J. Dairy Sci. 89:3552–3562

Effects of Dairy Cow Diet Forage Proportion on Duodenal Nutrient Supply and Urinary Purine Derivative Excretion

J. M. Moorby,*[1] R. J. Dewhurst,†[2] R. T. Evans,* and J. L. Danelón†
*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK
†Faculty of Agronomy, University of Buenos Aires, Avenida San Martín 4453, (C1417 DSQ) Buenos Aires, Argentina

ABSTRACT

Four mature Holstein-Friesian dairy cows were used in a 4 × 4 Latin square change-over design experiment made up of four 4-wk periods to investigate the relationship between microbial protein flow to the duodenum and excretion of purine derivatives (PD) in the urine. Four dietary treatments based on ad libitum access to ryegrass silage were offered, with a standard dairy concentrate included at different forage:concentrate (F:C) ratios, calculated on a dry matter basis: 80:20, 65:35, 50:50, and 35:65. Feed intakes increased as the proportion of concentrate in the diet increased, despite a concurrent decrease in silage intake. Increased feed intake led to increased nutrient flow to the duodenum. Milk yields increased as the diet F:C ratio decreased, with cows offered the 35:65 diet yielding nearly 8 kg/d more milk than cows offered the 80:20 diet; the concentrations of milk fat decreased and milk protein increased with a decreasing F:C ratio. Purine derivative excretion in the urine increased with an increasing proportion of concentrate in the diet, and there was a strong linear relationship between total PD excretion (allantoin and uric acid) and microbial N flow to the duodenum: microbial N (g/d) = 19.9 + 0.689 × total PD (mmol/d); R = 0.887. This strengthens the case for using PD excretion as a noninvasive marker of microbial protein flow from the rumen in dairy cows.
Key words: dairy cow, nitrogen partitioning, purine derivative, rumen function

INTRODUCTION

Microbial protein synthesis in the rumen is often the main component of the metabolizable protein supply in dairy cows (NRC, 2001). Many studies have estimated microbial protein flow from the rumen using inert marker techniques and cannulated animals. However, these invasive techniques are difficult to carry out and have not led to robust models for predicting microbial protein synthesis as part of rationing models (Titgemeyer, 1997; Dewhurst et al., 2000a), even though models that are currently used to predict microbial protein synthesis in this way are successful in most production settings. We have focused on less-invasive and noninvasive techniques for estimating microbial protein synthesis because they have the potential for development of novel diagnostic tests for commercial application of research. Urinary excretion of purine derivatives (**PD**) offers a way of estimating microbial N flow from the rumen (Johnson et al., 1998; González-Ronquillo et al., 2004) because most feed purines are broken down by rumen microbes (McAllan and Smith, 1973). Therefore, the majority of purines absorbed from the small intestine, degraded, and excreted in the urine are of microbial origin (McAllan, 1980). Previous studies have investigated the effects of a range of supplements and forage types (Johnson et al., 1998) and feed restrictions (González-Ronquillo et al., 2004), but not the effects of widely different forage-to-concentrate (**F:C**) ratios on the relationship between urinary PD excretion and duodenal microbial protein flow. It is possible that changes in purine metabolism would invalidate the use of urinary PD excretion for comparisons of such widely different diets.

We hypothesized that decreasing the F:C ratio of the diet of dairy cows would increase nutrient intake and generate an increased microbial protein flow to the duodenum that in turn would increase the excretion of PD in the urine. Therefore, the objective of this experiment was to investigate this relationship, stimulating differences in microbial protein yield from the rumen by offering diets differing in a wide range of F:C ratios to alter feed intake and the supply and utilization of nutrients in the rumen.

MATERIALS AND METHODS

Cows and Experimental Design

All procedures used in this experiment were licensed and regulated by the UK Home Office under the Ani-

Received September 14, 2005.
Accepted April 28, 2006.
[1]Corresponding author: jon.moorby@bbsrc.ac.uk
[2]Current address: Agriculture and Life Sciences Division, Lincoln University, Canterbury, New Zealand.

Table 1. Ingredients of the concentrate feed used in the experiment

Ingredient	Total content, % as fed
Wheat	30
Rapeseed meal	15
Corn gluten feed	15
Extracted linseed meal	12
High protein extracted sunflower meal	11
Sugar beet pulp	6
Molasses	6
Extracted palm kernel meal	2
Other[1]	3

[1]Vegetable oil, calcium carbonate, vitamin and mineral premix (7,500 IU of vitamin A/kg, 2,250 IU of vitamin D_3/kg, 10 IU of α-tocopherol/kg, 0.30 mg of sodium selenite/kg, and 40 mg of cupric sulfate/kg), salt, calcined magnesite.

mals (Scientific Procedures) Act of 1986. Four Holstein-Friesian dairy cows previously prepared with simple cannulas in the rumen (Bar-Diamond, Parma, ID) and proximal duodenum in midlactation [starting at a mean of 90 (SD = 33.6) DIM; mean BW of 627 kg (SD = 53.3)] were used in a 4 × 4 Latin square change-over experimental design with 28-d periods. The first 2 wk of each period were used for adaptation to the diets, and the last 2 wk were used for measurements. Animals were housed in individual stalls, and had free access to fresh water and a trace mineral lick (Red Baby Rockies, Tithebarn Ltd., Cheshire, UK). Cows were milked twice per day, at approximately 0800 and 1600 h, and all milk yields were recorded.

Dietary Treatments

Four dietary treatments were used, comprising the same second-cut ryegrass silage and standard dairy concentrate (Table 1), and were offered at 4 F:C ratios (DM basis): 80:20, 65:35, 50:50, and 35:65. Diet F:C ratios were achieved by measuring ad libitum silage DMI on a daily basis (silage was offered to allow at least 10% refusals), and allocating the appropriate amount of concentrate to each animal based on a rolling average of their silage DMI from the previous 3 d of the experiment. To reduce the chance of rumen acidosis, sodium bicarbonate was added to all diets at the rate of approximately 1.7% of total DM, mixed in with the concentrate ration, which was offered in 2 equal portions per day, one at each milking. At the start of each experimental period, concentrate allocations were gradually changed to the new F:C ratio in steps of 25% of the difference between the previous and new quantities of concentrate over the course of 6 d, with cows being offered the intermediate allocations for 2 d each.

Measurements and Sample Analysis

Silage and concentrate samples were collected and composited over each week of the experiment, stored frozen at –18°C, and freeze-dried prior to analysis according to the methods described by Dewhurst et al. (2000b).

The first 6 d of the third week of each experimental period were used for the collection of urine and feces for diet digestibility, N partitioning, and urinary PD output measurements. Samples for the calculation of diet digestibility and N partitioning were taken as described by Moorby et al. (2000) from total daily productions of urine and feces collected using an externally applied collection apparatus. Diet ME (Mcal/kg) density was calculated as 0.0037 × digestibility of the OM expressed as a proportion of the DM (Agricultural and Food Research Council, 1993). Daily collections of urine were preserved by acidification (using 1.5 L of 2 *M* sulfuric acid) and subsampled (1% of daily collection) to produce a composite sample for each animal. Feces were also subsampled (5% of daily production) daily after thorough mixing, and composited. Urine and feces were stored at 4°C during the week, and approximately 100-mL subsamples of the mixed composite urine samples were stored frozen for analysis. A further 100 mL of mixed composited urine was diluted with 400 mL of tap water and stored frozen for PD analysis. Urinary PD were measured as described by Dewhurst et al. (1996). Milk samples were taken (1% of milk produced from a.m. and p.m. milkings) and stored at 4°C between milkings, and were composited over the course of the 6-d measurement period. The N concentrations of milk, feces, and urine were measured as described by Moorby et al. (2000), and the milk CP concentration was calculated as N × 6.38. Milk samples were analyzed for fat and lactose by near-infrared spectroscopy (National Milk Records, Yeovil, Somerset, UK).

The final week of each experimental period was used for the measurement of digesta flow from the rumen as described by Dewhurst et al. (2003), with analysis of samples as described by Dewhurst et al. (2000b). Rumen pH was recorded manually with a benchtop pH meter (model EW-59003-25; Cole-Parmer Instrument Co. Ltd., London, UK) using strained digesta at 0900, 1000, 1100, 1300, 1500, 1700, and 2200 h, and the samples taken at these times were analyzed for concentrations of ammonia N and VFA. During the digesta flow measurement periods, ytterbium acetate (mean 584 mg Yb/d; SD = 41.0) and chromium ethylene diamine tetraacetic acid (CrEDTA; mean 2,562 mg of Cr/d; SD = 272.2) were continuously infused into the rumen as particulate and liquid markers, respectively, to allow estimation of digesta flows at the duodenum (Faichney,