The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets

A. R. Castillo, E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby, and J. France

Department of Agriculture, University of Reading, Earley Gate, P.O. Box 236, Reading RG6 6AT; U.K.

ABSTRACT: An experiment was conducted to examine the effect that various isoenergetic diets, containing different quantities of soluble carbohydrate and fiber and different types of starch, have on nitrogen (N) balances. Six lactating dairy cows in early to midlactation consuming grass silage diets with not less than 600 g/kg total DMI as forage were used in the experiment. Four concentrates were prepared that had higher amounts of either fiber, soluble sugars, corn (low degradable starch source), or barley (high degradable starch source). Overall N utilization by the cows was poor, rarely exceeding 0.30 g milk N/g of dietary N intake. Fecal N outputs accounted for more than half of total N excreted in all treatments except for diets supplemented with high degradable starch, in which urinary N excretion was significantly higher compared with the other treatments. Milk yield was unaffected by concentrate type, averaging 19.9 kg/d, but milk protein content decreased from 32.9 for starch-based diets to 30.9 and 30.0 g/kg for the soluble sugar- and fiber-based diets, respectively. The efficiency of N utilization improved in the low degradable starch treatment, which had lower N excretion (65%) and higher protein concentration in milk. Furthermore, feeding cows corn-based concentrates reduced urinary N excretion by almost 30% compared with barley-based concentrates; therefore, feeding corn-based diets is recommended for the reduction of nitrogen pollution in lactating dairy cows.

Key Words: Dairy Cows, Energy Intake, Grass Silage, Nitrogen Metabolism, Pollution

Introduction

The efficiency with which dietary N is utilized by dairy cows is generally low and, in part, may be related to an impaired utilization of N in the rumen. A major determinant of microbial protein synthesis is the availability of energy-yielding substrates (Dijkstra et al., 1998). In theory, energy-rich concentrates comprising several carbohydrates with different rates and extent of degradation in the rumen should be more efficient than simple concentrates in the capture of N degraded in the rumen from high-quality forages (Van Soest et al., 1991). However, under controlled conditions, Newbold and Rust (1992) showed that rumen bacteria were not affected by an asynchronization of N and energy supply, concluding that bacterial growth had the capacity to recover quickly from transient restrictions caused by deficits in N supply. These results agree with those of Henning et al. (1993), who concluded that merely improving the degree of synchronization between energy and N release rates in the rumen does not necessarily increase microbial yield.

Nitrogen excretion in feces and urine accounts for a high proportion of N intake, which may be more than 70% of the daily N consumption (Tamminga and Vers-tegen, 1996). Environmental pollution from N as ammonia (Jarvis, 1994) and nitrates is regarded as a major potential problem worldwide (Smith and Frost, 2000). Nutritional manipulations might be a way of significantly increasing N utilization and decreasing N pollution by dairy cows (Tamminga, 1992).

The objective of this study was, therefore, to consider N utilization in lactating dairy cows receiving a significant proportion of their diet as grass silage and to establish ways in which the overall efficiency of N utilization might be improved by strategic supplementation of the diet. The aim of the study was also to consider environmental concerns arising due to excessive excretion of N by dairy cows.

©2001 American Society of Animal Science. All rights reserved.


1The authors would like to thank MAFF Environmental Protection Division for financial support.
2Present address: EEA Rafaela INTA, Casilla de correo 22, (2300) Rafaela, Santa Fe, Argentina.
3Correspondence: phone: +44 (0) 1189318498; fax: +44 (0) 1189 318297; E-mail: E.Kebreab@reading.ac.uk.
4Novus, Av. Carlos Gomes, 111 cj. 303/304 90480-003, Porto Alegre RS, Brazil.
Received April 10, 2000.
Accepted August 3, 2000.
### Materials and Methods

#### Animals and Diets

The experiment was carried out at the Centre for Dairy Research (CEDAR) in the University of Reading. Six multiparous Holstein-Friesian dairy cows from the main herd at CEDAR were used during early to midlactation. At the beginning of the trial, the average BW of the cows was 584 kg (SD = 74).

The diets were composed of early first-cut, partially wilted perennial ryegrass silage (*Lolium perenne*) prepared without additive and fed with four concentrate feeds. The concentrates were pelleted and offered at 7 kg/d on top of grass silage (10 kg/d) in two equal meals daily for each cow on a DM basis. The total amounts of silage and concentrates offered were isoenenergetic with respect to ME and based on a predicted DMI of 17 kg/d (AFRC, 1993), with 600 g/kg total DMI derived from grass silage. An additional 10% of silage and concentrates were offered to the cows in case they consumed more than the predicted DMI.

The four concentrate mixes (as treatments) were formulated using different carbohydrate sources to provide different rates or extents of carbohydrate availability in the rumen (Table 1). The treatments were designated as follows: high NDF (HNDF), low degradability starch (LDS), high degradability starch (HDS), and soluble sugars (SS). These four supplements were formulated to contain 160 g CP/kg DM.

#### Experimental Procedure

The experiment was planned as an incomplete 3-way blocked design of four periods and six cows. The experiment originally had six treatments, but two of those treatments dealt with levels of protein supplementation and are reported in the companion article. Within each experimental period, wk 1 to 3 were used for dietary adaptation and to determine daily DMI and milk production. During wk 4 (sampling and N balance week), total feces and urine were collected daily from each animal for 6 d, in addition to measurements of feed intake, feed refusal, and milk production. Methods of collection and sampling (milk, feces, and urine) were described by Sutton et al. (1997). Samples of feeds, feces, and urine were stored frozen and dried at 60°C as appropriate for subsequent analyses.

Dry matter and CP degradability of the experimental feeds was estimated using the in situ technique (Ørskov and McDonald, 1979). In each period, three polyester bags per incubation time for each concentrate and two polyester bags per incubation time for each grass silage were placed for 5, 12, 24, 48, and 72 h in the rumen of one fistulated dairy cow fed a 60:40 grass silage:concentrate diet with 200 g CP/kg DM in the concentrate. Bags containing the feeds to determine the water-soluble fraction and bags with the residues from the different incubation times were washed with cold water (room temperature) using a washing machine on a gentle-cold program. Effective degradability was calculated according to AFRC (1993) using a passage rate of 0.08/h.

#### Chemical Analysis

For determination of total N content, silage, milk, urine, and feces were analyzed from thawed samples. Thereafter, all samples, except urine and milk, were dried at 60°C for 96 h and ground through a 1-mm screen before subsequent analysis. Nitrogen content in feeds, milk, feces, and urine was determined using total...
combustion (FP 2000, Leco Instruments, Stockport, U.K.). Silage was corrected for DM based on oven drying and correction for the concentration of volatile components, according to Porter et al. (1984). Neutral detergent fiber content was determined according to the method of Goering and Van Soest (1970) with the amendments as proposed by Van Soest et al. (1991). The determinations of starch content in concentrate feeds was based on the enzymatic method of MacRae and Armstrong (1968). Distilled water was used to extract water-soluble carbohydrate (WSC) from feeds, and the quantity was determined according to the methodologies of Somogyi (1952) and Bailey et al. (1992). Organic matter content was obtained by difference after ashing at 550°C for 16 h. The ether extract in feeds was determined by using petroleum ether according to standard methodologies (MAFF, 1986). Protein, fat, and lactose contents in milk were estimated by infrared techniques (Foss Electric UK, Ltd.) using externally calibrated milk samples.

Statistical analysis

Data were analyzed statistically using the General Linear Models procedure of SAS (SAS Inst. Inc., Cary, NC). Two methods of analysis were considered. First, all six treatments were used, and the effect of energy supplementation in the four treatments was assessed using orthogonal contrasts. A second method using Latin square design limited to the four treatments was used, with the sources of variation being periods, cows, and treatments. Although the error degree of freedom in the latter analysis was lower, the results were similar to those of the first method of analysis. The second method was adopted because it did not compromise the statistical analysis, and it offers a more concise and clear comparison of the four energy supplementation treatments. The overall means obtained were adjusted means or least squares means. Treatment least squares means were assessed for significant differences at the $P < 0.05$ probability level using LSD for mean comparison.

Results

Feed Composition

The chemical composition of the forage, including the estimates of in situ degradability (Table 2), indicated a moderate-quality grass silage, with low CP and high NDF content. However, whereas the effective rumen-degradable DM content was only 449 g/kg DM, the estimated effective rumen-degradable protein content was much larger (702 g/kg CP).

The chemical composition and the in situ digestion estimates of the concentrates (Table 2) reflected the ingredients used in this experiment (Table 1). The supplements were formulated to contain 160 g CP/kg DM; apart from LDS, which had slightly less concentration of CP (139 g/kg), this specification was achieved. However, because of the low CP content in the grass silage, the CP concentrations in the diets (mean 143 g CP/kg DM) were lower than planned.

The rate of DM degradation in concentrate feeds ranged from 0.06/h in LDS to 0.12/h in the SS supplement, with intermediate values for HNDF and HDS. The effective DM degradabilities in the supplements of contrasting energy sources were in accordance with the rates of DM degradation for SS and HNDF. However, the greater rate of DM degradation for HDS vs LDS did not translate into a greater effective DM degradability (592 and 578 g/kg DM, respectively). This is partly due to the relatively large water-soluble fraction, which is assumed to be 100% degradable, and perhaps partly because of inaccurate estimation by the equation used (AFRC, 1993). Effective CP degradability was lowest for LDS (570 g/kg) and highest for HDS (646 g/kg). A range in the rate of CP degradation from 0.08 (SS) to 0.18/h (HDS), was also observed.

Dry Matter and Organic Matter Intake

Silage, concentrate, and total DMI were similar in all treatments, with an average intake of 9.1, 7.0, and 16.1 kg/d, respectively (Table 3). The refusals were free of concentrate and consisted wholly of grass silage. Dry matter digestibility was higher for LDS and HDS treatments. The lower DM digestibility for HNDF and SS was related to greater NDF plus ash contents of these concentrates (455 and 408 g/kg DM, respectively) compared with the other concentrates (range 231 to 326 g/kg DM). There were no differences in OM digestibility, which ranged from 727 (SS) to 737 g/kg (HDS). Organic matter intake was greater on HDS and LDS compared with SS, with HNDF being intermediate, largely due to differences in OM content of the concentrates.

Milk Production and Composition

Milk yield was unaffected by concentrate type, averaging 19.9 kg/d (Table 4). However, milk protein concentrations differed significantly, although fat and lactose concentrations were unaffected by the treatments. Milk protein concentrations were higher on LDS and HDS, with no differences ($P = 0.66$) between them, averaging 32.9 g protein/kg milk. The SS treatment was 1 g protein/kg milk higher than HNDF, but the difference was not significant.

The differences in milk protein concentration were not reflected in milk protein yields, although similar trends were observed but not large enough to be significant. Milk lactose and fat yields were not affected by the treatments imposed.

Nitrogen Balance

Nitrogen balance data are presented in Table 5 as total N excreted and as a proportion of total N intake. Although the trend in N intake was in accordance with
Table 2. Chemical composition and in situ degradability of feeds

<table>
<thead>
<tr>
<th>Item</th>
<th>Grass silage</th>
<th>Concentratea</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HNDF</td>
<td>LDS</td>
<td>HDS</td>
<td>SS</td>
<td></td>
</tr>
<tr>
<td>DM, g/kg fresh weight</td>
<td>287</td>
<td>838</td>
<td>831</td>
<td>835</td>
<td>813</td>
<td></td>
</tr>
<tr>
<td>OM, g/kg DM</td>
<td>917</td>
<td>886</td>
<td>947</td>
<td>943</td>
<td>857</td>
<td></td>
</tr>
<tr>
<td>CP, g/kg DM</td>
<td>133</td>
<td>154</td>
<td>139</td>
<td>163</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>NDF, g/kg DM</td>
<td>559</td>
<td>341</td>
<td>178</td>
<td>193</td>
<td>265</td>
<td></td>
</tr>
<tr>
<td>Ash, g/kg DM</td>
<td>83</td>
<td>114</td>
<td>53</td>
<td>57</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Starch, g/kg DM</td>
<td>NDb</td>
<td>139</td>
<td>558</td>
<td>488</td>
<td>24.9</td>
<td></td>
</tr>
<tr>
<td>Water-soluble carbohydrates, g/kg</td>
<td>80.4</td>
<td>163.9</td>
<td>56.7</td>
<td>64.4</td>
<td>340.8</td>
<td></td>
</tr>
</tbody>
</table>

In situ degradabilityc

<table>
<thead>
<tr>
<th>Item</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, g/kg</td>
<td>210</td>
<td>375</td>
<td>422</td>
<td>424</td>
<td>504</td>
</tr>
<tr>
<td></td>
<td>629</td>
<td>464</td>
<td>389</td>
<td>305</td>
<td>478</td>
</tr>
<tr>
<td></td>
<td>0.049</td>
<td>0.076</td>
<td>0.057</td>
<td>0.098</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td>449</td>
<td>605</td>
<td>578</td>
<td>592</td>
<td>789</td>
</tr>
<tr>
<td>CP, g/kg</td>
<td>579</td>
<td>132</td>
<td>87</td>
<td>102</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td>289</td>
<td>776</td>
<td>765</td>
<td>792</td>
<td>598</td>
</tr>
<tr>
<td></td>
<td>0.061</td>
<td>0.116</td>
<td>0.138</td>
<td>0.179</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>702</td>
<td>594</td>
<td>570</td>
<td>646</td>
<td>641</td>
</tr>
</tbody>
</table>

aTreatments were concentrates containing high in NDF (HNDF), low degradable starch (LDS), high degradable starch (HDS), and soluble sugars (SS) offered to cows.

bND = not determined.

cIn situ degradability, a = water-soluble fraction; b = potentially degradable fraction; c = fractional rate of degradation of the b fraction per hour; ED = effective degradability calculated as a + [bc/(c + r)], where r is the rate of passage from the rumen per hour.

dietary CP concentration, the differences were not large enough to be significant. There were no significant differences in total N excreted as feces and secreted in milk, but amounts of total N excreted in urine were affected (P = 0.05) by the treatments. The supplement based on high degradability starch had a 30% (38 g N/d) higher excretion of N in urine compared with other treatments. Treatments HNDF, LDS, and SS had values that were not significantly different from each other.

The proportions of N excreted in feces or secreted in milk and N balance in relation to total N intake were not affected by the treatments (P > 0.05). There were, however, differences in the proportion of dietary N excreted in urine, with HDS being greater (P = 0.05) than other treatments. The lowest proportions of dietary N in urine occurred on LDS, but HNDF and SS supplements also had low values, which were not significantly different from LDS.

Discussion

The objective of this study was to establish differences in the carbohydrate component of dietary concentrates and to evaluate their effect on N utilization, with specific reference to silage dietary N. The grass silage used was similar to the average silage quality described in MAFF (1992) and updated by Steen et al. (1998),

Table 3. Intake and digestibility of DM and OM in cows fed various supplements

<table>
<thead>
<tr>
<th>Item</th>
<th>HNDF</th>
<th>LDS</th>
<th>HDS</th>
<th>SS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silage</td>
<td>9.1</td>
<td>9.0</td>
<td>9.2</td>
<td>9.1</td>
<td>0.23</td>
</tr>
<tr>
<td>Concentrates</td>
<td>7.0</td>
<td>7.1</td>
<td>7.0</td>
<td>6.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Total DMI</td>
<td>16.1</td>
<td>16.0</td>
<td>16.2</td>
<td>16.0</td>
<td>0.22</td>
</tr>
<tr>
<td>DM digestibility, g/kg</td>
<td>697x</td>
<td>712y</td>
<td>716y</td>
<td>701x</td>
<td>3.18</td>
</tr>
<tr>
<td>OM intake, g/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silage</td>
<td>8.2</td>
<td>8.2</td>
<td>8.4</td>
<td>8.3</td>
<td>0.22</td>
</tr>
<tr>
<td>Concentrates</td>
<td>6.2y</td>
<td>6.7x</td>
<td>6.6y</td>
<td>6.0x</td>
<td>0.04</td>
</tr>
<tr>
<td>Total OM intake</td>
<td>14.5y</td>
<td>14.9y</td>
<td>15.0y</td>
<td>14.2x</td>
<td>0.16</td>
</tr>
<tr>
<td>OM digestibility, g/kg</td>
<td>729</td>
<td>731</td>
<td>737</td>
<td>727</td>
<td>4.40</td>
</tr>
</tbody>
</table>

aTreatments were concentrates containing high in NDF (HNDF), low degradable starch (LDS), high degradable starch (HDS), and soluble sugars (SS) offered to cows.

x,y,zWithin a row, means lacking a common superscript letter differ (P < 0.05).
who evaluated silage composition from a wide range of situations and origins. The lack of any effect of energy source on silage DMI was in accordance with Petit and Tremblay (1995) and Keady and Murphy (1998), who, working with dairy cows, compared diets based on grass silage supplemented with different nonstructural carbohydrates.

Total fecal N excretion and the proportion of apparent fecal N were unaffected by carbohydrate type in concentrate, which was in accordance with Peyraud et al. (1995), who concluded that the amount of N excreted in feces of dairy cows is relatively constant because it consists of mainly undigested material and metabolic fecal N.

The results of this study agree well with those of Petit and Tremblay (1995) with regard to urinary N excretion. The most pronounced effect was the increase in urinary N excretion of cows fed the high degradable starch diet. It is reasonable to expect that the HDS diet would support greater microbial protein synthesis than the LDS diet by providing more rumen-fermentable energy. Although HDS concentrates can induce rumen acidosis, which might reduce microbial protein synthesis, evidence from earlier work (Gasa et al., 1991) suggests that this is unlikely to be a problem in diets containing such high proportions of grass silage. It is possible that, due to increase in the rate of starch degradation in the rumen, some of the N was absorbed as ammonia and excreted in urine or more amino acid was absorbed but deaminated and excreted in urine.

Reynolds et al. (1997) suggested that, when corn rather than barley is used, there is a possibility of increased starch digestion in the postruminal tract, which could stimulate microbial protein synthesis in the cecum and, hence, increase fecal output of N. In turn, this could possibly manipulate or redirect the output of N from urine to feces through changes in gut-tissue N interactions. Utilization of urea in the hindgut could create a draw in urea N that could then reduce urine N excretion. Mills et al. (1999 a,b) also stated that, as more starch is fermented in the large intestine, a greater loss of microbial biomass occurs in feces. That is supported by this study, in which a decrease of 112 g/kg N intake in urine was observed in the LDS diet.
compared with HDS with a concomitant increase of 60 g/kg N intake in feces. In terms of N pollution by dairy cows, urinary N is less desirable due to its greater tendency to leaching (Pakrou and Dillon, 1995) and volatilization as ammonia, the major source of which is urea from urine (Van Horn et al., 1996).

Tamminga (1992) suggested that N in dairy diets should not exceed 30 g/kg DM for a dairy cow producing 6,250 kg milk/yr for environmental reasons. Later, Tamminga and Verstegen (1996) recommended that total dietary N should be kept to a minimum of 24 g N/kg DM in order to avoid impaired ruminal digestion and because the formulation of diets with low N content is not easy to achieve. Nitrogen intake in this experiment was low: 22 g N/kg DM on average. The results indicate that it is possible to achieve lower N losses without any adverse effect on milk production by using mixtures of energy sources including low degradability starches and balancing the diets according to animal requirements and level of milk production. A higher efficiency of N utilization was noted for cows fed the LDS supplement, which had an intake of 21 g N/kg DM, against an average of 23 g N/kg DM for the other energy sources.

The absence of differences in total milk N output when comparing the different energy sources is probably related to differences in nonprotein N content in milk (DePeters and Ferguson, 1992), which was not measured in the present trial. However, in agreement with Phipps et al. (1987), Sloan et al. (1988), and Aston et al. (1994) and irrespective of the extent of rumen degradation, the starch-based concentrates improved milk protein concentration compared with soluble sugar- or highly fermentable fiber-based supplements. The lowest protein concentration in milk in this trial was on the high-fiber supplement, which agrees with other studies (Aston et al., 1994; Gonda et al., 1996). These results are consistent with other publications on starch utilization in dairy cattle (Nocek and Tamminga, 1991; Reynolds et al., 1997).

**Implications**

It is possible to improve N utilization in dairy cows by decreasing N intake on balanced diets. The amount and form of N excretion can be manipulated by changing the source of energy in the concentrate, without effects on milk yield and quality. Specifically, supplements with a high proportion of low degradable starch decreased urinary N excretion likely due to increased fecal N excretion. With less N absorbed, the efficiency of N utilization would be higher and the amount of N excreted in urine would be less.

**Literature Cited**


Dijkstra, J., J. France, and D. R. Davies. 1998. Different mathematical approaches to estimating microbial protein supply in rumi-


