Effect of reducing nitrogen fertilizer on grassland on grass intake, digestibility and milk production of dairy cows

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Abstract

To quantify the effect of a reduction in nitrogen fertilizer on grass intake and animal performance, four zero-grazing experiments were carried out, two in spring/early summer and two in late summer. Grass was fertilized at three levels of N fertilizer, 450, 300 and 150 kg/ha per year and harvested daily at dry matter yields between 1500 and 2000 kg/ha. Grass was fed ad libitum to three groups of 12 dairy cows in mid lactation. Reducing fertilizer N decreased crude protein content and in-vitro digestibility, but increased sugar content in grass. Overall, in the spring experiments, a reduction in N fertilization from 450 to 150 kg/ha per year did not affect grass intake. In one of the experiments carried out in spring, net energy intake of cows offered 150N grass was lower, resulting in lower milk yields. In late summer, cows consumed less 150N grass and produced less yields of milk, fat and protein compared to the other treatments. Except for milk production differences in S, a reduction of N fertilizer from 450 to 300 kg/ha per year did not affect intake or milk production.

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1. Introduction

In intensive grassland management systems, high nitrogen (N) application increases grass growth and as a consequence grass can be harvested, either by grazing or by cutting, in earlier stages of maturity (vegetative, pre- and early bloom) giving high nutritive values and maximum voluntary intake (VI) of grass (Minson, 1990). However, the disadvantages of using high levels of N fertilizer to the environment become more and more apparent. Under grazing conditions, grass is usually vegetative containing high amounts of rapidly rumen degradable N which is poorly utilized. This may result in high N-losses to the environment, hence causing ammonia volatilisation and nitrate leaching (Tamminga, 1992). Therefore, the use of less N fertilizer on grassland becomes possibly more attractive.

The effects on grass production, chemical composition and nutritive value of using lower amounts of N fertilizer are well documented and reviewed by

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Minson (1990). However, little is known about the effects of reduced amounts of N fertilizer on VI and animal performance of dairy cows during the growing season. Most experiments in literature were carried out with sheep offering very mature grass. It is uncertain whether the results of those experiments can be extrapolated to dairy cows grazing grass in earlier stages. When grass is harvested on the same day after regrowth, N fertilizer has no consistent effect on VI, indicating that the effect of N on VI is strongly related to stage of maturity (Minson, 1990). Therefore, more information is needed about the effects of reducing N fertilizer on VI and milk performance of dairy cows offered grass cut at similar dry matter (DM) yields.

The objective of our experiments was to quantify the effects on VI and milk performance of dairy cows fed indoors with grass grown at three different annual levels of N application (450, 300 and 150 kg/ha) during two different periods (spring/early summer and late summer). In order to minimize the effect of stage of maturity on VI and to connect with the practical grazing situation, VI of grass is tried to compare at DM yields between 1500 and 2000 kg/ha for the three N treatments.

2. Materials and methods

2.1. Cows and treatments

Experiments were carried out in May–July (spring/early summer) of 1991 (S₁) and 1992 (S₂) and in August–September (late summer) of 1992 (A₂) and 1993 (A₃). Before the start of each experiment 36 multiparous Holstein–Friesian crossbred dairy cows in mid lactation were allocated to 12 blocks of three cows on the basis of calving date, milk yield and milk composition. Means of the stage of lactation and milk production data of the 12 cows per treatment at the start of the experiments, are given in Table 1. Cows were housed in tie stalls on rubber mats and had free access to water. Within each block cows were allotted at random to one of the three treatments. Treatments were three rations containing fresh grass harvested daily and fertilized with either 450 (N₄₅₀), or 300 (N₃₀₀), or 150 (N₁₅₀) kg N/ha per year. Cows received daily 1 kg in the early and 2 kg compound feed in the late summer experiments which amount was offered in two equal portions during milking. This compound feed contained sugar beet pulp (20%), citrus pulp (20%), maize gluten feed (22.5%), palm kernel expeller (15%), soybean hulls (8%) and other ingredients. Faecal output was estimated by marker technique. Therefore, 4 g Cr₂O₃ per kg was added to another compound feed (Cr compound) containing sugar beet pulp (15%), maize gluten feed (20%), palm kernel expeller (17.5%), soybean hulls (14%), linseed expeller (8%), coconut expeller (8%), wheat (7.5%) and other ingredients. Cows received 1 kg of Cr compound per day in two equal portions after milking. Chemical composition and nutritive value of the commercial and Cr compound feeds did not differ between years. They contained per kg DM: 93 and 101 g ash, 178 and 163 g CP, 50 and 46 g CF, 21

<table>
<thead>
<tr>
<th>Experiment</th>
<th>S₁</th>
<th>S₂</th>
<th>A₂</th>
<th>A₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₁₅₀</td>
<td>N₃₀₀</td>
<td>N₄₅₀</td>
<td>N₁₅₀</td>
<td>N₃₀₀</td>
</tr>
<tr>
<td>LS (d)</td>
<td>133</td>
<td>122</td>
<td>136</td>
<td>173</td>
</tr>
<tr>
<td>Milk (kg/d)</td>
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<td>28.9</td>
<td>29.3</td>
<td>28.7</td>
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<td>Fat (g/kg)</td>
<td>46.5</td>
<td>47.1</td>
<td>46.4</td>
<td>45.7</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>35.4</td>
<td>35.6</td>
<td>35.5</td>
<td>36.6</td>
</tr>
<tr>
<td>LW (kg)</td>
<td>621</td>
<td>648</td>
<td>621</td>
<td>594</td>
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</tbody>
</table>
and 103 g starch, 347 and 383 g NDF, 97 and 70 g DVE (Tamminga et al., 1994) and 1061 and 1032 VEM (Van Es, 1978) for the commercial and Cr compound, respectively. The experiments carried out in spring/early summer started on 13 May in S90 and on 18 May in S92 and lasted for 10 weeks. The experiments in late summer started on 27 July in A92 and on 2 August in A93 and lasted for eight weeks. For all experiments an adaptation period of two weeks is included.

2.2. Meteorological and agronomy aspects

Rainfall was measured daily using a Lambrecht rain gauge. Temperature was measured every 10 min with thermometers consisting of platinum electrodes. Grass was grown on clay soils and contained 70% Lolium perenne, 20% Poa trivialis and 5% Poa annua. About 30 ha was divided into 26, 22 and 20 numbered plots (300 m × 12 m per plot) each receiving an annual application of 150, 300 and 450 kg N/ha, respectively. In early spring these plots received respectively 20, 50 and 65 kg N/ha prior to the first cut. Each day from mid-April onwards, three grass plots (starting with plot number 1 of each N treatment) were mown and directly harvested after which each plot received an application of 30, 60 and 90 kg/ha for the second cut. Plots were mown at target yields between 1500 and 2000 kg DM/ha. The spring experiments started when the first plot of the 150N regime (second cut) had reached that target DM yield. Fertilization and mowing activities were recorded on a grassland calendar. Third and fourth cuts received the same amount of N as the second cut. The remaining harvest received 20, 40 and 60 kg N/ha for the three N treatments, respectively. Grass growth was based on weekly grass DM yields obtained from nine plots (3 per N level) using an Agria motor scythe (Meijs, 1981). Due to the difference in cutting height between the Agria motor scythe and the cyclo mower (3 versus 6 cm cutting height), the nine plots were also cut with the Agria just before and after the harvest of the whole plot by the cyclo mower. Data of grass growth were used to perform the strategy of harvesting the swards at DM yields between 1500 and 2000 kg/ha.

During a drought in 1992 (A92), all plots were irrigated with 24 mm water in calendar week 27 and the third harvest of 150N plots was fertilized with an extra supply of 40 kg/ha.

2.3. Sampling and measurements

Grass was harvested daily with a cyclo mower starting at 09.00h, analyzed for DM content by a rapid method described by Meijs (1981) and weighed in 13/14 portions of 1.4 kg DM grass for each cow. After milking in the afternoon about eight grass portions were offered during the time between 16.00h and 21.00h. The remainder portions were stored at 3°C and offered after the morning milking between 07.00h and 16.00h. After the adaptation period, intake was measured individually on four consecutive days in each week by weighing grass refusals once daily directly before milking in the afternoon. On these days, grass samples were taken which were oven dried at 70°C and analyzed for DM, ash, Kjeldahl N, crude fibre, sugar and neutral detergent fibre (NDF) according to methods described by Van Vuuren et al. (1993). In-vitro organic matter digestibility (in-vitro \( d_{\text{OM}} \)) was estimated according the method of Tilley and Terry as modified by Van der Meer (1986). Samples of grass refusals were collected daily per N treatment, which were analyzed for DM and ash content. At the start, middle and end of the experiments, samples of the compound feeds were taken and analyzed for the same components as grass. In addition, crude fat and starch were analyzed in the compound feeds according the methods described in Van Vuuren et al. (1993). Samples of the Cr compound were taken every week and analyzed for DM, ash and Cr (see determination method by Van Vuuren et al. 1993). The energy value of the feed stuffs was calculated as net energy for lactation and expressed in VEM units according to Van Es (1978). Protein value was calculated as true protein digested in the small intestine (DVE) and the degraded protein balance in the rumen as OEB (Tamminga et al., 1994) based on CP content and day of harvesting.

To estimate total apparent digestibility, faecal samples were taken individually from nine cows per N treatment during six weeks in the spring/early summer and five weeks in the late summer experiments. During three consecutive days per week, each
dung patch excreted between 06.30h to 15.00h was sampled and pooled to one sample per cow per week. Samples were dried at 70°C and analyzed for DM, ash, NDF and Cr content and Kjeldahl N was analyzed in a fresh subsample. The fixed amount of Cr ingested each day and the Cr content in the faeces assuming a Cr recovery of 94%, were used to estimate daily faecal output per cow per week (faecal output = ingested Cr/faecal Cr content).

Cows were milked twice daily with milk yields being recorded at each milking. Milk samples were taken during six consecutive milkings per week for fat and protein analyses, which were determined by infrared analysis (Melkcontrolestation Noord-Nederland, Leeuwarden).

2.4. Statistical analyses

Intake, digestibility of nutrients and milk performance data were analyzed in a completely randomised block design and were subjected to analysis of variance, using per experiment the model: $Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$, where $\mu =$ mean, $\alpha_i =$ effect of block $i$, $\beta_j =$ effect of treatment $j$, and $e_{ij} =$ variation within a block. Data from the experiments carried out in the same season were pooled and analyzed by including the effect of year in the model described above. Statistical analysis was carried out using Genstat (Genstat 5 Committee, 1993). Treatment means were compared by Student’s $t$-test. In experiment A$_{93}$ one cow was infected by a severe form of mastitis and was assigned as a missing value in the statistical analysis of the data. The effect of N fertilizer on chemical composition and nutritive value of grass was tested for significance using a linear regression model with N treatment as the only variable.

3. Results

3.1. Weather conditions during the experiments

For the spring experiments, in 1991 (S$_{91}$) weather was relatively cold and wet, whereas in 1992 (S$_{92}$) temperature was high and the experimental period was characterised by drought with occasional rain showers (Fig. 1). Also the late summer experiment of 1992 (A$_{92}$) was carried out in hot weather conditions with drought, whereas in 1993 (A$_{93}$), weather was cold with high amounts of rainfall especially at the end of the experiment.

Due to the drought in 1992, crown rust was observed, notably at the 150N plots. During harvest-
The mean age of grass in S91 was five days lower than in S92 (Table 2), which reflected the better growing conditions in spring of 1991 as compared with 1992. Grass growth in A93 was not limited by drought which resulted in a higher growth rate in contrast to A92 especially at the highest level of N fertilizer. However, except for N150 in A92, days of regrowth differed not markedly between the late summer experiments which means that in A92 DM yield of grass was lower than in A93.

Within the spring experiments, the reduction of N fertilizer resulted in a systematic increase of up to 2.5 growing days to achieve 1500–2000 kg DM/ha. In the late summer experiments, grass was harvested at similar days after regrowth for the three treatments, except for N150 in A92 which was retarded by seven days.

3.3. Chemical composition and nutritive value

Chemical composition and nutritive value of grass samples are shown in Table 2. Because of dry weather conditions, DM content of grass cut in S92 was higher than in the other experiments (194 versus 145 g DM content). In A92 sugar content and in-vitro $d_{OM}$ of grass were relatively low as compared to the other experiments.

Grass DM, sugar and crude fibre (except in S91) content increased significantly ($P < 0.05$) with decreasing N application. Ash content was not affected by fertilizer N. The CP content decreased significantly ($P < 0.05$) with about 80 to 90 g/kg DM in spring and 60 to 70 g/kg DM in late summer when N application decreased from 450 to 150 kg/ha per year. The content of NDF was not affected by N fertilizer except in A92, where NDF increased significantly ($P < 0.05$) with decreasing N application. In all experiments VEM, DVE and especially OEB declined significantly ($P < 0.05$) by using lower amounts of N fertilizer with no seasonal effect. Except in S91, in-vitro $d_{OM}$ of 150N grass was significantly lower than of 450N and 300N grass with the most markedly difference in A92 (73.7% for N150 versus 77.7% and 76.3% for N450 and N300 grass, respectively).

3.4. Feed intake and digestibility of nutrients

Both compound feeds were consumed according the amounts offered daily (1.8 and 2.7 kg DM/cow in spring and late summer, respectively). When year effect is accounted for, no distinct effect of N fertilizer on VI was observed in spring whereas in late summer, but VI was reduced significantly on N150 compared with the other treatments (15.3 versus 16.2 kg DM). When compared within each experiment, a systematic reduction in VI with decreasing N fertilization was only obvious in A93 (Table 3). If grass intake was expressed per kg metabolic weight ($W^{0.75}$), treatment effects were significant only in S92 and A93. In A92, the extra N on the 150N plots resulted in an increase in VI during the last two weeks of this experiment. Further, VI of cows fed 450N grass in A92 was unexpectedly low in two of the six weeks thereby reducing the mean VI. Nevertheless, grass VI of cows fed N150 was markedly higher than of cows fed 150N grass during A92.

Differences in NDF intake reflected the differences in DM intake. Differences in kVEM, DVE and OEB intakes were a result of differences in VEM, DVE and OEB contents and differences in DM intake. In general, the intake of VEM, DVE and OEB decreased with a reduction in N fertilizer especially from 300 to 150 kg N/ha per year. In all experiments the apparent in-vivo digestibility of OM ($d_{OM}$), N ($d_N$) and NDF ($d_{NDF}$) was significantly ($P < 0.05$) lower on diet N150 compared with diet N450. For these response variables, the results of N100 varied between the other two treatments and differed more significantly from N150 than from N450. The in-vitro $d_{OM}$ calculated for the total ration was not different between N treatments following the in-vitro $d_{OM}$ differences of grass shown in Table 2.

3.5. Yield, composition of milk and liveweight

From the overall analysis with allowance for year effect, daily milk yield per cow did not differ significantly in spring between treatments whereas in
Table 2
Mean days after regrowth and mean content of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), sugar, DVE\(^1\), OEB\(^2\), VEM\(^3\) and in-vitro digestibility (in-vitro \(d_{\text{OM}}\)) of grass, fertilized with different amounts of N (450, 300 and 150 kg/ha/year), and offered to dairy cows in four zero-grazing experiments (\(S_{91}, S_{92}, A_{92}\) and \(A_{93}\)).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>(S_{91})</th>
<th>(S_{92})</th>
<th>(A_{92})</th>
<th>(A_{93})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days after regrowth (d)</td>
<td>20</td>
<td>22</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Grass</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DM (g/kg)</td>
<td>138(^a)</td>
<td>147(^b)</td>
<td>163(^c)</td>
<td>3.1</td>
</tr>
<tr>
<td>DM composition (g/kg)</td>
<td>108</td>
<td>105</td>
<td>102</td>
<td>1.7</td>
</tr>
<tr>
<td>Ash</td>
<td>250(^a)</td>
<td>200(^b)</td>
<td>163(^c)</td>
<td>4.7</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>214</td>
<td>222</td>
<td>219</td>
<td>2.4</td>
</tr>
<tr>
<td>NDF</td>
<td>462</td>
<td>467</td>
<td>459</td>
<td>3.6</td>
</tr>
<tr>
<td>Sugar</td>
<td>92(^a)</td>
<td>125(^b)</td>
<td>159(^c)</td>
<td>4.3</td>
</tr>
<tr>
<td>DVE(^1)</td>
<td>106(^a)</td>
<td>99(^b)</td>
<td>93(^c)</td>
<td>0.9</td>
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<tr>
<td>OEB(^2)</td>
<td>86(^a)</td>
<td>39(^b)</td>
<td>11(^c)</td>
<td>4.4</td>
</tr>
<tr>
<td>Net energy</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VEM(^3)/kg DM</td>
<td>1029(^a)</td>
<td>996(^b)</td>
<td>976(^c)</td>
<td>5.8</td>
</tr>
<tr>
<td>In vitro (d_{\text{OM}}) (%)</td>
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<tr>
<td></td>
<td>83.6</td>
<td>83.2</td>
<td>82.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

a, b, c: Means in the same row and experiment with different superscripts differ significantly (\(P < 0.05\)).

\(^1\)DVE = True protein digested in small intestine (Tamminga et al., 1994).

\(^2\)OEB = Degraded protein balance in the rumen (Tamminga et al., 1994).

\(^3\)1 kVEM = 6.9 MJ net energy lactation (Van Es, 1978).
Table 3
The effect of lowering N fertilizer on voluntary grass intake (VI in kg DM/d and g DM/kg LW$^{0.75}$) and total intake (grass + compound) of NDF, net-energy (VEM) and protein (DVE) and on the apparent digestibilities of OM, N and NDF

<table>
<thead>
<tr>
<th>Experiment</th>
<th>N$_{450}$</th>
<th>N$_{300}$</th>
<th>N$_{150}$</th>
<th>S.E.</th>
<th>N$_{450}$</th>
<th>N$_{300}$</th>
<th>N$_{150}$</th>
<th>S.E.</th>
<th>N$_{450}$</th>
<th>N$_{300}$</th>
<th>N$_{150}$</th>
<th>S.E.</th>
<th>N$_{450}$</th>
<th>N$_{300}$</th>
<th>N$_{150}$</th>
<th>S.E.</th>
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<tbody>
<tr>
<td>DM-intake of grass</td>
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</tr>
<tr>
<td>VI (kg/d)</td>
<td>15.4</td>
<td>15.9</td>
<td>16.0</td>
<td>0.5</td>
<td>17.2</td>
<td>16.7</td>
<td>16.6</td>
<td>0.4</td>
<td>15.4$^{ab}$</td>
<td>16.1$^{b}$</td>
<td>15.0$^{a}$</td>
<td>0.4</td>
<td>16.7$^{a}$</td>
<td>16.6$^{a}$</td>
<td>15.5$^{b}$</td>
<td>0.4</td>
</tr>
<tr>
<td>VI (g/kg LW$^{0.75}$)</td>
<td>123</td>
<td>122</td>
<td>126</td>
<td>4.2</td>
<td>137$^{a}$</td>
<td>132$^{b}$</td>
<td>129$^{b}$</td>
<td>3.8</td>
<td>129</td>
<td>130</td>
<td>124</td>
<td>3.9</td>
<td>140$^{a}$</td>
<td>135$^{a}$</td>
<td>127$^{b}$</td>
<td>3.5</td>
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<tr>
<td>NDF (kg/d)</td>
<td>7.8</td>
<td>8.0</td>
<td>8.0</td>
<td>0.2</td>
<td>8.9</td>
<td>8.7</td>
<td>8.8</td>
<td>0.2</td>
<td>8.6$^{a}$</td>
<td>9.2$^{b}$</td>
<td>8.7$^{b}$</td>
<td>0.2</td>
<td>8.6$^{a}$</td>
<td>8.6$^{b}$</td>
<td>8.1$^{b}$</td>
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<td>VEM</td>
<td>17.7</td>
<td>17.7</td>
<td>17.5</td>
<td>0.5</td>
<td>18.5$^{a}$</td>
<td>17.4$^{b}$</td>
<td>16.7$^{a}$</td>
<td>0.3</td>
<td>17.2$^{a}$</td>
<td>17.3$^{a}$</td>
<td>15.4$^{b}$</td>
<td>0.3</td>
<td>19.3$^{a}$</td>
<td>18.8$^{a}$</td>
<td>17.2$^{a}$</td>
<td>0.4</td>
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<tr>
<td>DVE (g/d)</td>
<td>1809$^{a}$</td>
<td>1742$^{b}$</td>
<td>1668$^{b}$</td>
<td>50</td>
<td>1643$^{b}$</td>
<td>1675$^{b}$</td>
<td>1507$^{a}$</td>
<td>33</td>
<td>1736$^{a}$</td>
<td>1711$^{a}$</td>
<td>1444$^{c}$</td>
<td>31</td>
<td>1948$^{a}$</td>
<td>1880$^{b}$</td>
<td>1658$^{c}$</td>
<td>39</td>
</tr>
<tr>
<td>OEB (g/d)</td>
<td>1343$^{a}$</td>
<td>630$^{b}$</td>
<td>175$^{c}$</td>
<td>28</td>
<td>934$^{a}$</td>
<td>433$^{b}$</td>
<td>135$^{c}$</td>
<td>19</td>
<td>1239$^{a}$</td>
<td>955$^{b}$</td>
<td>380$^{c}$</td>
<td>22</td>
<td>1406$^{a}$</td>
<td>853$^{c}$</td>
<td>443$^{c}$</td>
<td>27</td>
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<tr>
<td>Total diet apparent digestibility (%)</td>
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<td>In-vivo</td>
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<tr>
<td>d$_{OM}$</td>
<td>79.9$^{a}$</td>
<td>79.4$^{a}$</td>
<td>77.1$^{b}$</td>
<td>0.5</td>
<td>77.9$^{a}$</td>
<td>76.6$^{b}$</td>
<td>76.6$^{b}$</td>
<td>0.5</td>
<td>76.3$^{a}$</td>
<td>75.9$^{a}$</td>
<td>73.1$^{b}$</td>
<td>0.5</td>
<td>77.4$^{a}$</td>
<td>76.4$^{a}$</td>
<td>75.9$^{b}$</td>
<td>0.8</td>
</tr>
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<td>d$_{N}$</td>
<td>78.1$^{a}$</td>
<td>75.5$^{b}$</td>
<td>68.5$^{c}$</td>
<td>0.7</td>
<td>76.5$^{a}$</td>
<td>72.6$^{b}$</td>
<td>69.2$^{c}$</td>
<td>0.8</td>
<td>75.2$^{a}$</td>
<td>73.5$^{a}$</td>
<td>68.6$^{b}$</td>
<td>0.7</td>
<td>74.3$^{a}$</td>
<td>71.0$^{a}$</td>
<td>69.8$^{b}$</td>
<td>1.1</td>
</tr>
<tr>
<td>d$_{NDF}$</td>
<td>77.3$^{a}$</td>
<td>75.1$^{b}$</td>
<td>70.1$^{c}$</td>
<td>0.8</td>
<td>75.3$^{a}$</td>
<td>72.9$^{b}$</td>
<td>71.5$^{b}$</td>
<td>0.8</td>
<td>75.5$^{a}$</td>
<td>74.2$^{a}$</td>
<td>70.5$^{b}$</td>
<td>0.9</td>
<td>76.1$^{a}$</td>
<td>73.1$^{a}$</td>
<td>70.5$^{a}$</td>
<td>1.1</td>
</tr>
<tr>
<td>In-vitro</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d$_{OM}$</td>
<td>83.3</td>
<td>83.5</td>
<td>82.7</td>
<td>–</td>
<td>80.6</td>
<td>80.0</td>
<td>78.8</td>
<td>–</td>
<td>78.1</td>
<td>76.9</td>
<td>74.7</td>
<td>–</td>
<td>80.6</td>
<td>80.7</td>
<td>79.3</td>
<td></td>
</tr>
</tbody>
</table>

a, b, c: Means in the same row and experiment with different superscripts differ significantly ($P < 0.05$).
Table 4
Mean milk yield and composition, liveweight (LW) and liveweight change during the experiments of cows offered different rations (\(N_{450}\), \(N_{300}\) and \(N_{150}\)) in four experiments (\(S_{01}\), \(S_{02}\), \(A_{02}\) and \(A_{03}\)).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>(S_{01})</th>
<th>(S_{02})</th>
<th>(A_{02})</th>
<th>(A_{03})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N_{450})</td>
<td>(N_{300})</td>
<td>(N_{150})</td>
<td>(N_{450})</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk (kg/d)</td>
<td>22.1</td>
<td>22.1</td>
<td>21.7</td>
<td>0.7</td>
</tr>
<tr>
<td>FPCM (kg/d)</td>
<td>24.1</td>
<td>23.6</td>
<td>23.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>1028</td>
<td>985</td>
<td>985</td>
<td>42</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>782</td>
<td>794</td>
<td>784</td>
<td>27</td>
</tr>
<tr>
<td><strong>Milk composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>46.6</td>
<td>44.6</td>
<td>45.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>35.4</td>
<td>35.9</td>
<td>36.2</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Liveweight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LW (kg)</td>
<td>620(^a)</td>
<td>651(^b)</td>
<td>629(^a)</td>
<td>6</td>
</tr>
<tr>
<td>LW change (kg/week)</td>
<td>+1.1</td>
<td>+2.1</td>
<td>+1.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

\(a, b, c\): Means in the same row and experiment with different superscripts differ significantly \((P < 0.05)\).
late summer cows on N$_{150}$ produced significantly less milk than the cows in the other treatments (21.2 on N$_{150}$ versus 24.6 and 24.4 kg milk on N$_{450}$ and N$_{300}$, respectively). When compared within each experiment, milk yield on treatment N$_{150}$ was significantly ($P < 0.05$) lower than on N$_{450}$ (Table 4), except in S$_{91}$. In S$_{92}$ cows on N$_{300}$ produced significantly less milk than cows on N$_{450}$ (21.5 versus 23.7 kg milk). In late summer, cows on N$_{300}$ produced significantly more milk than cows on N$_{150}$, but no difference between N$_{300}$ and N$_{450}$ was observed. Yields of FPCM, fat and protein followed the differences in milk yield. Except in S$_{92}$ and A$_{93}$ where milk protein content between treatments was significantly affected in a non-consistent way, no differences in milk constituents between treatments were observed. Mean liveweight (LW) of cows in S$_{91}$ fed N$_{300}$ was significantly higher than of those in the other groups. In spring, live weight changes were positive in contrast to experiment A$_{93}$ where live weight changes were tended to be negative.

4. Discussion

4.1. Effect of N fertilizer on chemical composition and nutritive value of grass

The consequences of the use of older grass in S$_{92}$ compared with S$_{91}$ was that contents of grass CP, DVE, OEB, VEM and $d_{OM}$ were lower and contents of grass NDF and sugar were higher in S$_{92}$. The differences in chemical composition and nutritive value between A$_{92}$ and A$_{93}$ were more related to differences in growing conditions due to drought, in agreement with the research of Deinum (1966).

Differences in chemical composition and nutritive value between grass harvested in spring and late summer are usually caused by blooming of grass in spring/early summer, which does not occur in late summer in temperate regions (Minson, 1990). In our experiments, no large differences between spring and late summer grass were observed probably caused by our strategy of grassland management with a high frequency of defoliation at a relatively young stage.

The observed effects of a reduction in N fertilizer on chemical composition and $d_{OM}$ were in agreement with the results of Deinum (1966) and Wilman and Wright (1983), but the effects in our experiments were smaller. One of the factors that influence the magnitude of the effects is stage of maturity. This is emphasised in A$_{92}$ where the differences in chemical composition and nutritive value were much larger caused by a substantial difference in growing days for 150N grass (Table 2). Also Salette (1982) and Peyraud and Astigarraga (1998) stated that NDF content and $d_{OM}$ value of grass are more influenced by stage of maturity than by N fertilization.

Nutritive value decreased with decreasing levels of N application. The reduction of DVE and OEB with decreasing N fertilizer reflected the decrease in CP content. The reduction in DVE ranged between 11 and 18 g/kg DM and was much smaller than the reduction in OEB, which decreased between 54 (A$_{93}$) and 75 (S$_{91}$) g/kg DM. This agrees with estimates based on nylon bag studies (Valk et al., 1996; Van Vuuren et al. 1991). The reduction in VEM content with decreasing N fertilizer was mainly caused by the reduction in digestible CP which is an arithmetical component in the VEM equation (Van Es, 1978). In A$_{92}$, also the reduction in OM digestibility attributed to the decrease in calculated VEM content.

4.2. Effect of N fertilizer on grass intake and digestibility

Voluntary intake is a result of many interactions between the feed, the animal and its environment (e.g., Minson, 1990; Ketelaars and Tolkamp, 1992). In our experiments, VI of cows fed grass fertilized with different N levels was compared at the same time and place. So, within each experiment, VI was influenced by differences in the chemical and nutritive value of grass due to differences in level of N fertilizer. Between experiments, VI could also be affected by animal, climatic or seasonal factors.

In spring, level of N fertilizer had no marked effect on VI of cows fed grass, harvested at nearly similar stages of maturity. This is in agreement with the literature reviewed by Minson (1990) and Peyraud and Astigarraga (1998). Although, $d_{OM}$ differed significantly between treatments in a range between 76.6% to 79.9% (Table 3), VI was not affected. This agrees with Conrad et al. (1964) who found no relationship between $d_{OM}$ and VI at $d_{OM}$ levels higher than 70%.

In late summer, the reduction in N fertilizer from
450 and 300 to 150 kg N/ha per year decreased VI. This was observed both when grass was harvested at different stage of maturity ($A_{92}$) and when grass was harvested at more or less similar stage of maturity ($A_{93}$). Similarly to the spring experiments, these differences in VI could not be explained by the observed differences in $d_{\text{OM}}$ and $d_{\text{NDF}}$. Forbes (1995) states that the rate of degradation of NDF is a better predictor for intake than digestibility. During our experiments subsamples of grass were incubated in-situ (Valk et al., 1996). From these results it is concluded that a reduction in N fertilizer decreased the rate of NDF degradation in both spring and late summer. We can not explain why the positive relationship between VI and rate of NDF degradation observed in late summer was not observed in the spring experiments. It can only be speculated if in spring a possible negative influence on VI attributed to the decrease in the rate of degradation, was compensated by the markedly increase in sugar content of 150N improving palatability (Peyraud and Astigarraga, 1998). This phenomenon was probably not observed in late summer due to the fact that sugar content was only slightly increased compared to the increase in spring.

Based on the critical level of 140 g CP/kg DM in grass for dairy cows to maintain VI (Peyraud and Astigarraga, 1998), the low CP content of 150N grass in $S_{92}$ (131 g/kg DM) was probably just sufficient, even though the OEB intake was negative. A negative OEB suggest a deficiency of undegradable protein in the rumen for optimum microbial synthesis which could reduce rate of OM degradation and consequently VI (Forbes, 1995). However, this negative OEB intake had no effect on VI probably because DVE intake exceeded the requirement by 20% resulting in high N recycling from the blood urea pool to the NH$_3$-N pool in the rumen (Tammenga et al., 1994).

4.3. Effect of N fertilizer on milk production

Most of the variation in FPCM between treatments within each experiment could be related to variation in grass kVEM intake which was calculated by multiplying grass DM intake with VEM content of grass. Due to the large number of experiments and the variation within each experiment, it can be demonstrated how FPCM reacts on differences in kVEM intake caused by differences in DM intake and/or VEM content of grass. Therefore, the relative contribution of DM intake (intake effect) and grass VEM content (quality effect) to the difference in kVEM intake from high to low N treatment was calculated. For example, if DM intake decreased from 17 to 16 kg/d and grass VEM content decreased from 950 to 900, the difference in kVEM intake was 1.75 ($= 17 \times 0.95 - 16 \times 0.90$). The
relative contribution of DM intake was 51% \[ = (17 - 16) \times 0.900 / 1.75 \times 100 \] and the contribution of VEM content was 49% \( = 100 - 51 \).

In spring, the reductions in kVEM intake from high to low N treatment were mainly due to a reduction in VEM content (Table 2), whereas in late summer these differences were mainly caused by a reduction in DM intake (Table 3). The relative contributions of VEM content to the reductions in kVEM intake between treatments were related to the observed difference in FPCM production expressed as kg FPCM per kVEM difference (Fig. 2). This figure clearly demonstrates a decrease in the response on FPCM when the relative contribution of VEM content to the difference in kVEM intake increases. So, the calculated difference in VEM content of high and low fertilized grass must be smaller based on the observed response in FPCM production.

Within each experiment DVE intake increased at higher levels of N fertilizer. However, for all treatments in all experiments, DVE intake was always more than 20% above DVE requirement (Tammenga et al., 1994). So, it cannot be expected that differences in DVE intake influenced milk yield or milk composition. It seems also unlikely that the negative OEB of 150N grass in S42 influenced milk performance because also for this treatment DVE was fed above requirement and DM intake was maintained.

5. Conclusions

In late summer, reducing the amount of N fertilizer on grassland from 450 to 150 kg/ha per year decreased grass intake and milk yield of dairy cows, even when grass was harvested in an early stage of maturity at DM yields between 1500 and 2000 kg/ha. In spring, no effect of N fertilizer on grass intake was observed. Despite this, in one experiment kVEM intake of cows fed 150N grass was reduced significantly, resulting in lower milk yields. Except in one experiment, the reduction in N fertilizer from 450 to 300 kg/ha per year did not affect intake and animal performance. The lowest digestibilities and DM intakes were obtained when a reduction in N fertilizer was associated with the use of older grass.

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References


