumen fistulated cows were included in each treatment except those given restricted pasture (RP) and enabled *in sacco* incubations of pasture and silage mixtures fed to each cow to be conducted. Pasture used in incubations was obtained by cutting pasture five centimetres above ground level before grazing, and frozen prior to chopping at around 2 cm length and mincing for *in sacco* incubations. The pasture was incubated in all cows as a single constituent and also combined with sulla and maize silages in similar proportions to the diet eaten by cows.

Mixtures used for *in sacco* incubations comprised about 60% pasture DM and 40% silage DM. The four silage mixtures (PMS, PSM, PS, and PM) were only incubated in cows which were fed the same dietary mixture; i.e. the two cows fed pasture with maize silage were used to incubate pasture and maize silage; those fed PMS incubated PMS *in sacco*. Duplicate bags of dietary mixtures were removed at each time from all cows as well as duplicated bags of ryegrass pasture (81% ryegrass+19% white clover). The

only exception were cows fed full pasture, where duplicate bags of pasture were removed at each sampling time from the two cows.

In sacco incubations for each diet were prepared by chopping pasture and sulla silage to 2 cm length, mixing frozen chopped pasture, sulla and maize silages as required, and mincing to resemble chewed forage as described by Chaves (2003). Samples were weighed into *in sacco* bags and also used to determine DM, chemical composition, and distribution of particle size. A total of 28 bags (four per lingerie bag) were placed in the mid-ventral rumen of cows, and were removed at 2, 6, 9, 12, 24, 48, and 72 h. Bags, including 0 h samples which were not put in the cow, were washed, dried at 60°C and analysed to determine DM, CP, NDF and ADF content to calculate rates of disappearance during digestion. *In sacco* calculations and data analyses were done according to Chaves (2003).

The disappearance of DM, NDF and ADF were analysed using a non-linear model described by López et al. (1999). The effective degradability (E) was calculated from soluble and degradable pools and kinetic parameters, by fitting equations to *in sacco* data assuming a fractional passage rate (k_p) of 0.06 and 0.08 h⁻¹. A k_p value of 0.08 h⁻¹ was used (AFRC, 1992) for comparison with high producing dairy cows (Hoffman et al., 1998; Kolver et al., 1998) even though intakes in this study were relatively low.

Disappearance of CP was calculated using a similar procedure but additional definition was applied in relation to the degradability of protein and CP content of the DM. The RDP and RUP values for the diets (percent of CP) were estimated from the model describing degradation and ruminal escape of feed proteins (Ørskov and McDonald, 1970; NRC, 2001) using the equations:

$$\text{RDP} = A + B (k / (k + k_{\text{DM}}))$$

$$\text{RUP} = B (k_{\text{DM}} / (k + k_{\text{DM}})) + C$$

Where k (% h⁻¹) is the fractional rate of protein degradation, k_{DM} is the DM passage rate (% h⁻¹), and C is the undegradable fraction. The equation to estimate DM passage is $k_{\text{DM}} = 3.054 + 0.614 \text{ DMI}$, where DMI is expressed as a percentage of BW (NRC, 2001).

The metabolizable protein system (AFRC, 1992) for defining ruminal degradation was used to calculate protein degradability parameters. Effective rumen degradability of CP (ERDP, g kg⁻¹ DM) was calculated as: $\text{ERDP} (\text{g kg}^{-1} \text{ DM}) = \text{CP} ((0.8 \times A) + (B \times k) / (k + k_p))$. Here values for effective degradability of CP and ERDP were calculated using outflow rates of digesta DM from the rumen of 0.06 or 0.08 h⁻¹, which approximate to the outflow rates in cows producing less than 15 l milk d⁻¹ or cows fed a good quality diet that enabled a rapid passage through the rumen (Wales

Table 1. Chemical composition of pasture cut at 5 cm above ground for each dietary treatment, and of maize and sulla silages averaged over the three week experimental period

| | Composition of pasture offered to cows on treatments | | | | | | Maize silage | Sulla silage |
|-----------------------------|--|------|------|------|------|------|--------------|--------------|
| | FP ^a | RP | PMS | PSM | PS | PM | | |
| DM ^b (%) | 19.4 | 20.2 | 20.5 | 18.2 | 19.9 | 18.4 | 35.9 | 34.1 |
| CP (% DM) | 21.7 | 21.5 | 21.0 | 21.7 | 21.0 | 20.6 | 6.6 | 15.7 |
| NSC (% DM) | 9.2 | 9.1 | 8.5 | 8.9 | 9.4 | 9.0 | 41.4 | 3.6 |
| Lipid (% DM) | 4.0 | 3.9 | 4.0 | 4.0 | 3.9 | 3.8 | 2.9 | 5.2 |
| NDF (% DM) | 43.9 | 44.1 | 46.3 | 45.8 | 45.4 | 46.5 | 39.1 | 50.0 |
| ADF (% DM) | 24.4 | 24.9 | 25.4 | 24.8 | 25.2 | 25.9 | 23.8 | 40.8 |
| Ash (% DM) | 10.1 | 10.2 | 9.9 | 9.9 | 9.6 | 9.9 | 4.0 | 10.1 |
| Lignin (% DM) | 4.0 | 4.2 | 3.7 | 4.0 | 4.0 | 4.3 | 3.4 | 8.0 |
| OMD (%) | 75.6 | 74.9 | 74.1 | 74.8 | 73.6 | 74.6 | NA | 65.9 |
| ME (MJ kg ⁻¹ DM) | 10.7 | 10.6 | 10.5 | 10.6 | 10.5 | 10.6 | 10.8 | 10.5 |
| pH | | | | | | | 3.9 | 3.9 |
| Lactic acid (% DM) | | | | | | | 2.3 | 11.4 |
| Ammonia-N (% total N) | | | | | | | 0.9 | 4.7 |
| Total CT (% DM) | | | | | | | | 3.5 |

¹FP: full pasture; RP: restricted pasture; PMS (RP+4 kg maize silage cow⁻¹ d⁻¹+2 kg sulla silage cow⁻¹ d⁻¹); PSM (RP+4 kg sulla silage cow⁻¹ d⁻¹+2 kg maize silage cow⁻¹ d⁻¹); PS (RP+6 kg sulla silage cow⁻¹ d⁻¹); PM (RP+6 kg maize silage cow⁻¹ d⁻¹).

²DM: dry matter; CP: crude protein; NSC: non-structural carbohydrates; NDF: neutral detergent fibre; ADF: acid detergent fibre; OMD: organic matter digestibility; ME: metabolizable energy; CT: condensed tannins.

et al., 1999). Relationships between degradability, nutritive characteristics of the diet *in sacco* samples and ERDP were analysed by regression (SAS, 2006) using the model:

$$\text{ERDP (g kg}^{-1}\text{ DM)} = a + bX,$$

where X is the effective degradability of DM or NDF content of diet *in sacco* residues.

Rumen samples

Rumen fluid was also collected 5 times during the first day of the *in sacco* incubation to check the pattern of rumen metabolite concentration over 12 h from 07:00 h (pre-feeding) to 19:00 h. Rumen fluid samples were collected twice daily on the measurements days before morning and afternoon milking. On each occasion, about 1 kg of rumen contents was taken from the mid-ventral rumen and strained through a cheese cloth to collect 100 ml of rumen fluid. Rumen pH was determined at collection (PHM210, Radiometer Pacific Limited, Copenhagen) before samples were centrifuged and prepared to determine ammonia (Chaney and Marbach, 1962) and VFA (Attwood et al., 1998) concentrations.

Statistical analysis

Data from *in sacco* incubations were expressed as degradation curves using a non-linear least-square procedure (PROC NLIN; SAS, 2006) to provide estimates for A, B, and k. Evaluations were made for DM, CP, NDF, and ADF for soluble (A) and degradable (B) pool, and rate of degradation (k) according to López et al. (1999). For each degradation parameter (A, B, k and E) fixed model

effects tested differences between diets, cow within diet effects upon pasture digestion kinetics and cow effects. A general linear model procedure of SAS (PROC GLM; SAS, 2006) was used for analysis of the milk parameters and LW change with uniformity period data as a covariate (Woodward et al., 2002). Rumen ammonia and VFA concentrations were analysed using mixed model procedure of SAS (PROC MIXED; SAS, 2006) to calculate treatment means. Rumen pH analysis included day, treatment and treatment×day interaction. Effects were declared significant at p<0.05 unless otherwise noted.

RESULTS

Pasture and silage composition

The quality of pasture on offer is presented on Table 1 and prevented any benefits associated with silage quality to be expressed even though feed availability was very low. The CP concentration in pasture exceeded that in sulla silage, which had a higher NDF concentration than pasture. The main effect of silage supplementation was through provision of additional ME although the NSC content of maize silage did alter diet composition.

Dry matter of pasture cut to 5 cm above ground level ranged from an average of 18.2 to 20.5% across treatments. There was no significant change in pasture quality over the four week duration of the trial, with average DM of 18.6% in week three and 17.9% in week four. Crude protein was 21.1 and 21.4% in weeks three and four with NDF contents of 47.3 and 43.3% in the respective periods.

The mean values of CP and NDF for the pastures offered to each treatment group were consistent, averaging

Table 2. Daily pasture and silage allowances, dry matter intakes (DMI), substitution rate (SR), milk production, and live weight (LW) change over 4 weeks trial for cows given six dietary treatments (Average nutritional composition of diets eaten by cows)

| | Cow feeding treatments ^a | | | | | | LSD ^b | p |
|--|-------------------------------------|-------|------|------|------|------|------------------|--------|
| | FP | RP | PMS | PSM | PS | PM | | |
| Pasture allowance | 38 | 18 | 18 | 18 | 18 | 18 | | |
| Silage allowance | 0 | 0 | 6 | 6 | 6 | 6 | | |
| Total DMI (kg cow ⁻¹ d ⁻¹) | 15.7 | 10.4 | 14.6 | 14.4 | 13.9 | 14.3 | 2.3 | 0.001 |
| Pasture DMI (kg cow ⁻¹ d ⁻¹) | 15.7 | 10.4 | 8.8 | 9.0 | 8.7 | 8.8 | 1.6 | 0.001 |
| Silage DMI (kg cow ⁻¹ d ⁻¹) | | | 5.8 | 5.4 | 5.2 | 5.5 | 0.3 | 0.001 |
| Pasture utilization ^c (%) | 41 | 58 | 49 | 50 | 48 | 49 | 3.8 | 0.003 |
| Milk lactose (kg cow ⁻¹ d ⁻¹) | 0.83 | 0.64 | 0.71 | 0.66 | 0.66 | 0.67 | 0.05 | 0.002 |
| Milk fat (kg cow ⁻¹ d ⁻¹) | 0.73 | 0.57 | 0.56 | 0.56 | 0.54 | 0.55 | 0.06 | 0.001 |
| Milk protein (kg cow ⁻¹ d ⁻¹) | 0.57 | 0.42 | 0.46 | 0.44 | 0.44 | 0.46 | 0.02 | 0.001 |
| Milk (kg cow ⁻¹ d ⁻¹) | 17.2 | 13.2 | 14.3 | 13.7 | 13.7 | 13.7 | 1.00 | 0.001 |
| Milksolids (kg cow ⁻¹ d ⁻¹) | 1.30 | 0.99 | 1.02 | 1.00 | 0.98 | 1.01 | 0.07 | 0.001 |
| LW change (kg cow ⁻¹ d ⁻¹) | -3.8 | -12.4 | 1.0 | -4.5 | -0.8 | -1.4 | 3.40 | 0.060 |
| Substitution rate (silage for pasture) | | | 0.28 | 0.26 | 0.33 | 0.29 | | |
| Dietary composition | | | | | | | | |
| DM (%) | 17.4 | 15.9 | 26.5 | 24.6 | 25.2 | 25.4 | 4.85 | 0.005 |
| CP (% DM) | 21.7 | 21.5 | 16.4 | 18.4 | 19.0 | 15.4 | 3.91 | 0.035 |
| NSC (% DM) | 9.2 | 9.1 | 16.8 | 11.8 | 6.9 | 22.0 | 0.78 | <0.001 |
| Lipid (% DM) | 4.0 | 3.9 | 3.8 | 4.2 | 4.3 | 3.6 | 0.29 | 0.006 |
| NDF (% DM) | 43.9 | 44.1 | 44.8 | 45.8 | 47.5 | 42.7 | 6.94 | 0.647 |
| ADF (% DM) | 24.4 | 24.9 | 26.9 | 28.6 | 31.3 | 24.5 | 3.00 | 0.007 |
| Ash (% DM) | 10.1 | 10.2 | 8.4 | 9.3 | 10.0 | 7.4 | 0.69 | <0.001 |
| ME (MJ kg ⁻¹ DM) | 10.7 | 10.6 | 10.6 | 10.6 | 10.6 | 10.6 | 0.49 | 0.994 |

^a FP: full pasture; RP: restricted pasture; PMS (RP+4 kg maize silage cow⁻¹ d⁻¹+2 kg sulla silage cow⁻¹ d⁻¹); PSM (RP+4 kg sulla silage cow⁻¹ d⁻¹+2 kg maize silage cow⁻¹ d⁻¹); PS (RP+6 kg sulla silage cow⁻¹ d⁻¹); PM (RP+6 kg maize silage cow⁻¹ d⁻¹).

^b Least significant difference (p<0.05) for treatments within rows.

^c kg DM pasture eaten kg⁻¹ DM pasture offered×100.

21.2% and 45.3% of the DM, respectively (Table 1). Predicted organic matter digestibility (OMD) exceeded 73% for all pastures cut at 5 cm above ground level. Maize and sulla silages contrasted in chemical composition, with concentrations of 6.6 and 15.7% DM for CP and 39.1 and 50.0% DM for NDF for maize and sulla silage, respectively. The low NSC concentration in sulla silage (3.6% DM) was related in part to the high lactic acid content. Sulla silage contained 3.5±0.3% DM of condensed tannins.

Cow performance

Maize, sulla, and silage mixtures increased DMI but milk and milksolid (MS) productions were similar to the cows offered restricted pasture as a sole diet (Table 2). The main impact of silages was to maintain LW. For cows given the RP treatment, there was insufficient feed available to maintain both milk production and LW. Cows fed the full pasture treatments had significantly higher milk and MS yields than the silage supplemented cows (p<0.001) and LW lost was higher for RP than for all other treatments, and cows on PMS treatment had higher LW change than cows on FP and PSM treatments.

Cows given the high pasture allowance consumed 5.3 kg more pasture DM and produced additional 4.0 kg milk compared to RP cows; difference in milk production would

have been greater if LW changes were similar. The RP treatment group also lost 12.4 kg of LW during the three week feeding period. Milk composition was not affected by treatment (data not shown).

Pasture intake by cows given the four silage supplements were about 1.6 kg DM d⁻¹ lower than those on RP. On average for all silage supplemented cows, 5.5 kg silage DM was consumed giving a substitution rate (SR) of 1.6/5.5 = 0.29 (the reduction in kg pasture intake per kilogram of supplement, kg kg⁻¹; Table 2). All supplements had a similar SR (0.26 to 0.33) which showed provision of silages resulted in a substantial increase in feed intake when pasture allowance was 18 kg DM cow⁻¹ d⁻¹.

Pasture DM on offer ground level averaged 3,141±35 kg ha⁻¹ (mean±SD) for all treatments, with post grazing residuals of 1,525±132 kg ha⁻¹ for cows on restricted pasture allowance with silages and 1,814±110 kg DM ha⁻¹ for cows on full pasture allowance. Cows on restricted pasture as a sole diet left 1,298±290 kg residual DM (data not shown). Pasture DM utilization was 58% for RP and 49% across the four silage supplemented treatments (Table 2). Cows on unrestricted pasture allowance consumed 41% of on the pasture offered.

Although all silages were acceptable, with refusals of 0.2 to 0.8 kg DM cow⁻¹ d⁻¹, the preferred supplement by

cows comprised 4 kg maize silage and 2 kg sulla silage $\text{cow}^{-1} \text{d}^{-1}$. The refusals from this last treatment group comprised mainly sulla stem and proportions refused increased with the amount of sulla silage offered (about 0.6 kg with PSM treatment and 0.8 with PS treatment). The treatment PS had the lowest proportion of sulla silage eaten

compared to other supplemented treatments ($p < 0.001$; Table 2).

There were significant differences between diets in the concentration of most constituents (Table 2). Provision of silages increased the dietary DM concentration ($p = 0.005$). The supplementary silages were intended to meet cow

Table 3. *In sacco* degradation characteristics of dry matter (DM), crude protein (CP) and fibre (NDF and ADF), in cows fed full pasture or restricted pasture plus four silage supplements

| | A ^a (%) | B (%) | k (h ⁻¹) | C (%) | E _{6%} (%) | E _{8%} (%) |
|---------------------------|--------------------|-------|----------------------|-------|---------------------|---------------------|
| DM | | | | | | |
| FP ^b | 43 | 39 | 0.070 | 18 | 64 | 61 |
| PMS | 42 | 43 | 0.049 | 15 | 61 | 58 |
| PSM | 43 | 38 | 0.068 | 19 | 63 | 60 |
| PS | 46 | 36 | 0.084 | 17 | 68 | 65 |
| PM | 45 | 36 | 0.054 | 19 | 62 | 59 |
| Model p ^c | 0.676 | 0.196 | 0.002 | 0.145 | <0.001 | <0.001 |
| Forages p | 0.676 | 0.068 | 0.001 | 0.145 | <0.001 | <0.001 |
| Cow within pasture diet p | | 0.343 | 0.003 | 0.139 | <0.001 | <0.001 |
| Cow p | | 0.515 | 0.006 | 0.169 | <0.001 | <0.001 |
| r ² | 0.52 | 0.89 | 0.99 | 0.91 | 0.99 | 0.99 |
| CP | | | | | | |
| FP | 56 | 36 | 0.121 | 8 | 80 | 78 |
| PMS | 61 | 32 | 0.070 | 7 | 79 | 77 |
| PSM | 65 | 26 | 0.084 | 8 | 81 | 79 |
| PS | 66 | 28 | 0.090 | 6 | 83 | 81 |
| PM | 56 | 33 | 0.128 | 11 | 79 | 77 |
| Model p | 0.001 | 0.147 | 0.068 | 0.037 | 0.040 | 0.056 |
| Forages p | 0.001 | 0.046 | 0.020 | 0.017 | 0.013 | 0.020 |
| Cow within pasture diet p | | 0.169 | 0.518 | 0.033 | 0.162 | 0.175 |
| Cow p | | 0.807 | 0.127 | 0.283 | 0.109 | 0.139 |
| r ² | 0.74 | 0.91 | 0.94 | 0.96 | 0.96 | 0.94 |
| NDF | | | | | | |
| FP | 22 | 50 | 0.053 | 28 | 45 | 41 |
| PMS | 13 | 64 | 0.033 | 23 | 35 | 31 |
| PSM | 11 | 56 | 0.054 | 33 | 37 | 33 |
| PS | 27 | 43 | 0.058 | 30 | 49 | 46 |
| PM | 14 | 53 | 0.037 | 33 | 35 | 31 |
| Model p | <0.001 | 0.070 | 0.080 | 0.257 | <0.001 | <0.001 |
| Forages p | <0.001 | 0.015 | 0.025 | 0.150 | <0.001 | <0.001 |
| Cow within pasture diet p | | 0.392 | 0.339 | 0.426 | <0.001 | <0.001 |
| Cow p | | 0.395 | 0.188 | 0.285 | <0.001 | <0.001 |
| r ² | 0.94 | 0.94 | 0.94 | 0.87 | 0.99 | 0.99 |
| ADF | | | | | | |
| FP | 23 | 49 | 0.050 | 28 | 44 | 41 |
| PMS | 14 | 62 | 0.031 | 25 | 34 | 30 |
| PSM | 14 | 52 | 0.053 | 33 | 38 | 34 |
| PS | 26 | 44 | 0.065 | 31 | 49 | 45 |
| PM | 13 | 53 | 0.030 | 34 | 32 | 29 |
| Model p | <0.001 | 0.137 | 0.029 | 0.339 | <0.001 | <0.001 |
| Forages p | <0.001 | 0.056 | 0.009 | 0.224 | <0.001 | <0.001 |
| Cow within pasture diet p | | 0.238 | 0.167 | 0.688 | <0.001 | <0.001 |
| Cow p | | 0.302 | 0.083 | 0.268 | <0.001 | <0.001 |
| r ² | 0.94 | 0.91 | 0.96 | 0.84 | 0.99 | 0.99 |

^a Kinetics are defined by soluble (A), degradable insoluble (B), and undegradable residue ($C = 100 - A - B$) as well as fractional disappearance rate (k), and effective degradability (E) which takes in account the effect of passage from the rumen. Passage rate of 0.05 h⁻¹, 0.06 h⁻¹, and 0.08 h⁻¹ were used.

^b FP: full pasture; PMS (RP+4 kg maize silage $\text{cow}^{-1} \text{d}^{-1}$ +2 kg sulla silage $\text{cow}^{-1} \text{d}^{-1}$); PSM (RP+4 kg sulla silage $\text{cow}^{-1} \text{d}^{-1}$ +2 kg maize silage $\text{cow}^{-1} \text{d}^{-1}$); PS (RP+6 kg sulla silage $\text{cow}^{-1} \text{d}^{-1}$); PM (RP+6 kg maize silage $\text{cow}^{-1} \text{d}^{-1}$).

^c p: probabilities assessing goodness of fit for the overall model and tests of forage, cow/diet and cow effects.

Table 4. Rumen degradable protein (RDP) and rumen undegradable protein (RUP) as a percentage of crude protein concentration, and rate of dry matter passage from the rumen (k_{DM}) (NRC, 2001) for pasture and mixtures of pasture and silages incubated *in sacco*

| | RDP | RUP | k_{DM} (h^{-1}) |
|----------------------|--------------------------------|--------|-----------------------|
| | ----- % of crude protein ----- | | |
| FP ^a | 81.4 | 18.6 | 0.049 |
| PMS | 79.9 | 20.1 | 0.050 |
| PSM | 82.1 | 17.9 | 0.049 |
| PS | 83.8 | 16.2 | 0.049 |
| PM | 79.7 | 20.3 | 0.049 |
| Model p ^b | 0.034 | 0.034 | |
| Forages p | <0.001 | <0.001 | |
| Cow/diet p | 0.149 | 0.149 | |
| Cow p | 0.106 | 0.106 | |
| r^2 | 0.96 | 0.96 | |

^aFP: full pasture; PMS (RP+4 kg maize silage cow⁻¹ d⁻¹+2 kg sulla silage cow⁻¹ d⁻¹); PSM (RP+4 kg sulla silage cow⁻¹ d⁻¹+2 kg maize silage cow⁻¹ d⁻¹); PS (RP+6 kg sulla silage cow⁻¹ d⁻¹); PM (RP+6 kg maize silage cow⁻¹ d⁻¹).

^bp: probabilities assessing goodness of fit for the overall model and tests of forage, cow/diet and cow effects.

requirements for crude protein (CP), and the values in Table 2 show this was achieved with the exception of the PM diet which was only slightly less than the desirable 16-17% CP in diet for cows in mid lactation (NRC, 2001). The non-structural carbohydrate concentration in the diet was highest when maize silages were fed reaching 22% of dietary DM but was lowest with sulla (6.9%). Dietary lipid averaged 4.0% for all treatments and ash was lowest for cows fed high proportions of maize silage. There was no difference ($p>0.05$) between diets in concentration of NDF and estimated ME (Table 2), although ADF concentrations were higher ($p<0.001$) in treatments where sulla silage was fed.

All silage treatments provided a similar level of nutrition, indicated by milk yield, and that 86-96% of silage offered was eaten, the responses to performance of cows given full pasture were due to higher feed intakes. In contrast, the cows offered 18 kg pasture DM d⁻¹ (RP treatment) only ate 10.4 kg DM which was 58% of DM on offer.

Although there was no difference in milk production between the individual silage supplement treatments, the PMS resulted in the highest milk yield without LW loss (Table 2). This diet also met the cow requirement for CP and provided 16.8% DM of non-structural carbohydrates, suggesting a relatively good nutritive value despite its relatively high NDF concentration (44.8% DM). The PM diet also achieved a similar level of milk production, probably because the high pasture CP content complemented the low protein in maize silage.

In sacco incubations

Data presented here are averaged for incubation of

pasture in each of the 10 fistulated cows and for pasture/silage mixtures incubated in two cows fed each treatment: PMS, PSM, PS, and PM. The key tests of significance are indicated on each table as follows: fit of the model ("Model"), comparison between pasture/silage mixtures regardless of the cow diet ("Forage") and differences between cows based on *in sacco* digestion of pasture only ("Cow within pasture diet"). Effects of individual cows on degradation rates were also tested in the model ("Cow").

DM digestion

The amount of DM in the soluble "A" fraction was similar for all diets (Table 3; $p = 0.676$) and the slowly degradable B fraction was slightly higher ($p = 0.068$) for the PMS (B = 43) than other diets. The PS was most rapidly degraded ($E_{6\%} = 68$) and the PM and PMS were slowly degraded ($E_{6\%} = 62$ and 61, respectively; Table 3). The rate of pasture DM degradation rate ($k = 0.070$) was intermediate and higher than PMS, PSM and PM diets ($p<0.001$). There was difference due to cow within pasture diet ($p = 0.003$). Significant differences in k values between cows ($p = 0.006$) and also between forages ($p<0.001$) were found.

CP digestion

The effective degradability of CP (Table 3) was higher than that of DM for all diets, probably because about 61% of CP was solubilized and the undegradable CP fraction was smaller than that for DM. Diets with a high proportion of sulla silage (PS) had higher ($p<0.001$) effective degradability and a high proportion of maize silage (PM) lowered effective degradability. Cow within pasture diet had no effect on protein degradability ($p = 0.518$).

In contrast to DM disappearance, the rate of protein degradation *in sacco* was reduced when sulla was mixed with pasture (Table 3; $p = 0.020$), possibly in response to the protection of CP conferred by condensed tannins in sulla. Reduced protein degradation rate is likely to increase protein availability for absorption and increase nutritive value for cows. Calculations from NRC (2001) showed an average of 81.4% RDP and 18.6% RUP across all diets including pasture. Diets containing sulla had the highest proportion of RDP (Table 4).

Forage mixtures varied in NDF content from 42.7 to 47.5% DM (Table 2) and when analysed by regression the concentration of NDF accounted for 68% of the variation in effective rumen degradability for protein (ERDP) across diets. These data demonstrate a linear positive ($p<0.001$) relationship between dietary NDF concentration and ERDP across the five diets incubated *in sacco*:

$$\text{ERDP (g kg}^{-1}\text{ DM)} = -352 + 1.1(\pm 0.18) \times \text{NDF} \\ (r^2 = 0.68; \text{Root MSE} = 11.1; \text{CV} = 8.05\%; p < 0.001).$$

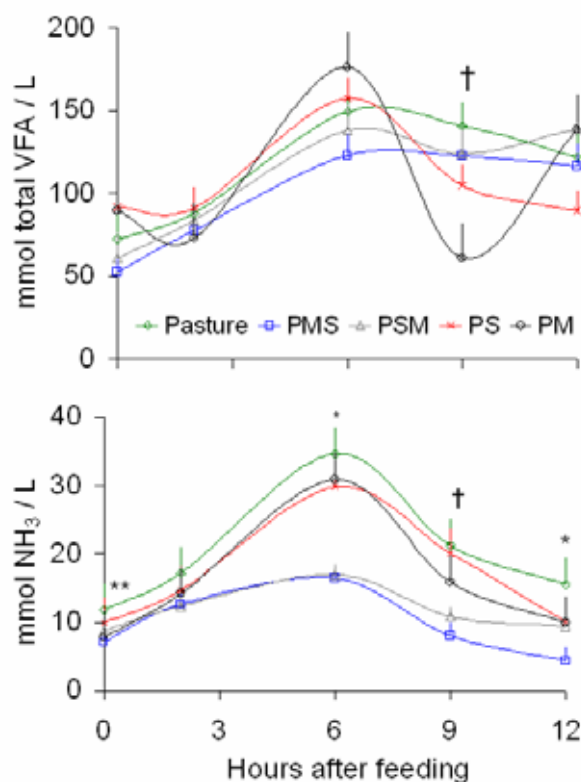


Figure 1. Pattern of rumen fluid a) ammonia and b) total VFA concentration between 07:00 h and 19:00 h, averaged from fistulated cows fed full pasture (Pasture) and four silages supplements [PMS (restricted pasture, RP+4 kg maize silage $\text{cow}^{-1} \text{d}^{-1}$ +2 kg sulla silage $\text{cow}^{-1} \text{d}^{-1}$); PSM (RP+4 kg sulla silage $\text{cow}^{-1} \text{d}^{-1}$ +2 kg maize silage $\text{cow}^{-1} \text{d}^{-1}$); PS (RP+6 kg sulla silage $\text{cow}^{-1} \text{d}^{-1}$); PM (RP+6 kg maize silage $\text{cow}^{-1} \text{d}^{-1}$)] on the first day of the *in sacco* incubation. † $p \leq 0.10$, * $p < 0.05$ and ** $p < 0.01$.

Fibre digestion

Although there were no differences in NDF concentration between the pasture/silage mixtures (Table 2) when they were incubated *in sacco* there were differences in rates of NDF and ADF digestion ($p < 0.05$; Table 3). Both NDF and ADF were present mainly in the slowly degradable fraction (B) and undegradable residues. Rates of fibre degradation were slowest for mixtures containing a high proportion of maize silage and tended to be most rapid for PS (Table 3).

The fibre effective degradability for pasture and pasture supplemented with 6 kg DM sulla silage $\text{cow}^{-1} \text{d}^{-1}$ were about 30% higher than other supplemented cows treatments (Table 3). Incubation of pasture showed that cow within pasture diet did not affect rates of fibre degradation when pasture was incubated *in sacco* ($p = 0.339$ for NDF and 0.167 for ADF).

Rumen pH, ammonia, and VFA concentrations

Rumen liquor pH showed higher values ($p < 0.001$) prior to AM feeding (mean 6.7 ± 0.1) compared to after the PM

milking (16:00 h) which averaged 5.6 ± 0.1 . There was no effect of diets on either morning or afternoon pH, or diurnal pattern (data not shown).

Mean concentrations of rumen VFA were similar across treatments, averaged 101 mmol/l, with a proportion of about 69% acetate, 17% propionate, and 11% butyrate. There was no treatment effect on concentration or molar proportion of VFA (Figure 1). The ratio of acetate:propionate averaged 4.1 and was similar for all diets. The diurnal variation in VFA concentrations showed peak concentrations about 6 hours after morning feeding (Figure 1). The diurnal range in total VFA concentrations was greatest with the PM diet and least with PMS. Dietary effects on the extent of diurnal variation was similar for acetate and n-butyrate but diets containing sulla appeared to have least diurnal variation in concentrations of minor VFA (data not shown). In contrast, the pasture diet resulted in highest concentrations of rumen ammonia and lowest values were measured when maize silage was included in the diet. Cows fed either pasture or pasture plus sulla silage had higher rumen NH_3 concentrations than other supplemented treatments ($p = 0.030$).

Ammonia concentrations followed a similar diurnal pattern as VFA but the variation was much smaller with PMS and PSM, than other diets. This can be explained by grazing behaviour because cows fed PMS and PSM chose to eat supplements first, followed by pasture while cows given PS or PM grazed pasture first and ate supplements after grazing.

DISCUSSION

The impact of silage supplementation has been examined in terms of production response and extent of pasture substitution by silage. Interpretation has been based on DM and nutrient intake and *in sacco* digestion kinetics. This trial was constrained by the high quality pasture on offer, which exceeded the quality of silage supplements in some instances.

The extent of production response will depend upon the amount of pasture available, which was probably insufficient here, and the nutritional value of the diet. Substitution of pasture by supplements can be an important consideration when optimising production against feeding costs and it is important to ask whether both the extent of substitution and the response to supplementation for several silages and mixtures can be addressed in a single trial. An initial requirement will be an accurate measurement of cow intakes.

Dry matter intake prediction

Estimation of cow intakes is difficult, whether on a group (Berchielli et al., 2000) or on an individual basis

using indigestible markers and faecal sampling. A brief evaluation of intakes measured in the current study and Woodward et al. (2002) has been made by comparison with predictions of DMI using the AAC equations for dairy cows (AAC, 1990). Inputs for the model are cow breed, days of pregnancy, LW and LW change, age, condition score, milk production and composition, energy content and digestibility of the feeds, and initial estimate of DMI. DMI predictions were highly correlated with actual values for DMI ($p < 0.001$; $r^2 = 0.61$) and showed that DMI was underestimated by $0.12 \text{ kg cow}^{-1} \text{ d}^{-1}$ across all diet treatments. The over prediction of intakes for cows given restricted pasture as a sole diet suggest an inability of the model to account for minimal residual DM when pasture allowance is low. When pasture on offer was only $18 \text{ kg DM cow}^{-1} \text{ d}^{-1}$ it would be impossible for cows to eat $15 \text{ kg DM cow}^{-1} \text{ d}^{-1}$. Model predicted DMI when pasture was offered at a moderate to high allowance or when grazing cows were offered silage supplements, were close to DMI measured in this experiment. This is reassuring and suggests the pre and post-grazing pasture cuts enabled an accurate estimate of group intakes. The allocation of very low quantities of pasture does not enable good model prediction and excessive feed restriction is inappropriate for maintenance of milk.

Pasture allowance and substitution

Similar cow responses to all silage supplements in this experiment suggests ME was more limiting than other nutrients and differences in silage composition were too small to affect milk production. The relation between intake and pasture allowance is generally curvilinear (Delagarde et al., 2001). Pasture intake increases with an increasing allowance until it reaches a plateau. Hodgson (1990) suggested that the herbage allowance should be two to three times the maximum daily herbage intake of the animals, but higher allowances result in pasture wastage associated with low utilization. A high allowance is synonymous with a low utilization, but high residual DM does not always equate to wastage. In fact allowances below about $40 \text{ kg DM cow}^{-1} \text{ d}^{-1}$ in spring are likely to limit intakes even though only 40% of available feed may be utilised (Matthews et al., 1999). High allowances will benefit cow health and production but stocking rate may be increased to manage pasture quality and prevent wastage (Brookes, 2003).

The acceptability of both supplements and pasture may provide an insight into the nutritional balance required by the cows, and suitability of silages for supplementing pasture. In this study the cows ate the silage mixtures (PMS and PSM) in preference to pasture and this resulted in lower 12 h fluctuations in rumen ammonia and VFA concentrations (Figure 1). Acceptability is likely to affect both DMI and the extent of substitution. Other factors

include quality and quantity of pasture and supplements, cow demand for nutrients and consequences of grazing close to the ground when pasture availability is restricted (Delagarde et al., 2001; Ribeiro Filho et al., 2005).

In a study where dairy cows were grazing on restricted pasture allowance and supplemented with grass silage, supplementation had a significant effect of increasing forage intake and maintenance of the milk fat percentages ($p > 0.05$) but did not increase milk production or gross energy efficiency (Sung and Okubo, 2001).

Rumen degradation *in sacco*

Understanding the impact of forages impact digestion, as well as the effect on intake potential has provided the base for prescriptive increased forage inclusion in animal diets (Bull, 2000).

When maize silage was added to pasture, the extent and rate of DM degradation declined, whereas the reverse was true for sulla. With highly productive cows, the rate and extent of DM degradation will affect performance and sulla silage may be more appropriate than maize silage, especially as CP degradation rate was reduced when sulla silage was incubated with pasture. Slower CP degradation is consistent with the presence of CT (Broderick and Albrecht, 1997; Burke et al., 2002).

Pasture incubation in cows given five diets allowed cow/diet effects to be measured, and different diets affected degradability of pasture DM and fibre (NDF and ADF). There appeared to be less DM and fibre degradation when pasture was incubated in cows offered diets including maize silage but addition of sulla to cow diets increased effective degradability. Effects of diet on CP degradation were less apparent. Impact of diet on degradation rates has been observed by Mertens and Waghorn (unpublished) who observed that diets based on maize silage reduced rates of both maize and lucerne degradation relative to diets based on lucerne. Results reported here support this observation and show that the effects of diet on effective rumen degradation were greater with higher intakes and/or high outflow rates (Table 3). The effect of diet on *in sacco* degradation requires more investigation.

Why did this study use different ways to assess dietary protein? Metabolizable protein is an important component of diet quality and is the sum of digestible microbial true protein and digestible undegraded feed protein (AFRC, 1992). Digestible microbial true protein is calculated from the microbial CP supply which is, in turn, calculated from the total fermentable energy in the diet, providing there is sufficient effective rumen degradability protein (ERDP) for unrestricted microbial growth. The degradation coefficients (A, B, and k) of feed proteins incubated in dacron bags in the rumen are required for the determination of ERDP.

Clark et al. (1997) suggest there is limited

understanding of the nutritional constraints to dairy production from summer pastures, but effects of grass maturation and pasture supply can limit MP for milk production. There is little information on rumen degradability of forage CP (Barrell et al., 2000; Burke et al., 2000; Chaves, 2003) so several approaches (AFRC, 1992; NRC, 2001) were used to evaluate this problem. Proportion of RDP and RUP differed across diet treatments (Table 4) and estimates of ERDP showed strong positive relationship with dietary CP content.

Crude protein degradation characteristics reported here are in agreement with an Australian study of perennial pastures through the year (Wales et al., 1999). These authors showed that summer pasture in Victoria, Australia, provided a surplus of MP of 0.14 to 0.23 kg cow⁻¹ d⁻¹ compared to 0.48 to 1.21 kg cow⁻¹ d⁻¹ in spring and suggested MP was unlikely to limit milk production of cows eating 17 kg pasture DM d⁻¹ and producing up to 30 kg milk d⁻¹. These data support the argument that the good quality pasture available for both trials presented here resulted in insufficient energy rather than protein for cows producing 1.0-1.2 kg milksolids d⁻¹.

Rumen digesta and cow feeding behaviour

Diets did not affect concentration or molar proportions of VFA. The 84% increase in dietary NSC concentration when maize silage was added to pasture did not affect either propionate concentration or acetate:propionate ratio. Although there were no effects of diet on rumen VFA, diets with pasture, maize and sulla silages (PMS and PSM treatments) provided a stable rumen environment possibly due to feeding behaviour. The lower variation over the 12 h period from 07:00-19:00 h for VFA was also evident for NH₃ (Figure 1), probably because the cows given these treatments ate supplements first (PMS and PSM) and then grazed pasture. Cows grazed pasture first with PM and PS diets. The PMS treatment was the most acceptable diet for the cows, with least refusals and provided the highest NSC dietary concentration. Visual observations suggest the cows preferred mixtures of sulla and maize silages compared either silage fed alone, but the short duration of the trial and restricted pasture availability prevented further evaluation of silage effects on total DMI or performance.

CNCPS diet evaluation

The CNCPS model was used to evaluate dietary mixtures of maize and sulla silages with pasture to identify limitations to performance and predict rumen degradation parameters.

The model provided useful information concerning microbial growth, rumen passage rates, nutrient limitations and predictions of performance of cattle supplemented with

single silages (Chaves and Kolver, 2004). Silages mixtures were fed to provide a more balanced nutrient supply to dairy cows in this experiment and ruminal measurements indicated some effects of diet on fermentation. The CNCPS model has been used to further evaluate these diets and to identify sources of variation in cow performance. This information will enable improved dietary formulations for grazing cows given supplements. Feed composition collected in this experiment and degradation rates from CNCPS feed library for pasture diets were used to evaluate pasture diets with the Cornell model (CNCPS).

Metabolizable energy was the first limiting nutrient for cows fed pasture alone and metabolizable protein (MP) was the first limiting nutrient when cows were fed pasture plus silages. At least 50% of the total MP was of microbial origin for all diets, with highest percentage for the maize silage treatment (PM: 68% of MP of microbial origin; 886 g d⁻¹). The recommendation for ruminal N balance is 100 to 110% of requirements (Fox et al., 2003) and this was easily achieved from all diets (123 to 207% of requirement).

Development of rations that will improve efficiency of nutrient use and reduce nutrient wastage on farm are requirements for economic and environment sustainability. Provision of silage mixtures attempted to balance nutrient supply with demand and the CNCPS simulations predicted nutrient requirements, balances, excretion of N and urea cost for each treatment group. Higher concentration of CP in pasture resulted in high cost of excreting excess N (2.9% and 3.3% of ME intake for FP and RP, respectively). This represents an increased cow maintenance cost, associated with removal of excess nitrogen, and the high predicted MP from undegraded feed (652 g d⁻¹) with the FP diet was a association with the highest N excretion (204 g d⁻¹). This will have a negative impact on the environment in terms of nitrogen leaching and generation of nitrous oxides (Carran, 2002) relative to diets containing maize and sulla silages.

Although CNCPS provided useful information concerning nitrogen fluxes with similar predictions of total digestible intake protein (DIP) to effective CP degradability measured *in sacco*, the predictions of mean rumen pH differed from observed values (not presented).

Future progress to maximise milk production from cows grazing pasture and supplemented with silages should investigate the ability of diets to alter rate of fermentation and be nutritionally balanced. Studies should also test both low and high pasture allowances to obtain information on choice of nutrient from pasture versus the supplement. This information will provide a better understanding of substitution than can be obtained with a single pasture allowance and a focus on choice and cow behaviour may provide a better understanding of rumen stability and cow performance.

CONCLUSION

Dairy farmers face the task of maintaining a desired level of production often when the quality of pasture is less than optimal. As pasture is likely to provide the cheapest source of nutrients, it is important to maximise feed intake at minimal cost. Silage supplements can be used to fill summer feed deficits when pasture quality declines due to maturation of ryegrass. To achieve positive responses from supplements, supplements should be of higher nutritive value than pasture and the supplements must be chosen to complement the pasture on offer. The differences between digestion kinetics of maize and sulla silage supplements demonstrate the importance of selecting an appropriate supplement to complement the pasture on offer.

The hypothesis was not proven, but the low pasture allowance may have prevented a response due to insufficient ME intake. Dietary mixtures did reduce diurnal variation in rumen fermentation parameters, without affecting changes in milk or milksolid production.

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