Influence of dietary rumen-degradable protein supply on rumen characteristics and carbohydrate fermentation in beef cattle offered high-grain diets

S.M. Martín-Orue, J. Balcells*, F. Vicente, C. Castrillo

Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Miguel Servet 177, Zaragoza, Spain

Received 28 October 1999; received in revised form 19 April 2000; accepted 31 July 2000

Abstract

Four crossbred Holstein–Friesian heifers (initial live weight 306 ± 6.1 kg) fitted with rumen and duodenal cannulae were randomly allocated to one of two dietary treatments in a double 2 × 2 crossover design. Both diets were composed of (g/kg as fed) 250 barley straw and 750 concentrate. The concentrate consisted of (g/kg as fed) 655 corn and 225 barley (Diet C) and 225 corn and 655 barley (Diet B), respectively. During Period 1, two heifers were given Diet C and the other two heifers were given Diet B and all four heifers were infused intraruminally, during four sequential 16-day intervals, with four levels of effective rumen degradable protein (ERDP). ERDP was given as an iso-nitrogenous mixture of urea and casein at 0, 25, 50 or 75 g/kg of concentrate intake. Animals offered Diet B ate more DM, OM and NDF than those offered Diet C (97.6, 89.9 and 37.6 g/kg versus 94.4, 87.3 and 31.9 g/kg metabolic live weight (W0.75), respectively (P < 0.05). Starch digestion did not differ significantly between diets, but fibre was better digested in Diet C than in Diet B, i.e. 56.5% versus 47.5%, 51.3% versus 36.4% and 50.5% versus 40.2% for arabinose, xylose and cellulose–glucose digestibilities, respectively (P < 0.05). Mean rumen ammonia concentrations increased linearly from 29.1 mg/l when no ERDP was infused to 184.5 mg/l when ERDP was infused at the highest level. Ruminal pH was lower (P < 0.05) in animals offered Diet B than those offered Diet C (97.6, 89.9 and 37.6 g/kg versus 94.4, 87.3 and 31.9 g/kg metabolic live weight (W0.75), respectively (P < 0.05). Starch digestion did not differ significantly between diets, but fibre was better digested in Diet C than in Diet B, i.e. 56.5% versus 47.5%, 51.3% versus 36.4% and 50.5% versus 40.2% for arabinose, xylose and cellulose–glucose digestibilities, respectively (P < 0.05). Mean rumen ammonia concentrations increased linearly from 29.1 mg/l when no ERDP was infused to 184.5 mg/l when ERDP was infused at the highest level. Ruminal pH was lower (P < 0.05) in animals offered Diet B than those offered Diet C (6.29 versus 6.46) and in ERDP-supplemented rather than unsupplemented diets (6.73 versus 6.28). However, pH never fell below 5.5. There were no differences in effective rumen degradability between Diets B and C, and increasing the ERDP supply promoted an increase in straw (P < 0.05) and corn (P < 0.1) DM disappearance from polyester bags. The diets without ERDP infusion were apparently deficient in degradable N because rumen microbial yield increased from 76.0 to 102.5 g N/d (P < 0.05) when ERDP infusion rate was increased from 0 to 25 g/kg of concentrate, irrespective of which type of grain concentrate was used. With further increases in ERDP, microbial yield maintained constant and there was no further...
effect on intake and digestion of DM, OM and fibre components. The rumen ammonia concentration promoting maximum microbial yield under the conditions of this experiment was approximately 81 mg/l. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Barley; Corn; Grain feeding; Rumen degradable protein; Rumen fermentation

1. Introduction

In the Mediterranean area, because of low pasture availability, diets are based on concentrates. Intensive systems for rearing beef cattle make use of high-grain diets supplemented with straw. High-grain diets may promote particular rumen characteristics such as latent acidosis, reduced rumination and saliva secretion and lower ratios of acetate to propionate in the volatile fatty acids (VFA) produced by rumen microorganisms (Beauchemin and Buchanan-Smith, 1990).

There is limited information concerning the requirements for rumen degradable N (RDN) when high-grain diets are given to steers, and most of that information has been obtained using urea as the source of RDN. However, few experiments have been conducted to determine whether pre-formed degradable true protein (e.g. casein) is required to generate optimum levels of rumen fermentation and microbial growth. Considering the positive effect of peptides, amino acid and/or other growth factors on starch-fermenting bacteria (Argyle and Baldwin, 1989) a source of degradable true protein may be needed to optimise rumen fermentation of high-grain diets (Russell et al., 1992).

Grain protein is the major source of N in cereal-based diets. Barley protein is highly fermentable in the rumen whereas the N fraction in corn is more resistant and tends to escape ruminal fermentation (AFRC, 1993). Therefore, any response in rumen fermentation to different supplemental protein sources may depend on the type of grain being ingested.

The objective of this experiment was to estimate the degradable-N requirements of yearling steers ingesting concentrates based mainly on either barley or corn grain. The optimum level was established by analysing the effect of supplying increasing rates of intraruminal infusion of ERDN (as an iso-nitrogenous mixture of urea and casein on: (1) digestive characteristics and rate, site and extent of digestion, and (2) rumen microbial production. A brief account of these results has been reported previously elsewhere (Martín-Oruè et al., 1998).

2. Material and methods

2.1. Animals and diets

Four crossbred heifers (average initial live weight (W) $306 \pm 6.1$ and final W $402 \pm 7.1$ kg) were each surgically fitted with a simple cannula in the rumen (6.5 cm i.d.)
and a T-shape cannula in the proximal duodenum (5 cm distal from the pylorus). They were allowed to recover for 2-month before the experiment was started. The animals were housed in 10 m² pens and given continuous access to drinking water. A double 2 × 2 crossover design was used, with each animal being randomly assigned to one of two experimental diets. These diets, consisting of 75% concentrate and 25% of straw, were offered to the animals twice daily (8.00 and 17.00 h). The concentrate was formulated with different percentages of corn and barley, i.e. 65.5–22.5% Diet C, and 22.5–65.5% Diet B, respectively (see Table 1). Concentrate ingredients were ground to pass a 6 mm sieve and straw was chopped to 10 cm. The heifers were given unrestricted access to a mixed diet during an adaptation period when voluntary intake was recorded. This intake (approximately 2.2% kg/kg W) was then the ration offered throughout the experiment. In addition, the heifers receiving both basal diets were continuously infused into the rumen, during sequential 16-day intervals, with four levels of effective rumen degradable protein (ERDP) as an iso-nitrogenous mixture of urea and casein, i.e. 0, 25, 50 or 75 g ERDP/kg of concentrate offered. Two animals (one from each experimental diet) received these ERDP infusions at rates in a decreasing and two in an increasing sequence.

2.2. Experimental treatment

In each of the two experimental periods, heifers were stepped up to the experimental diet for 30 days before the start of the four sequential infusion. Each infusion interval
lasted for 16 days which included 6 days for adaptation to the ERDP supply and 10 days for sample collection. At the beginning of each infusion interval the weight of the animals was recorded. Activities during each infusion interval were as follows: ERDP was then infused continuously into the rumen for the whole 16 days; digesta flow markers were infused from days 3 to 14; a digestibility trial was performed from days 7 to 10; the kinetics of dry matter (DM) disappearance from polyester bags was determined on days 7 to 8; collection of duodenal digesta was performed on days 13 to 14 and rumen liquor was sampled for isolation of bacteria, protozoa counting and characterisation of rumen fermentation on days 15 and 16.

Casein was diluted daily in 2 l of alkaline solution (with NaOH to pH 12) and then pH was adjusted to 6–7 with 2 M HCl. Urea and ammonium sulphate (13% w/w) were diluted in 0.5 l of tap water. Casein and urea solution were then mixed and, during marker infusions, 0.25 l of chromium ethylene diamine tetra-acetic acid (Cr-EDTA) was added and the final solution made up to 3 l using tap water. This solution was infused continuously into the rumen (2 ml/min). Cr-EDTA (120 mg Cr/kg DMI) was prepared as described by Downes and McDonald (1964). Ytterbium acetate was diluted in distilled water and infused intraruminally via a separate tube (to avoid Yb precipitation) (0.45 ml/min; 50 mg Yb/kg DMI). All the ingredients were continuously infused using a peristaltic pump (Minipuls, Gilson, Villers le Bel, France). Infusion of the solution onto the side of the rumen was prevented by inserting 30 cm of tubing inside the rumen. During the digestibility trial, faeces were collected from the concrete floor every two hours. All samples collected were bulked and homogenised each day, sampled (5% on a fresh matter basis), and stored in plastic bags at −20°C. Urine was collected daily under sulphuric acid (to maintain pH below 3) by means of external separators glued to the vulva. After recording weight and specific gravity, two individual sub-samples (1% of total urine collected) were stored at −20°C.

Corn, barley and straw were incubated in polyester-bag to determine kinetics of dry matter disappearance. Samples of each ingredient were ground through a 3 mm screen and 5 g was placed in 15 × 20 cm polyester bags (70 × 40 μm mesh size and 1700 pores per cm²) and suspended in the rumen in duplicate sets. Twenty-four bags were placed in the rumen of each animal immediately before feeding (8.00 h), and six bags (3 duplicate ingredients) were removed after 4, 12, 24 and 36 h. After removal, the bags were rinsed in a washing machine, squeezed by hand and dried at 60°C for 48 h. In addition, two bags of ingredients were included in the washing process to determine disappearance at zero time. Duodenal digesta samples (250 ml) were collected at 6 h intervals for 48 h and refrigerated. These samples were pooled at the end of each collection period and centrifuged (1000 × g for 5 min) and the supernatant and solids (Faichney, 1975) were freeze-dried separately for subsequent analyses.

Finally, rumen fluid was sampled by means of a manual vacuum pump at 0, 1, 2, 4, 6, 8, 12, 24 and 36 h after the end of marker infusion (after the 8.00 h feeding). The pH in these samples was recorded immediately and the fluid was then filtered through four layers of surgical gauze to remove coarse particles. Three samples were taken from the filtered rumen fluid: one sample (40 ml) was frozen and stored for Cr determination and the other two samples were acidified, one with HCl (25 ml 0.2 M HCl was added to 25 ml rumen fluid) and the other with H₃PO₄ (1 ml of 0.5 M H₃PO₄, 50 mM 4-methyl-valerate
per 4 ml of rumen fluid) and stored at −20°C until analysis for ammonia-N and VFA concentrations, respectively. Changes in pH, and ammonia and VFA concentrations were determined during the 12 h after the feeding time at 8.00 h. The Two additional samples were taken at 24 and 36 h for Cr determination and to enable estimation of liquid outflow rate. Samples for isolation of a microbial fraction were taken at 0, 2, 6 and 12 h after the 8.00 h feeding time from the ventral sac of the rumen. Samples for each animal were pooled and the resulting composite sample was squeezed through four layers of surgical gauze plus 50 μm nylon filter to trap particulate matter. A bacterial fraction from the liquid phase was isolated by differential centrifugation procedures: First, an slow-speed centrifugation (500 × g for 5 min) was used to precipitate particulate material and protozoa, and the resulting supernatant was centrifuged at 20000 × g for 20 min at 4°C to deposit a bacterial fraction. This deposit was then resuspended in physiological saline solution and again centrifuged at 20000 × g for 20 min at 4°C. The washed microbial pellet was freeze-dried for subsequent analyses. Samples for protozoa counting were taken 4 h after the 8.00 h feeding time and preserved by mixing them with an equal volume of 18% (v/v) formaldehyde.

2.3. Analytical procedures

Dry matter (DM) was determined by drying samples to a constant weight at 105°C, and organic matter (OM) by ashing at 550°C for 8 h. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) concentrations in feeds were determined by the procedures of Goering and Van Soest (1975). Starch-rich samples were pre-treated with amylase to hydrolyse starch before fibre analysis. Neutral monosaccharide components of diets and digesta samples were analysed by gas liquid chromatography (GLC) as described by Theander (1991) and starch content was analysed after enzymatic hydrolysis with thermostable α-amylase and amyloglucosidase. The glucose released was determined using glucose oxidase (Theander and Westerlund, 1986). Total N content of feed, digesta, faeces, incubation residues and non-ammonia N (NAN) were determined by the Kjeldahl method, using Se as the catalyst. Ammonia-N was analysed by the colorimetric method described by Chaney and Marbach (1962) after centrifugation of an acidified rumen liquor sample (13000 × g for 30 min). Purine bases (PB), used as a microbial marker, were analysed in digesta and bacterial extracts after perchloric acid hydrolysis (Martín Orúe et al., 1995). Cr and Yb concentrations were determined by atomic absorption spectrometry: either after solubilization of these markers from ashed samples (Siddons et al., 1985), or directly in the supernatant fraction from rumen liquid after centrifugation at 2500 × g for 20 min. VFA concentrations in deproteinized rumen fluid were determined by GLC, using the method of Jouany (1982). Protozoa were counted by the method used by Dehority (1993).

2.4. Calculations and statistical analysis

Nutrient flow to the duodenum was calculated by reference to Cr-EDTA and Yb-acetate as fluid and particle markers, respectively, following the procedure of Faichney (1975). Liquid outflow rate was obtained from the dilution curve of Cr concentration in
the rumen fluid once Cr-infusion was stopped. Duodenal PB were assumed to be microbial in origin and microbial flow was calculated using the bacterial PB/N ratio.

Kinetics of DM and N disappearance from polyester-bags were described by the non-linear equation proposed by Ørskov and McDonald (1979), viz. \( d = a + b \left(1 - e^{-ct}\right) \) where \( d \) represents the proportion of N or DM that disappears during time \( t \), and \( a, b \) and \( c \) are constants (fitted by an iterative least-square procedure) that are considered to represent the rapidly soluble fraction, the potentially degradable fraction and its fractional rate of degradation, respectively. The fitted equation was constrained so that \((a + b)\) did not exceed 1000 g/kg. Effective degradability \( (dg) \) values for DM and N \( (dg = a + [bc/(c + k)]) \) was calculated assuming a fractional outflow rate from the rumen \( (k) \) of 5%/h.

Results were analysed as a randomised block design with the animals considered as a random variable. Data were subjected to a split plot as follows. Main plot effects: Diet (D), Animal (A) and Period (P) were compared with the first error term (E1, Interaction: animal × period). Sub-plot effects: Level of ERDP supplementation (within every animal and diet, L) and their interactions, were compared with the residual error term (E2). The carry-over effect of a previous treatment (increasing or decreasing order or ERDP supply, T) was also compared with E1. The sum of squares of supplementation level (L) was split up into one set of orthogonal contrasts: (1) unsupplemented versus supplemented diets; (2) Level 1 versus Levels 2 and 3; and (3) Level 2 versus Level 3. All the analyses followed the procedures of Steel and Torrie (1980).

3. Results

Chemical composition of the straw and the concentrates used are shown in Table 1. The animals remained in good health throughout the experiment. In general, the diets were well accepted by the animals. Refusals amounted to <5% of the feed offered except for two animals (A3 and A4) during one of the infusion periods when refusals amounted to more than 20% of the feed offered. The corresponding data were considered as missing values in the statistical analyses. The average daily gains of the four animals during the experiment were: 703 ± 40.4, 512 ± 72.9, 401 ± 40.3 and 590 ± 43.9 g/d. Growth rates were 637 ± 52.7 and 614 ± 54.4 g/d for animals fed Diet C and B, respectively, and did not differ significantly between diets.

3.1. Intake and digestibility of carbohydrates

Intake and duodenal flow of DM, OM, NDF and digestibility in the rumen and total gastrointestinal tract are shown in Table 2. Because the interactions between the basal diet and rate of ERDP infusion were not significant, only the main effects are indicated in the tables. Intake of DM tended \( (P < 0.1) \) to be higher for animals which were fed Diet B than for animals fed the Diet C. These differences were statistically significant when intake was expressed in terms of metabolic live weight (97.6 versus 94.4 g DM per kg \( W^{0.75} \) for barley and corn diets, respectively \( (P < 0.05) \)). In none of the cases were the differences modified by ERDP supply.
Table 2
Daily intake (kg per day), duodenal flow (kg per day) and digestibility (%) in the rumen and in the total gastrointestinal tract (TGIT) of dry matter (DM), organic matter (OM) and neutral detergent fibre (NDF) and digested organic matter intake (DOMI, kg per day) in four heifers fed corn and barley concentrates supplemented with different levels of effective rumen degradable protein

<table>
<thead>
<tr>
<th>Diets</th>
<th>Level of infusion</th>
<th>RSD1</th>
<th>RSD2</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DM</th>
<th>C</th>
<th>B</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td>7.45</td>
<td>7.81</td>
<td>7.59</td>
<td>7.55</td>
<td>7.59</td>
<td>7.78</td>
<td>0.314</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>4.60</td>
<td>4.97</td>
<td>4.72</td>
<td>4.65</td>
<td>5.18</td>
<td>4.60</td>
<td>1.027</td>
<td>0.814</td>
</tr>
<tr>
<td></td>
<td>41.9</td>
<td>38.2</td>
<td>41.4</td>
<td>38.7</td>
<td>38.8</td>
<td>40.9</td>
<td>2.28</td>
<td>8.39</td>
</tr>
<tr>
<td></td>
<td>60.3</td>
<td>66.5</td>
<td>60.6</td>
<td>64.2</td>
<td>64.1</td>
<td>64.8</td>
<td>8.52</td>
<td>4.10</td>
</tr>
<tr>
<td>OM</td>
<td>6.89</td>
<td>7.20</td>
<td>7.04</td>
<td>6.98</td>
<td>7.00</td>
<td>7.15</td>
<td>0.257</td>
<td>0.283</td>
</tr>
<tr>
<td></td>
<td>3.92</td>
<td>4.29</td>
<td>4.08</td>
<td>3.91</td>
<td>4.47</td>
<td>3.97</td>
<td>0.823</td>
<td>0.733</td>
</tr>
<tr>
<td></td>
<td>46.7</td>
<td>41.4</td>
<td>44.9</td>
<td>44.3</td>
<td>42.5</td>
<td>44.4</td>
<td>1.25</td>
<td>8.30</td>
</tr>
<tr>
<td></td>
<td>61.8</td>
<td>67.9</td>
<td>62.1</td>
<td>65.7</td>
<td>65.6</td>
<td>65.8</td>
<td>8.10</td>
<td>3.78</td>
</tr>
<tr>
<td></td>
<td>60.2</td>
<td>58.1</td>
<td>58.2</td>
<td>61.4</td>
<td>58.2</td>
<td>59.1</td>
<td>1.67</td>
<td>11.39</td>
</tr>
<tr>
<td></td>
<td>4.25</td>
<td>4.87</td>
<td>4.37</td>
<td>4.56</td>
<td>4.60</td>
<td>4.70</td>
<td>0.441</td>
<td>0.337</td>
</tr>
<tr>
<td>NDF</td>
<td>2.52</td>
<td>3.00</td>
<td>2.74</td>
<td>2.78</td>
<td>2.82</td>
<td>2.71</td>
<td>0.143</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>1.31</td>
<td>1.80</td>
<td>1.52</td>
<td>1.44</td>
<td>1.68</td>
<td>1.58</td>
<td>0.268</td>
<td>0.233</td>
</tr>
<tr>
<td></td>
<td>48.4</td>
<td>40.3</td>
<td>45.5</td>
<td>48.7</td>
<td>41.0</td>
<td>42.3</td>
<td>4.92</td>
<td>7.05</td>
</tr>
<tr>
<td></td>
<td>46.0</td>
<td>57.8</td>
<td>45.9</td>
<td>50.0</td>
<td>50.6</td>
<td>49.1</td>
<td>6.40</td>
<td>4.69</td>
</tr>
</tbody>
</table>

a RSD1: residual standard deviation of animal × period as error term; RSD2: residual standard deviation of residual error term; D: statistical significance of the diet effect; P: statistical significance of the ERDP level effect using the following orthogonal contrast: C1: level 0 versus 1–3; C2: level 1 versus 2 and 3.

b N.S.: not significant.

* $P < 0.10$.

** $P < 0.05$.

*** $P < 0.01$. 
Digestibilities of DM, OM and NDF were not affected by diet but increased significantly with increasing ERDP supply (60.6% versus 64.4%, 62.1% versus 65.7% and 45.9% versus 49.9% for DM, OM and NDF for unsupplemented versus supplemented diets, respectively). However, rumen DM and OM digestibilities were not significantly altered by ERDP supply, but were higher \( (P < 0.05) \) in Diet C than in Diet B (41.9 versus 38.3 and 46.7 versus 41.4 for rumen DM and OM, respectively).

Rumen digestibilities of starch and fibre are presented in Table 3. Values for galactose, rhamnose, mannose and fructose were not relevant, and thus, only glucose-cellulose, arabinose and xylose were considered as a fibre fraction. No significant differences were detected in the amount of starch apparently digested in the rumen. The fibre fraction, however, was apparently better digested in Diet C than in Diet B \( (P < 0.05) \).

3.2. Rumen fermentation parameters

Fig. 1 shows the changes in rumen NH₃ concentration throughout the day. Mean and minimum values of NH₃ concentration and pH, weighted with times, are given in Table 4. Total VFA concentration in rumen fluid, proportions of acetate, propionate and butyrate, the acetate-propionate ratio and dilution rate of the liquid phase are also indicated.

Continuous ERDP infusion did not overcome the daily variations in rumen ammonia concentration that occurred when the heifers received the unsupplemented basal diets, otherwise changes throughout the day increased with increasing ERDP infusion rates (Fig. 1). Maximum concentrations were reached 1 h after the 8.00 h feeding time and then decreased to minimum values after 5–6 h (approx.). However, daily patterns of ERDP concentrations over time were similar and changes in the ammonia concentration in relation to experimental treatments did not depend on the sampling time. Mean concentrations of rumen ammonia increased proportionally from 29 to 185 mg/l with increasing rates of ERDP supply.

Ruminal pH peaked at the 8.00 h (feeding time) and then fell to minimum values between 6 and 12 h later. The minimum pH was up to 5.5 and the average minimum pH tended \( (P < 0.10) \) to be lower with Diet B than Diet C (5.71 versus 5.89). Mean pH values were lower \( (P < 0.05) \) for animals offered Diet B than for animals offered Diet C (6.29 versus 6.37; \( P < 0.05) \). ERDP infusion affected pH values, which were lower in, ERDP-supplemented than unsupplemented diets (6.46 versus 6.28, C1: \( P < 0.05) \).

Ruminal VFA concentration was higher \( (P < 0.05) \) for animals which were fed barley than for those which were fed the corn diets and ERDP-availability caused an increase \( (P < 0.05) \) in total VFA. The proportion of individual VFA was also affected both by diet and ERDP supply. Diet B tended to generate \( (P < 0.1) \) higher concentrations of acetate whereas Diet C generated significantly higher propionate concentrations \( (P < 0.05) \). As a consequence, the acetate to propionate ratio was higher in barley than in corn diets \( (P < 0.001) \). ERDP supply also generated a significant increase in propionate \( (C1: P < 0.01) \) and a decrease in acetate to propionate ratio in ERDP-supplemented diets \( (C1: P < 0.01) \).

*Entodinium* species formed the main fraction of total ciliate protozoa (87.8%); *Epidinium* (4.6%), *Isotricha* (4.5%) and *Dasytricha* (3.1%) were also identified. Total
Table 3
Daily intake (g per day), duodenal flow (g per day) and apparent rumen digestibility (%) of the main carbohydrates in four heifers fed corn and barley concentrates supplemented with different levels of effective rumen degradable protein\(^a\)

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Intake</th>
<th>Flow duodenum</th>
<th>Apparent digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>B</td>
<td>0</td>
</tr>
<tr>
<td>Starch–glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>3564</td>
<td>3290</td>
<td>3483</td>
</tr>
<tr>
<td>Flow duodenum</td>
<td>1329</td>
<td>1014</td>
<td>1198</td>
</tr>
<tr>
<td>Apparent digestibility</td>
<td>65.8</td>
<td>70.5</td>
<td>66.2</td>
</tr>
<tr>
<td>Cellulose–glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>897.2</td>
<td>1030.1</td>
<td>955.2</td>
</tr>
<tr>
<td>Flow duodenum</td>
<td>445.6</td>
<td>637.1</td>
<td>548.4</td>
</tr>
<tr>
<td>Apparent digestibility</td>
<td>50.5</td>
<td>40.2</td>
<td>44.6</td>
</tr>
<tr>
<td>Arabinose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>161.9</td>
<td>185.7</td>
<td>177.4</td>
</tr>
<tr>
<td>Flow duodenum</td>
<td>70.5</td>
<td>101.9</td>
<td>87.3</td>
</tr>
<tr>
<td>Apparent digestibility</td>
<td>56.5</td>
<td>47.5</td>
<td>51.3</td>
</tr>
<tr>
<td>Xylose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake</td>
<td>431.4</td>
<td>491.3</td>
<td>459.9</td>
</tr>
<tr>
<td>Flow duodenum</td>
<td>210.6</td>
<td>315.0</td>
<td>261.6</td>
</tr>
<tr>
<td>Apparent digestibility</td>
<td>51.3</td>
<td>36.4</td>
<td>44.0</td>
</tr>
</tbody>
</table>

\(^a\) RSD1: residual standard deviation of animal × period as error term; RSD2: residual standard deviation of residual error term; D: statistical significance of the diet effect; \(P\): statistical significance of the ERDP level effect using the following orthogonal contrast: C1: level 0 versus 1–3.

\(^b\) N.S.: not significant.

\(* P < 0.10.\)

\(** P < 0.05.\)

\(*** P < 0.01.\)
Fig. 1. Rumen NH$_3$ concentration (mg/l) in four heifers, 0, 1, 2, 4, 6, 8 and 12 h after 8.00 h feeding, fed high concentrate diets based on corn (C) or barley (B), supplemented by continuous infusion of different levels of effective rumen degradable protein (ERDP) 0, 25, 50 and 75 g/kg of concentrate.
Table 4
Rumen NH$_3$ concentration, pH, VFA, protozoa concentration and outflow rate in four heifers fed corn and barley concentrates supplemented with different levels of effective rumen degradable protein

<table>
<thead>
<tr>
<th>Diets</th>
<th>NH$_3$ (mg/l)</th>
<th>Level of infusion</th>
<th>RSD1</th>
<th>RSD2</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>B</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Daily mean</td>
<td>102</td>
<td>111</td>
<td>29</td>
<td>81</td>
<td>133</td>
</tr>
<tr>
<td>Minimum</td>
<td>43.2</td>
<td>38.4</td>
<td>10.0</td>
<td>24.0</td>
<td>51.9</td>
</tr>
<tr>
<td>pH Daily mean</td>
<td>6.37</td>
<td>6.29</td>
<td>6.46</td>
<td>6.21</td>
<td>6.35</td>
</tr>
<tr>
<td>Minimum</td>
<td>5.89</td>
<td>5.71</td>
<td>5.97</td>
<td>5.70</td>
<td>5.83</td>
</tr>
<tr>
<td>VFA Total (mM)</td>
<td>108</td>
<td>112</td>
<td>101</td>
<td>116</td>
<td>116</td>
</tr>
<tr>
<td>Acetate (mol/100 mol)</td>
<td>62.1</td>
<td>63.9</td>
<td>64.0</td>
<td>62.7</td>
<td>61.7</td>
</tr>
<tr>
<td>Propionate (mol/100 mol)</td>
<td>21.7</td>
<td>20.0</td>
<td>18.7</td>
<td>21.7</td>
<td>22.8</td>
</tr>
<tr>
<td>Butyrate (mol/100 mol)</td>
<td>11.8</td>
<td>11.5</td>
<td>13.1</td>
<td>11.4</td>
<td>10.8</td>
</tr>
<tr>
<td>Acetate/Propionate</td>
<td>3.06</td>
<td>3.31</td>
<td>3.54</td>
<td>3.00</td>
<td>2.96</td>
</tr>
<tr>
<td>Total Protozoa (103/ml)</td>
<td>79.1</td>
<td>59.5</td>
<td>58.3</td>
<td>65.6</td>
<td>61.5</td>
</tr>
<tr>
<td>Entodinium (%)</td>
<td>86.4</td>
<td>89.2</td>
<td>86.1</td>
<td>87.0</td>
<td>89.9</td>
</tr>
<tr>
<td>Epidinium (%)</td>
<td>5.58</td>
<td>3.48</td>
<td>5.80</td>
<td>5.52</td>
<td>2.89</td>
</tr>
<tr>
<td>Liquid dilution rate (%/h)</td>
<td>9.44</td>
<td>9.78</td>
<td>10.16</td>
<td>9.66</td>
<td>9.41</td>
</tr>
</tbody>
</table>

* RSD1: residual standard deviation of animal × period as error term; RSD2: residual standard deviation of residual error term; D: statistical significance of the diet effect; P: statistical significance of the ERDP level effect using the following orthogonal contrast: C1: level 0 versus 1–3; C2: level 1 versus 2 and 3.

* N.S.: not significant.

* $P < 0.10$.

** $P < 0.05$.

*** $P < 0.01$.

**** $P < 0.001$. 
Fig. 2. Time course of the in situ DM and CP disappearance of corn, barley and straw incubated in the rumen of four heifers fed high concentrate diets based on corn (C) or barley (B) and supplemented by continuous infusion of different levels of effective rumen degradable protein (ERDP): 0, 25, 50 and 75 g/kg concentrate.
protozoa concentration and relative proportion of the different species were not affected by experimental treatment.

Fig. 2 shows the time course of the DM and crude protein (CP) disappearance of corn, barley and straw incubated in four heifers fed corn and barley concentrates supplemented with different levels of effective rumen degradable protein. Effective rumen degradability of the DM and CP in the different foodstuffs did not differ between Diet B and Diet C when these were not supplemented with ERDP; however, increasing the ERDP supply promote an increase in the DM degradability of straw (C1: \( P < 0.05 \)) and corn (C1: \( P < 0.10 \)) as well as a linear increase in protein effective disappearance of barley grain (\( P < 0.05 \)), but this effect was less clear in corn.

### 3.3. Duodenal N flow and rumen microbial synthesis

Although dietary N intake was higher (\( P < 0.05 \)) for animals which were fed Diet B than those on Diet C (Table 6), reflecting differences in both the CP content of the raw material and DM intake (Table 2), passage of total NAN to the duodenum did not differ significantly between diets (Table 6). It was only when unsupplemented diets were fed, did passage of N through the duodenum exceed N intake, suggesting that the recycling of endogenous N had a minimal effect on NAN flow.

Nitrogen and PB contents of the bacterial fractions isolated from the rumen liquid did not differ significantly between diets, and the mean PB/N ratio was 1.16 mmol/g.

### Table 5

<table>
<thead>
<tr>
<th>Diets</th>
<th>Level of infusion</th>
<th>RSD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>B</td>
<td>0</td>
</tr>
<tr>
<td>DM effective degradability&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Straw</td>
<td>29.1</td>
<td>27.1</td>
<td>24.5</td>
</tr>
<tr>
<td>Corn</td>
<td>63.6</td>
<td>64.5</td>
<td>62.2</td>
</tr>
<tr>
<td>Barley</td>
<td>75.8</td>
<td>76.0</td>
<td>75.1</td>
</tr>
<tr>
<td>CP effective degradability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>51.2</td>
<td>51.3</td>
<td>51.4</td>
</tr>
<tr>
<td>Barley</td>
<td>79.2</td>
<td>79.9</td>
<td>77.7</td>
</tr>
</tbody>
</table>

* RSD1: residual standard deviation of animal × period as error term; RSD2: residual standard deviation of residual error term; D: statistical significance of the diet effect; \( P \): statistical significance of the ERDP level effect using the following orthogonal contrast: C1: level 0 versus 1–3; C2: level 1 versus 2 and 3.

<sup>a</sup> Calculated from the model: \( \text{dg} = a + (bc/(c+k)) \), where \( \text{dg} \): effective degradability, \( a \): rapidly soluble fraction, \( b \): fraction that is subjected to degradation, \( c \): rate of degradation, \( k \): digesta passage rate of 5% per h.

<sup>b</sup> Effective degradability, calculated from the model: \( \text{dg} = a + (bc/(c+k)) \), where \( \text{dg} \): effective degradability, \( a \): rapidly soluble fraction, \( b \): fraction that is subjected to degradation, \( c \): rate of degradation, \( k \): digesta passage rate of 5% per h.

<sup>c</sup> N.S.: not significant.

\* \( P < 0.10 \).

\** \( P < 0.05 \).

\*** \( P < 0.005 \).
promoted a higher \( (P < 0.05) \) microbial N flow rate than Diet C and also a trended to produce a more efficient microbial net synthesis expressed in relation to OM apparently digested in the rumen. However, differences between Diet B and Diet C were not significant when microbial production was expressed in relation to OM truly digested in the rumen, or apparently digested in the total gastrointestinal tract.

Increasing the rate of infusion ERDP caused a linear increase in the N content of bacterial fractions isolated from the rumen liquid and also in the concentration of purine bases. Consequently the PB/N ratio was not affected by ERDN infusion rate \((1.14, 1.23, 1.06 \text{ and } 1.23 \text{ mmol/g; RSD 0.156, } P > 0.1)\). The rate of supply of ERDP modified the estimated microbial N flow through the duodenum, although this effect was not linear. Microbial N flow increased significantly at the first level of N supplementation, but did not increase further with higher rates of ERDP infusion. The efficiency of microbial protein outflow from the rumen was not affected by ERDP infusion. The mean value for the whole experiment was 35.8 \( \pm \) 8.5 g microbial N/kg OM apparently digested in the rumen (OMADR) and 23.9 \( \pm \) 3.9 g microbial N/kg OM truly digested in the rumen (OMTDR). However, when microbial production was related to OM apparently digested in the total gastrointestinal tract (TGIT), microbial synthesis efficiency was lower for the unsupplemented diets.

### Table 6

Intake of N, duodenal flow of non ammonia nitrogen (NAN), rumen microbial synthesis and efficiency of synthesis in four heifers fed corn and barley concentrates supplemented with different levels of effective rumen degradable protein $^a$

<table>
<thead>
<tr>
<th>Diets</th>
<th>Level of infusion</th>
<th>RSD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>B</td>
<td>0</td>
</tr>
<tr>
<td>N intake (g per day)$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total NAN</td>
<td>101.6</td>
<td>114.2</td>
<td>100.7</td>
</tr>
<tr>
<td>Microbial N</td>
<td>79.4</td>
<td>107.1</td>
<td>76.0</td>
</tr>
<tr>
<td>Efficiency of synthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/kg OMADR$^d$</td>
<td>29.6</td>
<td>42.1</td>
<td>31.3</td>
</tr>
<tr>
<td>g/kg OMTDR$^e$</td>
<td>21.6</td>
<td>26.3</td>
<td>20.5</td>
</tr>
<tr>
<td>g/kg DOMIf</td>
<td>18.8</td>
<td>21.8</td>
<td>17.3</td>
</tr>
</tbody>
</table>

$^a$ RSD1: residual standard deviation of animal \( \times \) period as error term; RSD2: residual standard deviation of residual error term; D: statistical significance of the diet effect; $P$: statistical significance of the ERDP level effect using the following orthogonal contrast: C1: level 0 versus 1–3; C2: level 1 versus 2 and 3

$^b$ Including effective rumen degradable protein infusion.

$^c$ N.S.: not significant.

$^d$ Organic matter apparently digested in the rumen.

$^e$ Organic matter truly digested in the rumen.

$^f$ Organic matter apparently digested in the total gastrointestinal tract.

* \( P < 0.10. \)

** \( P < 0.05. \)

*** \( P < 0.01. \)
4. Discussion

4.1. Effect of type of grain on digestibility and microbial protein synthesis

Barley and corn were chosen as the main components of the basal diets because of their different patterns of rumen starch and protein fermentation (McAllister et al., 1990). In general, starch and protein are more degradable in barley than in corn. Differences have been mainly attributed to the different properties of the protein matrix that limit, to a variable extent, the access of ruminal bacterial enzymes to starch granules (McAllister et al., 1993).

As pointed out previously, feed was offered twice daily, but the amount offered to each animal was restricted to the voluntary intake recorded before the experiments were started (approx. 2.2% W for both diets). These intakes are slightly lower than the potential intakes that would be predicted by ARC (1980). Nevertheless, heifers offered Diet B showed a higher DM intake than those offered Diet C, and the difference was significant when intake was expressed in terms of metabolic live weight basis. Other published studies do not fully support our findings because when large amounts of corn were replaced with barley, DMI decreased (McCarthy et al., 1989; Casper et al., 1990). Other workers found there were no differences in voluntary feed intake between corn and barley in finishing steers (Boss and Bowman, 1996) and dairy cows (Yang et al., 1997a). In this connection, de Visser and de Groot (1980) have suggested that the high fermentability of barley starch, at higher levels of dietary inclusion, provoke an abrupt decrease in ruminal pH that would limit microbial growth and fibre digestion, and consequently depress DMI. In our case, however, indirect evidence from our estimates of rumen variables and rumen microbial outflow indicates that the rate of fermentation of the barley did not limit the efficiency of rumen fermentation. The pH was lower in animals ingesting Diet B, in accord with previous findings (Boss and Bowman, 1996; Yang et al., 1997a) but the differences in the minimum values between Diet B and Diet C in our study were small. Moreover, the minimum pH values were not indicative of severe acidosis. Nevertheless, rumen VFA concentrations were also higher in barley diets, consistent with the faster rates of fermentation of barley DM, compared with corn DM in our polyester-bag experiments in which effective degradability differences were statistically significant (12.2 and 11.5 percentage units when incubated in the rumens of heifers given Diet C and Diet B, respectively).

Apparent digestibilities of DM and OM in the total gastrointestinal tract, did not differ between diets even though Diet B tended to be more highly digested than Diet C. Our results were consistent with previous reports which demonstrated a little or no effect of the type of cereal (corn versus barley) on total tract digestibility of OM and DM (McCarthy et al., 1989; Overton et al., 1995; Yang et al., 1997b). In relation to rumen digestibility, our data agree with results from other trials indicating that barley starch is more fermentable in the rumen than corn starch (Ørskov et al., 1971; Boss and Bowman, 1996) but its faster fermentation rate may be associated with a reduced rate of ruminal fermentation of fibre (de Peters and Taylor, 1985). Conversely, because the starch granules in corn are protected within a more resistant protein matrix, the rate of ruminal fermentation of corn starch is slower than for barley starch and this promotes a more
stable fermentation and higher rates of fibre digestion (Overton et al., 1995). The effect
seems to be mediated through pH whereby lower pH values affect adhesion and growth of
the microbial population (Chamberlain and Choung, 1995). However, even though pH
was apparently not a limiting factor in our experiment, differences in fibre digestion
between grains were still evident. This implies that other factors apart from sudden pH
variations, such as competition for fermentable substrates, may be the responsible for the
differences in rumen function and DMI.

4.2. Effect of supplemental degradable N

The mean weighted rumen ammonia concentrations for Diet B and Diet C when
unsupplemented with ERDP were 28 and 30 mg/l, respectively. These concentrations are
within the range of those reported previously in ruminants given high-grain diets without
N supplementation (mg/l: 12.6, Slyter et al., 1979; 16.2, Kang-Meznarich and Broderick,
1981; 20.3, Shain et al., 1994; 37.5, Milton et al., 1997) but are lower than minimum
values thought to be necessary to meet microbial requirements for degradable N (Satter
and Slyter, 1974; Balcells et al., 1993). Infusing ERDP allowed us to overcome these
restrictions. Concentrations reached with the top level of ERDP supply (185 mg/l)
exceeded most of the values associated with optimum microbial protein synthesis in vivo.
Although, Mehrez et al. (1977) found that even higher values (235 mg/l) were needed to
achieve the maximum level of DM disappearance from polyester bags. No interaction
diets × ERDP supply was observed in rumen ammonia concentration. The response in
rumen NH₃ concentration was proportional to the level of ERDP infusion and this means
that neither endogenous recycling nor microbial uptakes were unable to compensate for
differences in protein infusion. Our results differ from those of other workers (Milton
et al., 1997; Kang-Meznarich and Broderick, 1981) who found that rumen NH₃
concentration did not differ, when steers were given diets with urea concentrations
increasing from 0 to 0.5% or 1%. These differences between studies may be explained by
different N sources or the effects of other dietary ingredients. In this context, Streeter
and Mathis (1995) reported a linear increase in rumen NH₃ using a pre-formed protein
(fishmeal) and similar experimental conditions.

In our experiment, continuous infusion of ERDP did not maintain a constant rumen
NH₃ concentration. The concentration increased after the 8.00 h feeding time, when N
intake and ERDP-supply were coincident, and then decreased (Fig. 1). This pattern might
reflect a lack of synchronisation between energy and degradable N availability. A similar
limitation was described by Erdman et al. (1986) in cows receiving a continuous ruminal
infusion of urea, although Mehrez et al. (1977) obtained a fairly constant rumen NH₃
concentration in sheep by continuous feeding of the basal diet.

Bacterial growth is largely dependent on the amount of degradable N and fermentable
OM in the rumen. Most of the bacterial species can use ammonia as their sole N-source
although, a substantial improvement in microbial yield has been reported when pre-
formed amino acids are present for both in vitro (Argyle and Baldwin, 1989) and in vivo
experiments (Maeng and Baldwin, 1976). High-grain diets provide a source of ready
fermentable carbohydrates but the amount of ERDP supplied by the cereal may not
supply the microbes with an adequate amount of degradable N. Heifers which were fed
the unsupplemented diet (9.4 and 10.8% CP, for corn and barley diets, respectively) responded with an improvement in microbial outflow from the rumen (from 76.0 to 102.5 g/d) when 2.5% of CP per kg of concentrate was supplied, and the effect was independent of the type of grain used. Beyond 2.5%, microbial outflow was maintained at a constant value. Microbial yield efficiency showed a similar pattern although it tended to plateau at the third highest level of ERDP infusion. In general, changes were not statistically significant, probably because of the variability inherent in such measurements. Rumen ammonia concentration, as an indicator of the availability of ruminal degradable N, was modified by ERDP supply and our results indicated that rumen NH₃ concentrations of 29 mg/l were not able to sustain the maximum level of microbial production. The concentration required to achieve this would have been somewhere between 30 and 81 mg/l in the conditions of this experiment. The concentration of rumen ammonia required for optimal microbial production on various diets is not well defined. Several authors (Ørskov et al., 1971; Milton et al., 1997) have been described a lack of response to urea supplementation in high grain diets, whereas other workers obtained responses similar to ours. Slyter et al. (1979) found that microbial yield reached a plateau when rumen ammonia was around 22 mg/l and Kang-Meznarich and Broderick (1981) found that the maximal yield occurred between 33 and 85 mg ammonia per litre. Other authors, using protein-free diets (Hume and Bird, 1979) or sodium-treated straw (Balcells et al., 1993) as basal diets, found that microbial protein synthesis was stimulated by increasing ammonia concentration to between 88–133, or 114 mg/l rumen fluid, respectively.

In relation to the effect of pre-formed degradable protein on microbial yield, Rooke and Armstrong (1989) demonstrated an increase in microbial production when urea-N was replaced with casein-N. Similarly, Milton et al. (1997) found an increase in microbial protein synthesis and efficiency when urea-N was replaced with soyabean-N. From our results, it is not possible to define the effect of pre-formed protein on microbial yield. However, our results, using a mixture of pre-formed peptides and amino acids in addition to simple sources of ammonia (i.e. urea) did not enhance rumen fermentation and microbial growth more than has been found in similar feeding conditions with urea as the sole ERDP supplement.

In the present experiment, neither rumen digestion of DM, OM and fibre components, nor effective rumen degradability of barley and corn were significantly affected by ERDP supply. However, the fact that ERDP supply significantly increased the rate of DM disappearance of incubated straw, may confirm that ammonia N is essential for the growth of many bacterial populations in the rumen and especially for some cellulolytic species that cannot use any N sources other than ammonia. Rumen ammonia-N deficiency, thus, seems to act as a depressing factor by limiting the growth of cellulolytic bacteria.

5. Implications

The high concentrate diets consisting of cereal grains, used in intensive beef cattle system are lacking in degradable protein and this limits microbial fermentation and may affect ruminal fibre digestion. Effective rumen degradable protein enhances microbial
protein supply to the host animal without any considerable increase in digestion of OM and starch. In the conditions of the present experiment, barley is a source of high fermentable starch likely to maintain a higher level of microbial N supply to the duodenum than corn. No differences in the ERDP supply required to maintain the maximum microbial growth were detected between the two cereals.

Acknowledgements

This work has been supported by the CICYT Project AGF 94-0415-C02-01. We would like to thank to Dr. J. Nolan for the critical review of the manuscript.

References


