The effect of nitrogen fertilization level and protein supplementation on herbage intake, feeding behaviour and digestion in grazing dairy cows

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Abstract

An experiment is described to study the influence of nitrogen (N) fertilization and protein supplementation on the nutrition of grazing dairy cows. Two levels of N fertilization (0 and 60 kg N per ha per regrowth) and two levels of soyabean meal supplementation (0 and 2 kg day⁻¹) were factorially combined and compared in a 4 × 4 Latin square design using periods of 11 days. Eight fistulated Holstein cows were strip grazed on perennial ryegrass pastures at a constant daily herbage allowance — measured above 5 cm from ground level — of 20 kg OM cow⁻¹. The individual herbage OM intake was calculated using chromic oxide and faecal N and ADF contents. Grazing behaviour, duodenal digesta flow and ruminal fermentation patterns were also measured.

No interaction was found between fertilization and supplementation levels. Herbage OM intake was greatly depressed in unfertilized swards (−2.0 kg OM day⁻¹). This could be attributed to a decrease of the rate of intake due to the lower green leaf mass (1.6 vs. 2.3 t OM ha⁻¹). Herbage OM digestibility, non-ammoniacal N (NAN) flow into the duodenum and ruminal fermentations were reduced on unfertilized swards. However, the sites of OM digestion and NAN flow expressed g per kg digestible OM intake were similar at both fertilization levels. Cows receiving protein supplementation showed similar herbage intake, but higher total OM intake and NAN flow than unsupplemented cows. The low level of concentrate fed, and perhaps also its composition, may explain the additivity between herbage and concentrate. Treatments had no effect on total grazing time nor on mean rate of biting. © 1997 Elsevier Science B.V.

Keywords: Dairy cows; Grazing; Nitrogen; Fertilization; Protein supplementation; Intake; Grazing behaviour; Digestion; Perennial ryegrass

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1. Introduction

Current economic and environmental concerns require the control of nitrogen (N) inputs in intensive grassland systems in order to maximize N use efficiency and to reduce harmful N losses. Before recommending a decrease of N inputs at grazing, it is necessary to assess correctly the nutritional consequences on dairy cattle.

The effects of reducing N fertilization on grass productivity and quality are well established. Lowering N fertilization reduces herbage growth rate, tiller density and height and finally herbage mass for a given age of growth (Wilman, 1980). These agronomical changes are associated with a sharp decrease in herbage N content. Reducing N fertilization only has a small effect on OM digestibility when grass is harvested at the same age of growth. On average, digestibility decreases by 2 to 3 points (Demarquilly, 1977; Van Vuuren et al., 1992; Peyraud et al., 1996a), mainly because any decrease of the N content of herbage is compensated for by an increase in soluble carbohydrates which are highly digestible (Demarquilly, 1977).

Reducing N fertilization level has no effect on the quantity of grass voluntarily ingested by stall fed sheep and cattle (Demarquilly, 1977; Holmes and Lang, 1963; Peyraud et al., 1996a), but it could bring about a decrease of intake at grazing through the influence of the sward structure upon herbage intake (Allden and Whittaker, 1970; Jamieson and Hodgson, 1979a). However, this aspect is not yet quantified because published studies were carried out using high doses of fertilizer (Gordon, 1973) or small difference in herbage mass between the contrasting levels of N fertilization (Combellas and Hodgson, 1979; Peyraud et al., 1994).

Moreover, for dairy cows grazing on low N grass, protein supply may limit milk yield as observed in the experiments of Delaby et al. (1996). These authors showed that an iso-energetic replacement of cereals by slow degradable protein is more efficient on low than on high fertilized swards. Therefore, to maintain individual animal performances, a simple solution may be to adjust the protein content of the concentrate to the N fertilization level. However, the potential benefit of protected protein supplementation according to the level of N fertilization on herbage intake and nutrient supply in grazing dairy cows is not well documented and require further investigations.

The aim of the present study is to investigate the effect of N fertilization level on perennial ryegrass swards in interaction with protein supplementation on herbage intake and digestion in grazing dairy cows.

2. Materials and methods

2.1. Experimental treatments and design

Two levels of N fertilization and two levels of protein concentrate supplementation were compared using a $2 \times 2$ factorial arrangement of treatments. Nitrogen fertilization levels were 0 (designated as Low: L) and 60 (High: H) kg N ha$^{-1}$ per regrowth after a first application of 40 (L) and 80 (H) kg N ha$^{-1}$ on 19 March. Daily supplement levels were 0 (Unsupplemented: U) and 2 (Supplemented: S) kg concentrate per cow. Concentrate comprised 50% soyabean meal and 50% formaldehyde-treated soyabean meal on a fresh weight basis. Therefore, the four treatments were LU, LS, HU and HS.
The experimental design was a balanced 4 × 4 Latin square with experimental periods of 11 days. Animal measurements occurred during the last 6 days of each period. The experiment was carried out in May and June 1993.

2.2. Animals and pastures

Eight Holstein multiparous dairy cows were used, each fitted with a large rumen cannula (internal diameter 123 mm) and a T-shaped duodenal cannula. Before the experiment, the cows were grazed together day and night for 2 weeks on a perennial ryegrass pasture and were supplemented daily with 2 kg of a commercial concentrate (18% crude protein). Afterwards, they were paired off according to their pre-experimental milk production (25.0 ± 2.7 kg FCM), live weight (635 ± 59 kg) and lactation stage (156 ± 46 days). Each pair was assigned to a treatment sequence.

The experiment took place at Mésusseaume near Rennes (Brittany, France) on 3 to 5-year-old pure perennial ryegrass (Lolium perenne L.) pastures. Four paddocks (one per period) of approximately 1 ha each were used, sown with cultivar Belfort (Periods 1 to 3) and Vigor (Period 4). During the previous 2 years, the paddocks had been longitudinally divided and each sub-paddock had received one of the two experimental fertilizer treatments. There were also strip-grazed by the herd of the experimental farm. In spring 1993, prior to the experiment, the paddocks were not grazed. They were cut two to three times, so that the final cut in each paddock allowed a herbage regrowth of 32 days during the animal measurement period. Nitrogen fertilizer was applied immediately after each cut until the experiment.

2.3. Feeding and grazing management

During the experiment, cows were strip-grazed in two groups (L and H pastures), with members being changed between periods according to the Latin square design. Each cow was offered 20 kg OM daily, measured from 5 cm above ground level. A new area of pasture was given once daily after the morning milking. Back-grazing was prevented by electric fencing, while the area available to the cows corresponded to 2 days of grazing (additional walking and lying area). Daily areas to be offered to cows were determined three times per period by motorscythe cutting (see under), but also adjusted daily to compensate for herbage growth. Water and mineral blocks were always available.

Time spent out of the pasture was on average 3 h per day (milking), and total walking distance between pastures and milking parlour was 1.0 to 1.5 km per day. Experimental concentrate was given individually to cows in two equal amounts per day at milking times (07.00 h and 17.00 h). Refusals (only 4 days for one cow in Period 3) were placed directly into the rumen through the cannula. Concentrate was sampled once weekly throughout the experiment for chemical analysis.

2.4. Herbage measurements

For each period, measurements were made of the pre-grazing herbage mass up to 5 cm and to ground level, as well as the chemical composition of the herbage offered and selected, the morphological composition of the grass and the extended sward height.
The pre-grazing herbage mass was measured on Days 1, 5 and 9. At each time, three strips of $0.5 \times 5$ m were cut by motorscythe on L and H swards and the fresh weight was recorded before sampling for DM determination and subsequent analysis. Residual stubble mass after cutting was measured on each strip with a quadra (30 $\times$ 30 cm) using hand shears. Total herbage mass to ground level was calculated as the sum of amounts measured above and under the motorscythe cutting height.

The morphological composition of the herbage offered and the chemical composition of the herbage selected by the cows were measured on Days 5 and 9. Ten herbage handfuls in L and H swards were cut to ground level with hand shears, bulked and arranged correctly in a bag to keep the sward structure undamaged and then immediately frozen. Green leaf proportion in the total DM herbage mass was determined by manual separation from a first subsample prior to drying. The second subsample, with its original structure still preserved, was cut at a height corresponding to the mean post-grazing sward height (see below). The upper portion, considered as representative of the selected herbage, was dried before chemical analysis.

The mean sward height was measured before and after grazing, on areas grazed on Days 6, 8 and 9. At each time, a hundred tillers were taken at random and the extended height to ground level of the longest leaf and stem were measured.

2.5. Animal measurements

Individual herbage OM intake was determined by estimating faecal output and organic matter digestibility (OMD) of the herbage selected. Total faecal output was estimated by the dilution of chromic oxide ($\text{Cr}_2\text{O}_3$) in the faeces, assuming 100% recovery. The digestibility of herbage OM was estimated for the unsupplemented cows from faecal N and ADF contents as faecal indicators. The equation used had been established previously using herbage-based diets without supplements at the same experimental site (Comerón and Peyraud, 1993):

$$\text{OMD (g kg}^{-1}\text{OM)} = 0.791 + 0.0334 \text{FN} - 0.0038 \text{FADF}$$

$$(n = 24; R = 0.89; \text{RSD} = 0.013)$$

where FN is the faecal N content (g kg$^{-1}$ OM) and FADF is the faecal ADF content (g kg$^{-1}$ OM).

Each day, 18 g chromic oxide ($\text{Cr}_2\text{O}_3$) were introduced directly into the rumen. In practice, cows received at each milking time 200 g of a pelleted concentrate comprising 5% $\text{Cr}_2\text{O}_3$, wheat bran 40%, maize 25%, barley 15%, soyabean meal 11%, and molasses, 4%. Coloured plastic particles (80 g day$^{-1}$) for dung pat identification were given at the same time in the rumen. Each morning, from Day 8 to Day 12 (5 days collection), all dung pats were sampled at pasture and after milking in the parlour, bulked per cow and then dried for chemical analysis.

For herbage intake calculations, faecal OM output from herbage was estimated by subtracting indigestible OM attributable to concentrates (182 and 100 g kg$^{-1}$ OM for chromic oxide and experimental concentrates, respectively; INRA, 1989) from total measured faecal OM output. The herbage OMD measured on unsupplemented cows was also applied to supplemented cows assuming no digestive interaction between herbage
and concentrate. Nitrogen intake from herbage was calculated as the product of herbage OM intake and N content of the selected herbage.

Grazing and rumination activities were recorded automatically from jaw movements with the portable electronic device initially described by Brun et al. (1984). Records were repeated from Day 6 to Day 11 until at least two or three complete records per cow were obtained. Biting rate was measured by visual observation one day at the end of each period. Cows were individually observed for 2 min every 15 min in the morning from 08.00 h to 11.00 h and in the evening from 18.00 h to 22.00 h (i.e. main periods of grazing activity). Total bites per day was calculated as the product of grazing time and biting rate. Rate of intake and bite size were estimated indirectly by dividing daily herbage OM intake by grazing time and total bites per day, respectively.

Duodenal flow rates were measured using chromic oxide and polyethylene-glycol (PEG) as markers. Throughout the experiment, polyethylene-glycol was introduced into the rumen twice daily (200 g day$^{-1}$) during milking times as chromic oxide concentrate. For each cow, a total of eight samples (400 ml each) of duodenal contents were manually collected between Day 6 and Day 9 at 00.00, 04.30, 07.30, 10.30, 13.30, 17.30, 19.30 and 21.30 h (400 ml per sample). A first subsample of 50 ml was stored at +4°C and bulked according to cow and period until PEG analysis. The remaining subsample (350 ml) was bulked per cow, frozen at −16°C and subsequently freeze-dried for analysis. Duodenal flow was calculated by dividing daily quantities of Cr$_2$O$_3$ delivered and amount of PEG recovered in faeces (assuming total recovery for Cr$_2$O$_3$) by their respective concentrations in duodenal digesta. The mean value of the two estimates is presented here, according to Mambrini and Peyraud (1994).

Ammonia (NH$_3$), pH and volatile fatty acids (VFA) in the rumen were measured on Day 8. Ruminal fluid was sampled seven times at the following hours: 07.30, 09.30, 11.30, 13.30, 15.30, 17.30 and 21.30 h. At each time, a 50 ml sample was taken and the pH immediately measured. After straining, two subsamples were frozen for NH$_3$ and VFA analysis according to the procedure described by Mambrini and Peyraud (1994). Ruminal cellulolytic activity was estimated on Day 9 from in sacco DM disappearance of soyabean hulls (620 g NDF kg$^{-1}$ DM) after 24 h incubation in the rumen in duplicated nylon bags (6 × 11 cm, pore size 0.046 mm, 3 g of sample).

Individual milk production was measured each day. Milk fat and protein contents were determined each week on eight consecutive milkings. Cows were also weighed on the last day of each period.

2.6. Chemical analysis

Dry matter content was measured by drying 48 h at 80°C (herbage, concentrate and faeces samples) or after freeze-drying (duodenal samples). All samples were ground to a 0.8 mm screen before analysis. Organic matter, N, NDF and ADF in feedstuffs, faeces and duodenal samples, NH$_3$ and VFA in ruminal fluid, and PEG in faeces and duodenal samples were analyzed as described by Mambrini and Peyraud (1994). Chromic oxide was determined by the method of Mathieson and Davidson (1970) as modified for an auto-analyzer (Technicon) by Poncet and Rayssiguier (1980). The pepsin-cellulase DM digestibility of the herbage was determined according to Aufrère and Demarquilly (1989).
2.7. Statistical analysis

All animal data were analysed as a 4 × 4 Latin square design using the GLM procedure of Statistical Analysis Systems Institute Inc. (1987) according to the following model:

\[ Y_{ijkl} = m + C_i + P_j + F_k + S_l + (FS)_{kl} + e_{ijkl} \]

where \( m \) is mean; \( C_i \) is cow effect \((i = 1...8)\); \( P_j \) is period effect \((j = 1...4)\); \( F_k \) is fertilization effect \((k = 1,2)\); \( S_l \) is supplementation effect \((l = 1,2)\); \( (FS)_{kl} \) is interaction fertilization × supplementation effect; \( e_{ijkl} \) is residual term.

Mean milk data from Days 4 to 10 were retained for statistical analysis. The same procedure was used for herbage data, but the corresponding model included only mean values, period effect, fertilization effect and residual term.

3. Results

The experiment was carried out satisfactorily and all animal data are included in the statistical analysis. Total rainfall (164 mm) and mean temperature (15.1°C) over the 2 months of the experiment were close to seasonally normal climatic conditions. No significant interaction between fertilization and supplementation levels can be detected in animal measurements. Therefore, the effects of the two factors are presented separately here.

3.1. Sward measurements

Herbage mass above 5 cm, green leaf mass and extended tiller height were greatly reduced in unfertilized swards (Table 1) but the herbage mass to ground level and stem height were not affected by the level of fertilization. Lowering N fertilization decreased crude protein content and OMD estimated from pepsin-cellulase method of the grass, but not cell wall content.

Cows on unfertilized swards were given on average 48% more area than cows on fertilized swards. Herbage allowance above 5 cm (20.7 kg OM per day) and allowance of green leaves (18.0 kg OM per day) were similar for both fertilization levels, but the cows were given much more herbage as measured to ground level on unfertilized than on fertilized swards (Table 2). Post-grazing sward height tended to be lower \((P = 0.09)\) on unfertilized swards. On average, for both types of sward, crude protein content \((+34 \text{ g kg}^{-1} \text{ DM})\) and OMD estimated from pepsin-cellulase method \((+0.041)\) were higher on selected herbage (upper strata) than on herbage offered, but differences in chemical composition between fertilization levels were similar to those observed in herbage offered (Tables 1 and 2). The crude protein content of the selected herbage ranged between periods from 121 to 154 g kg\(^{-1}\) DM on unfertilized swards and from 200 to 222 g kg\(^{-1}\) DM on fertilized swards.

3.2. Fertilization

The OM digestibility of the herbage selected by the cows, estimated from faecal composition, was 0.777 and 0.809 on average for the low and high fertilization levels,
Table 1
Herbage mass, sward height and chemical composition of the herbage offered at the two levels of nitrogen fertilization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sward treatments</th>
<th>SED</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low fertilization</td>
<td>High fertilization</td>
<td></td>
</tr>
<tr>
<td>Herbage mass (t OM ha⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to ground level</td>
<td>5.0</td>
<td>5.0</td>
<td>0.08</td>
</tr>
<tr>
<td>above 5 cm</td>
<td>1.9</td>
<td>2.6</td>
<td>0.13</td>
</tr>
<tr>
<td>as green leaves</td>
<td>1.6</td>
<td>2.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Pre-grazing tiller height (mm)</td>
<td>281</td>
<td>348</td>
<td>10.0</td>
</tr>
<tr>
<td>Pre-grazing stem height (mm)</td>
<td>120</td>
<td>129</td>
<td>6.4</td>
</tr>
<tr>
<td>Analyses (g kg⁻¹ DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter (g kg⁻¹ fresh weight)</td>
<td>217</td>
<td>162</td>
<td>11.0</td>
</tr>
<tr>
<td>Organic matter</td>
<td>909</td>
<td>899</td>
<td>0.6</td>
</tr>
<tr>
<td>Crude protein</td>
<td>106</td>
<td>173</td>
<td>10.2</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>505</td>
<td>504</td>
<td>7.4</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>246</td>
<td>246</td>
<td>4.6</td>
</tr>
<tr>
<td>OM digestibility</td>
<td>0.752</td>
<td>0.781</td>
<td>0.0063</td>
</tr>
</tbody>
</table>

* In this and subsequent tables: SED, standard error of the difference; NS, not significant; * P < 0.05; * * P < 0.01; * * * P < 0.001. Above 5 cm. Calculated from pepsin-cellulase digestibility (Aufère and Demarquilly, 1989).

respectively (P < 0.05). Nitrogen fertilization level had no effect on total faecal OM output (Table 3). Herbage OM intake was lower on unfertilized than on fertilized swards (-2.0 kg, P < 0.001). Total grazing time was not affected by the level of fertilization, but cows on unfertilized swards grazed for a shorter time after afternoon milking than

Table 2
Grazing management and chemical composition of the herbage apparently ingested by cows at the two levels of nitrogen fertilization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sward treatments</th>
<th>SED</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low fertilization</td>
<td>High fertilization</td>
<td></td>
</tr>
<tr>
<td>Offered area (m² per cow per day)</td>
<td>114</td>
<td>77</td>
<td>5.7</td>
</tr>
<tr>
<td>Herbage allowance (kg OM per cow per day)</td>
<td>21.4</td>
<td>19.9</td>
<td>1.06</td>
</tr>
<tr>
<td>Post-grazing tiller height (mm)</td>
<td>135</td>
<td>151</td>
<td>6.2</td>
</tr>
<tr>
<td>Post-grazing stem height (mm)</td>
<td>98</td>
<td>109</td>
<td>4.4</td>
</tr>
<tr>
<td>Analyses (g kg⁻¹ DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic matter</td>
<td>911</td>
<td>907</td>
<td>3.7</td>
</tr>
<tr>
<td>Crude protein</td>
<td>135</td>
<td>212</td>
<td>7.2</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>509</td>
<td>526</td>
<td>16.0</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>240</td>
<td>232</td>
<td>11.4</td>
</tr>
<tr>
<td>OM digestibility</td>
<td>0.798</td>
<td>0.818</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

* Above 5 cm. Strata upper to post-grazing tiller height. Calculated from pepsin-cellulase digestibility (Aufère and Demarquilly, 1989).
Table 3

Effect of nitrogen fertilization level and protein supplementation on faecal output, herbage intake, grazing and rumination behaviour in grazing dairy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>SED</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LU</td>
<td>LS</td>
<td>HU</td>
</tr>
<tr>
<td>Total faecal OM output (kg day⁻¹)</td>
<td>2.87</td>
<td>3.22</td>
<td>2.97</td>
</tr>
<tr>
<td>Herbage OM intake (kg day⁻¹)</td>
<td>12.7</td>
<td>13.5</td>
<td>15.3</td>
</tr>
<tr>
<td>Total OM intake (kg day⁻¹)</td>
<td>13.0</td>
<td>15.5</td>
<td>15.6</td>
</tr>
<tr>
<td>Total DOM intake (kg day⁻¹)</td>
<td>10.1</td>
<td>12.3</td>
<td>12.6</td>
</tr>
<tr>
<td>Total grazing time (min day⁻¹)</td>
<td>510</td>
<td>519</td>
<td>546</td>
</tr>
<tr>
<td>Evening grazing time (min day⁻¹)</td>
<td>235</td>
<td>238</td>
<td>257</td>
</tr>
<tr>
<td>Rate of intake (g OM min⁻¹)</td>
<td>25.1</td>
<td>26.7</td>
<td>28.3</td>
</tr>
<tr>
<td>Rate of biting (bites min⁻¹)</td>
<td>54</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>Total bites (×1000 day⁻¹)</td>
<td>27.3</td>
<td>29.1</td>
<td>29.0</td>
</tr>
<tr>
<td>Estimated bite size (mg OM per bite)</td>
<td>469</td>
<td>482</td>
<td>534</td>
</tr>
<tr>
<td>Ruminating time (min day⁻¹)</td>
<td>515</td>
<td>477</td>
<td>455</td>
</tr>
<tr>
<td>Total chewing time (min day⁻¹)</td>
<td>1025</td>
<td>995</td>
<td>1001</td>
</tr>
</tbody>
</table>

* Treatments: LU, low fertilization and cows unsupplemented; LS, low fertilization and cows supplemented; HU, high fertilization and cows unsupplemented; HS, high fertilization and cows supplemented. In this and subsequent tables: Fert, fertilization effect; Suppl, supplementation effect; Interaction, fert × suppl interaction. Sum of digestible OM from herbage and concentrates. Grazing time from afternoon milking (18.00 h) to next morning milking (07.00 h).

Cows on fertilized swards (P < 0.05) (Table 3). Mean rate of biting and total bites per day were unaffected. Thus, the rate of intake and the estimated bite size were consistently lower on unfertilized swards. Cows on unfertilized swards ruminated on average 44 min more than cows on fertilized swards (P < 0.05). Total chewing time was unaffected by the fertilization level.

Organic matter entering the duodenum was lower (P < 0.05) on unfertilized than on fertilized swards. However, the level of fertilization had no effect on the proportion of digestible OM apparently digested in the rumen (Table 4). The quantity of non ammoniacal nitrogen (NAN) entering the duodenum was lower (P < 0.01) on unfertilized than on fertilized swards, but the NAN flow expressed in g per kg digestible OM intake was unaffected by level of fertilization (Table 4). Even though NAN flow was lower than N intake for cows on fertilized swards, it was higher than N intake for cows on unfertilized swards, indicating a net gain of N between the diet and the duodenum. When cows grazed on unfertilized swards, ruminal ammonia concentration was greatly reduced (P < 0.001), pH (P < 0.05) and total VFA (P < 0.07) also decreased. There were a slight but significant increase in butyrate (P < 0.001) and a decrease of the proportion of isocids (P < 0.001), but no effect on the molar proportions of acetate and propionate (Table 5). Ruminal cellulolytic activity was greatly depressed on unfertilized swards (P < 0.01).

On average, cows grazed on unfertilized swards produced 2.0 kg milk less than cows on fertilized swards (P < 0.001), without any change in milk fat content, but showing a
Table 4
Effect of nitrogen fertilization level and protein supplementation on OM and N digestion in grazing dairy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments a</th>
<th>SED</th>
<th>Significance b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LU  LS  HU  HS</td>
<td>Fert Suppl Interaction</td>
<td></td>
</tr>
<tr>
<td>Organic matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>entering the duodenum (kg day⁻¹)</td>
<td>6.1  7.2  7.0  7.7</td>
<td>0.42</td>
<td>*  **  NS</td>
</tr>
<tr>
<td>apparent total digestibility  b</td>
<td>0.778 0.791 0.809 0.818</td>
<td>0.0037</td>
<td>***  ***  NS</td>
</tr>
<tr>
<td>apparent ruminal digestion (%DOMI) c</td>
<td>68.0 66.9 67.7 66.3</td>
<td>2.61</td>
<td>NS  NS  NS</td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total intake (g day⁻¹) d</td>
<td>309 466 584 706</td>
<td>20.8</td>
<td>***  ***  NS</td>
</tr>
<tr>
<td>apparent total digestibility</td>
<td>0.678 0.726 0.795 0.807</td>
<td>0.0092</td>
<td>***  ***  NS</td>
</tr>
<tr>
<td>NAN entering the duodenum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g day⁻¹</td>
<td>394 518 472 570</td>
<td>30.0</td>
<td>**  ***  NS</td>
</tr>
<tr>
<td>g per kg DOMI  c</td>
<td>39.0 42.7 37.6 41.5</td>
<td>1.96</td>
<td>NS  *  NS</td>
</tr>
<tr>
<td>g per g N intake</td>
<td>1.29 1.13 0.81 0.81</td>
<td>0.052</td>
<td>***  *  *</td>
</tr>
</tbody>
</table>

a Treatments: LU, low fertilization and cows unsupplemented; LS, low fertilization and cows supplemented; HU, high fertilization and cows unsupplemented; HS, high fertilization and cows supplemented. b Total digestible OM intake/total OM intake. c DOMI, total digestible OM intake. d Total N intake calculated from N content of the selected herbage.

significant decrease of milk protein content (P < 0.05) (Table 6). Live weight was unaffected by the fertilization level.

3.3. Supplementation

Supplemented cows showed higher total faecal OM output, but similar herbage OM intake compared with unsupplemented cows (Table 3). Feeding concentrate numerically

Table 5
Effect of nitrogen fertilization level and protein supplementation on daily mean ruminal fermentation parameters and cellulolytic activity of the ruminal fluid in grazing dairy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments a</th>
<th>SED</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LU  LS  HU  HS</td>
<td>Fert Suppl Interaction</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.15 6.01 6.26 6.13</td>
<td>0.068</td>
<td>*  NS  *</td>
</tr>
<tr>
<td>NH3 concentration (mmol l⁻¹)</td>
<td>2.7 5.0 10.4 12.7</td>
<td>0.49</td>
<td>***  *  NS</td>
</tr>
<tr>
<td>VFA concentration (mmol l⁻¹)</td>
<td>101 111 106 117</td>
<td>3.7 0.07</td>
<td>***  NS</td>
</tr>
<tr>
<td>acetate (%)</td>
<td>63.4 63.7 63.6 62.8</td>
<td>0.76</td>
<td>NS  NS  NS</td>
</tr>
<tr>
<td>propionate (%)</td>
<td>21.0 20.4 20.8 21.2</td>
<td>0.54</td>
<td>NS  NS  NS</td>
</tr>
<tr>
<td>butyrate (%)</td>
<td>12.6 13.0 11.5 11.9</td>
<td>0.38</td>
<td>***  NS  NS</td>
</tr>
<tr>
<td>isoacids b (%)</td>
<td>1.7 1.7 2.5 2.5</td>
<td>0.08</td>
<td>***  NS  NS</td>
</tr>
<tr>
<td>Cellulolytic activity  c</td>
<td>59.9 62.5 69.6 64.5</td>
<td>2.55</td>
<td>*  NS  *</td>
</tr>
</tbody>
</table>

a Treatments: LU, low fertilization and cows unsupplemented; LS, low fertilization and cows supplemented; HU, high fertilization and cows unsupplemented; HS, high fertilization and cows supplemented. b Isoacids: isobutyrate + isovalerate. c Disappearance of soyabean hulls (% DM) after 24 h of in sacco incubation in the rumen.
Table 6
Effect of nitrogen fertilization level and protein supplementation on milk production, milk composition and live weight in grazing dairy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments a</th>
<th>SED</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LU</td>
<td>LS</td>
<td>HU</td>
</tr>
<tr>
<td>Milk production (kg day⁻¹)</td>
<td>22.7</td>
<td>25.3</td>
<td>25.0</td>
</tr>
<tr>
<td>Milk fat production (g day⁻¹)</td>
<td>778</td>
<td>883</td>
<td>884</td>
</tr>
<tr>
<td>Milk protein production (g day⁻¹)</td>
<td>619</td>
<td>717</td>
<td>702</td>
</tr>
<tr>
<td>FCM production (kg day⁻¹)</td>
<td>20.8</td>
<td>23.4</td>
<td>23.2</td>
</tr>
<tr>
<td>Milk fat content (g kg⁻¹)</td>
<td>34.3</td>
<td>35.0</td>
<td>35.3</td>
</tr>
<tr>
<td>Milk protein content (g kg⁻¹)</td>
<td>27.4</td>
<td>28.4</td>
<td>28.2</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>635</td>
<td>643</td>
<td>647</td>
</tr>
</tbody>
</table>

a Treatments: LU, low fertilization and cows unsupplemented; LS, low fertilization and cows supplemented; HU, high fertilization and cows unsupplemented; HS, high fertilization and cows supplemented.

increased herbage OM intake on unfertilized swards (+ 0.8 kg OM) but not on fertilized swards (− 0.4 kg OM), but the interaction was not significant (P = 0.19). Finally, total OM intake was significantly increased when cows were supplemented (P < 0.001). Grazing and rumination time were not affected by concentrate feeding, as well as mean rate of biting (Table 3).

Feeding concentrate increased daily OM duodenal flow (P < 0.001) but had no effect on apparent ruminal digestion of OM (Table 4). The NAN flow increased by 111 g per day on average when cows were supplemented (Table 4). Soyabean meal supplementation significantly decreased ruminal pH, increased total VFA and NH3 concentrations in the rumen, while it had no effect on the molar proportion of VFA (Table 5). Supplementation reduced ruminal cellulolytic activity for cows on fertilized swards, but tended to increase it for cows on unfertilized swards (interaction fertilization X protein supplementation: P < 0.05).

Cows produced 2.6 kg and 1.8 kg FCM more when they were supplemented on low and high fertilization levels, respectively (Table 6). Supplementation increased (P < 0.01) milk protein content but had no effect on milk fat content. Supplementation had no effect on live weight.

4. Discussion

4.1. Grazing management

In order to accurately compare fertilization levels, it was planned in the experimental design that the herbage allowance should be the same at the two levels of fertilization, as herbage allowance is well known to be a major factor regulating herbage intake at grazing (Le Du et al., 1979; Peyraud et al., 1996b). Such an objective was achieved by increasing the offered area on unfertilized swards in proportion to the difference between herbage mass measured on the two types of swards. Cows received an average
of 20 kg OM per day above 5 cm at both fertilization levels. Therefore, in the present study, the variations in herbage intake between different treatments are not linked to variations in the herbage allowance, but only to variations in sward structure and/or quality. Moreover, a daily herbage allowance of 20 kg OM per cow would be theoretically not limiting for intake according to the results of Peyraud et al. (1996b) established on well fertilized pastures with favourable sward structure for intake.

4.2. Fertilization

Lowering N fertilization had predictable effects on sward characteristics, with a major reduction in the sward height (−19%) and green leaf mass (−30%). In this context, the cows ingested a clearly smaller quantity (−13%, i.e. −2.0 kg OM per day) on unfertilized swards compared with fertilized swards, although they received similar herbage allowance. Such an effect of fertilization level on herbage intake was not observed by Combellas and Hodgson (1979) or Peyraud et al. (1994), who used in their experiments large herbage mass not limiting for intake. Stakelum (1986) and Peyraud et al. (1996b) have clearly demonstrated a specific effect of the herbage mass and/or green leaf mass on intake which is independent of the amounts offered. According to Eq. 7 proposed by Peyraud et al. (1996b), the variation of ingestion linked to variations in green leaf mass in the present study would be 2.7 kg OM, a value which is close to the total observed variation (2.0 kg OM).

This reduction in herbage intake on unfertilized swards was mainly mediated by a lower rate of intake which was in turn explained by a decrease in bite size. In effect, neither the total grazing time nor the rate of biting were affected by the level of fertilization. The estimated rate of intake and bite size were reduced by 10% and 12% respectively (i.e. −2.9 g OM min⁻¹ and −64 mg OM per bite). This result is in good agreement with numerous studies investigating the effect of sward characteristics on behavioural variables. As the sward height decreases, intake per bite decreases linearly (Allden and Whittaker, 1970; Jamieson and Hodgson, 1979b; Forbes and Hodgson, 1985). For sheep and with continuous grazing, any reduction in bite mass can be compensated at least in part by an increase in biting rate or grazing time (Allden and Whittaker, 1970, Penning et al., 1994). This compensation was not observed in the present study, a fact which may be linked to the animal species and/or grazing management. Forbes and Hodgson (1985) have shown that sheep but not cows are able to increase their grazing time when sward height is reduced. Jamieson and Hodgson (1979a) and Jamieson and Hodgson (1979b) have shown an increased grazing time in response to limiting sward conditions in continuous grazing but a lack of response under strip grazing. It is also possible that cows reached their maximum chewing capacity with more than 1000 min per day spent chewing. On unfertilized swards, one hour more was spent ruminating. If there is a limitation to chewing activity, this must have an effect on grazing time.

In the present experiment, grazing time after afternoon milking was significantly reduced even on unfertilized swards. Similar results have already been observed by Le Du et al. (1979) and Peyraud et al. (1996b) in the case of strip-grazing dairy cows with low herbage allowances, i.e. presence of limiting sward structures at the end of the day.
This suggests a real physical hindrance to prehension or a major loss of motivation in
the grazed animals, despite a similar residual sward height in both fertilization levels.
Cows may cease grazing earlier in the evening on unfertilized swards, not because the
height was strictly a limiting factor, but rather because stem-rich strata were more
rapidly attained than on fertilized swards. The estimated volume of defoliated stem,
calculated as the product of daily available area by the mean stem defoliation depth, is
moreover 50% higher on unfertilized than on fertilized swards.

Furthermore, reduced fertilization had a negative effect on sward quality as OMD and
crude protein content were reduced on herbage offered and selected by cows. Also
observed was a decrease of cellulolytic activity in the rumen linked to the lower
ammonia level. Herbage intake on unfertilized swards might have been reduced by
limiting ruminal digestion. However, digestion data show that the level of fertilization
little affected the nutritive value of the grass. The OMD remained high (0.777) and
duodenal flow of NAN per kg DOM intake did not vary due to net recycling of nitrogen
in the rumen. These results are in good agreement with previous data obtained with stall
feeding dairy cows (Van Vuuren et al., 1992; Peyraud et al., 1996a). Moreover, with
cows fed fresh grass indoors, Holmes and Lang (1963) and Peyraud et al. (1996a) have
shown that a similar reduction in OMD and crude protein content of grass as observed in
our experiment is insufficient to affect voluntary intake. In our experiment, it is obvious
that the lowered digestibility and CP content of unfertilized grass had only a secondary
effect in reducing the herbage intake, while a primary role was played by the sward
structure and its effect on behaviour.

4.3. **Supplementation**

For supplemented cows, herbage intake was calculated from the digestibility mea-
sured on unsupplemented cows and assuming no digestive interaction between herbage
and concentrate. This approach has been previously employed by Jennings and Holmes
(1984) and Kibon and Holmes (1987), who used high levels of concentrate. Moreover,
Delagarde and Peyraud (1995) have shown that the supplementation of fresh grass by 3
kg of rapidly fermentable cereal had no effect on herbage digestibility and ruminal
fermentations in stall-feeding cows. Thus, the hypothesis of calculation is probably
sound when concentrate is only slightly degradable and distributed in small doses, as
was the case in the present study.

Herbage intake, whether from fertilized or unfertilized swards, did not change when
the animals were supplemented by 2 kg of soyabean meal. This result is supported by
the observation that neither grazing time nor biting rate were modified by the supply of
concentrate. There are very few data in the literature concerning herbage intake and
protein supplementation of dairy cows on temperate pastures, since protein was gener-
ally not previously recognized to be the major limiting factor in milk production. Under
the condition of non-limiting herbage allowances, reported substitution rates with
energetic concentrates are highly variable, but fall in the range 0.4–0.7 for 3–6 kg of
concentrate and cows producing 15–25 kg FCM (Meijs and Hoekstra, 1984; Stakelum,
1986; Opatpatanakit et al., 1993).
Three factors may explain the absence of substitution in the present study. Firstly, the amount of distributed concentrate is low (2 kg) compared with levels reported in the literature and it is probable that the substitution rate diminishes with decreasing levels of concentrate, as found by Hijink et al. (1982). Secondly, the pre-experimental milk production level of the cows was 25 kg FCM day\(^{-1}\), which is among highest values reported for intake studies at grazing. Although the influence of the milk production level on substitution rate has not been demonstrated in the case of grazing, the lowest substitution rates reported (0–0.2) with non-limiting herbage allowances were obtained with cows producing more than 25 kg FCM (Jennings and Holmes, 1984; Kibon and Holmes, 1987; King et al., 1990). Finally, it is possible that the absence of substitution could be linked in part to the high protein content of concentrate even on fertilized swards. In effect, Andersen et al. (1988) have shown that young bullocks have a tendency to ingest more grass (18% CP, 81% OMD) when meat flour is incorporated into high-energy concentrates. According to Ketelaars and Tolkamp (1992), protein absorption could stimulate ingestion well beyond the N content of herbage limiting microbial fermentation in the rumen. In the present study, the soyabean meal, partially formaldehyde-treated, has increased the intestinal NAN supply and thus could have stimulate ingestion by improving the general nitrogenous nutritional status of cows, and thereby indirectly limit substitution (Seoane, 1995).

Even though statistically non significant, the effect of soyabean meal supplementation on herbage intake may be more marked on unfertilized compared with fertilized swards (+0.8 kg OM and -0.4 kg OM herbage intake, respectively). This difference may be partly related to the discrepancies in herbage intake between the two fertilization levels. According to Grainger and Mathews (1989), the substitution rate is positively correlated with the pasture intake at zero concentrate intake. Such an effect observed in feeding-stall experiments has been explained by Faverdin et al. (1991) in terms of the extent to which energy requirements are satisfied. In the present experiment, the energy balance (intake-requirements) of unsupplemented cows is negative for unfertilized swards (-1.8 UFL day\(^{-1}\); 1 UFL = 7.115 MJ NE\(_{\text{L}}\), INRA, 1989) and positive on fertilized swards (+0.7 UFL day\(^{-1}\)). Otherwise, the stimulating effect of nitrogenous supplementation on microbial activity in the rumen and the ingestion of poor quality forage has been known for a long time (Hoden, 1973) and also observed under grazing conditions (Caton et al., 1988). However, no significant increase was observed either in cellulolytic activity or in the ruminal OM digestibility when soyabean meal was fed. It is thus rather unlikely that soyabean meal can have improved the digestibility of unfertilized herbage. In other respects this lack of effect corroborates the method adopted here for the calculation of herbage intake. On the other hand, the increase of amino acids supplied to the duodenum with soyabean meal may stimulate ingestion to a greater extent on unfertilized swards than on fertilized swards, because of a much lower NAN flow when cows grazed on unfertilized swards.

4.4. Nutritive inputs, milk production and N excretion

Milk production data could be examined carefully, because of the short periods and the small number of cows in the experiment. However, there is an obvious trend and the
milk data well support the variations in intake and duodenal flow measurements. The major drops in the digestible OM intake and NAN flow from unfertilized swards accounts for the fall in milk production (−2.4 kg FCM for unsupplemented cows) and milk protein content (Coulon and Rémond, 1991). The response of milk production to soyabean meal supplementation was high for both fertilization levels (1.2 kg FCM per kg DM concentrate on average). This response is clearly due to an absence of substitution which is reflected in the strong rise of digestible OM intake and NAN flow when cows were supplemented. The experiment described here does not enable a quantification of the respective effects of energy and nitrogen supply on the response of milk production to soyabean meal supplementation observed in production trials (Delaby et al., 1996).

This experiment allows us to quantify the combined effect of N fertilization and supplementation on N excretion by the cows. Nitrogen output in milk varies in a narrow range (102, 119, 117, and 131 g day⁻¹ for LU, LS, HU and HS, respectively) comparing N intake (Table 4). Therefore, N restitutions were largely affected by the treatments. Faecal N excretion increased slowly with N intake (98, 126, 119, 136 g day⁻¹ for LU, LS, HU and HS, respectively). Assuming no N retention, N excreted in urine can be calculated by the difference between N intake and N in milk and faeces. Urinary N output increases sharply with N intake (130, 242, 380, 472 g day⁻¹ for LU, LS, HU and HS, respectively). For similar DOM intake, NAN duodenal flow and milk protein production, urinary N output was lower on LS than on HU treatment. This clearly shows that N emission by grazing cows could be managed without adverse effect on animal nutrition, and that feeding protected protein is suitable for overcoming the shortage of nutrients for cows on unfertilized swards.

5. Conclusion

The results of the present study show that, for a given herbage allowance, nitrogen fertilization and protein supplementation lead to an important modification in the total energy and protein supply of grazing dairy cows. The fall in intake and production on unfertilized swards is attributed mainly to a reduction in the herbage mass and sward height, whereas lowering N fertilization has only minor effects on the nutritive value of the grass. It may be possible to counterbalance this detrimental effect on herbage intake by increasing the herbage allowance on unfertilized swards as suggested by the results of Delaby et al. (1996). Other agronomical measures as an increase in growing days, thereby increasing herbage mass, might limit this negative effect on sward characteristics, but greatly reduce the nutritive value of grass. Soyabean meal supplementation does appear to greatly increase nutrient supply in particular when cows grazed on low fertilized swards where substitution was not observed.

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References


Jamieson, W.S. and Hodgson, J., 1979b. The effects of variation in sward characteristics upon the ingestive