Effect of Dietary Carbohydrate Composition and Availability on Utilization of Ruminal Ammonia Nitrogen for Milk Protein Synthesis in Dairy Cows

A. N. Hristov and J. K. Ropp

Department of Animal and Veterinary Science,
University of Idaho, Moscow, ID 83844-2330

ABSTRACT

A trial with four ruminally and duodenally cannulated, late-lactation dairy cows was conducted to investigate the effect of dietary carbohydrate (CHO) composition and availability on ruminal ammonia N utilization and transfer into milk protein. Two diets were fed at 8-h intervals in a crossover design. The diets differed in CHO composition: the ruminally fermentable nonstructural carbohydrates (RFSS) diet (barley and molasses) contained a larger proportion of ruminally available CHO in the nonstructural carbohydrate fractions and the ruminally fermentable fiber (RFNDF) diet (corn, beet pulp, and brewer's grains) contained a larger proportion of CHO in ruminally available fiber. Nitrogen-15 was used to label ruminal ammonia N and consequently microbial and milk N. Fermentation acids, enzyme activities, and microbial protein production in the rumen were not affected by diet. Ruminal ammonia concentration was lowered by RFNDF. Ruminal and total tract digestibility of nutrients did not differ between diets except that apparent ruminal degradability of crude protein was lower for RFNDF compared with RFSS. Partitioning of N losses between urine and feces was also not affected by diet. Milk yield and fat and protein content were not affected by treatment. Average concentration of milk urea N was lower for RFNDF than for RFSS. Proportion of milk protein N originating from ruminal microbial N (based on the areas under the ^{15}N -enrichment curves) was higher for RFNDF than for RFSS. Cumulative recovery of ^{15}N in milk protein was 13% higher for RFNDF than for RFSS indicating enhanced transfer of ^{15}N -ammonia into milk protein with the former diet. The results suggested that, compared to diets containing higher levels of ruminally fermentable starch, diets providing higher concentration of ruminally fermentable fiber may enhance trans-

fer of ruminal ammonia and microbial N into milk protein.

(**Key words:** dairy cow, carbohydrate, rumen ammonia, milk protein)

Abbreviation key: AIA = acid-insoluble ash, ANDF = available NDF, APE = atom percent excess, AUC = area under the curve, MN = microbial nitrogen, MUN = milk urea nitrogen, NSC = nonstructural carbohydrates, NSP = nonstarch polysaccharides, PDA = polysaccharide-degrading enzyme activities, RFNDF = ruminally fermentable fiber, RFSS = ruminally fermentable nonstructural carbohydrates, RS = reducing sugars, TFC = total fermentable carbohydrates.

INTRODUCTION

Efficiency of utilization of dietary N for milk protein synthesis by dairy cows has been calculated at 19 to 20% (Tamminga, 1992; MacRae et al., 1995). This low efficiency is partially due to ammonia N losses in the rumen (Tamminga, 1992). Ruminal ammonia concentration is inversely related to carbohydrate availability (Russell et al., 1983; Hristov et al., 1997; Heldt et al., 1999a). If energy is limiting in the rumen, microorganisms degrade feed protein to ammonia and ammonia uptake by ruminal microorganisms is inhibited (Nocek and Russell, 1988; Hristov et al., 1997). Microbial protein synthesized in the rumen supplies the majority of absorbable amino acids to the small intestine (NRC, 2001). Because rumen microbes form a large proportion of their cell protein from ammonia N (Hristov and Broderick, 1996), enhanced microbial protein synthesis in the rumen may result in more efficient transfer of ruminal ammonia N into body and milk proteins. Energy intake and carbohydrate availability are the primary factors regulating microbial protein yield in the rumen (Rohr, 1986; Lebzien et al., 1993). The Cornell Net Carbohydrate and Protein System distinguished several carbohydrate fractions in feedstuffs related to sugars, starch, and ruminally available or unavailable fiber (Sniffen et al., 1992). Ruminal microorganisms can derive energy for cell protein synthesis from all three

Received November 14, 2002.

Accepted January 24, 2003.

Corresponding author: A. N. Hristov; email: ahristov@uidaho.edu.

types of carbohydrates (Strobel and Russell, 1986), but the effect of carbohydrate type/rate of degradation on ammonia utilization in the rumen has not been adequately studied in vivo. Ammonia is the preferred N source of fiber-degrading bacteria in the rumen (Russell et al., 1992) and provision of ruminally degradable fiber may stabilize ruminal pH and enhance ruminal ammonia utilization (Firkins, 1997). Feng et al. (1993) reported significant reduction in ammonia concentrations in the rumen as the diet contained increasing levels of fiber with a moderate rate of fermentation.

The objective of this work was to investigate the effects of different dietary ratios of ruminally fermentable nonstructural carbohydrates to fiber on ruminal fermentation, nutrient digestibility, urinary N losses, milk yield and composition, and transfer of ruminal ammonia N into milk protein in dairy cows.

MATERIALS AND METHODS

Animals and Feeding

Four multiparous, late-lactation Holstein cows fitted with ruminal (Bar Diamond, Parma, ID) and simple T-type duodenal (Ankom Technology, Fairport, NY) cannulae were used in this experiment. The duodenal cannulae were placed on the ascending duodenum anterior to the pancreatic duct. The cows (average BW 715 ± 33.1 kg; average DIM 323 ± 19.5 d) were cared for according to the guidelines of the University of Idaho Animal Care and Use Committee and were grouped (two cows per group) and fed two diets in a crossover design. The two diets (Table 1) contained similar proportions of alfalfa forage but had either a higher concentration of ruminally fermentable nonstructural carbohydrates (**NSC**, as starch and sugars; Diet **RFSS**) or ruminally fermentable fiber (**RFNDF**) provided with the nonforage components. Dietary concentrations of carbohydrate fractions were analyzed and concentrations of protein fractions and energy content of the diets were estimated using the CPM Dairy program (version 2.0.23, University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY and William H. Miner Agricultural Research Institute, Chazy, NY). Since studying ruminal ammonia utilization and transfer was the focus of this trial, diets were formulated to have high CP content and provide high ammonia concentrations in the rumen. The diets were fed as total mixed rations three times a day at 0600, 1400, and 2200 h. Cows were fed at 90% of ad libitum intake determined before initiation of each experimental period. Diets were mixed in a Data Ranger (American Calan Inc., Northwood, NH). Refusals were collected and weighed daily; composited samples (per cow and per period) were analyzed for chemical composition.

Table 1. Diet composition (% of DM).

Ingredient	Diet ¹	
	RFSS	RFNDF
Alfalfa silage ²	26.7	34.0
Alfalfa hay, chopped	12.6	2.1
Forage, % of DM	39.3	36.1
Barley grain, rolled	38.6	...
Corn grain, cracked	...	20.0
Solvent soybean meal	9.7	8.2
Brewer's grains, dry	...	12.6
Beet pulp, dry	...	20.7
Molasses, dry	9.1	...
Blood meal	0.8	...
Mono calcium phosphate	0.5	0.4
Sodium bicarbonate	1.4	1.4
Mineral/vitamin mix ³	0.6	0.6
Composition, % of DM		
CP	19.5	19.1
NDF	26.1	34.1
ADF	13.5	17.7
Starch	26.7	17.7
Ether extract	1.6	2.5
Lignin	1.7	2.0
Ash	8.4	8.8
ANDF ⁴	22.0	29.2
NSC ⁵	47.8	40.3
NSP ⁶	21.2	22.6

¹RFSS, ruminally fermentable nonstructural carbohydrates; RFNDF, ruminally fermentable fiber.

²Alfalfa silage was 44% DM.

³Composition: 34.5–41.4% NaCl, 26.9–33.8% ground rice hulls, 27.9% barley, 0.24% MnSO₄, 0.21% ZnO₃, 0.052% Cu₂SO₄, 0.0014% Fe₂CO₃, 0.0059% CaI₂O₆.H₂O, 0.0010% CoCO₃, 932 KIU/kg Vitamin A, 818 KIU/kg Vitamin D, and 15 KIU/kg Vitamin E.

⁴Available NDF; calculated as: NDF – (Lignin $\times$ 2.4) (CPM Dairy).

⁵NSC (nonstructural carbohydrates); calculated as: 100 – CP – (NDF–NDFCP) – Ash – Ether extract (CPM Dairy).

⁶NSP (nonstarch polysaccharides); calculated as: NSC – starch.

Each experimental period consisted of 14 d adaptation to the diet and 7 d of sampling.

Markers

Ruminal ammonia N was labeled through continuous 3-d intraruminal infusion of 20 atom percent excess (**APE**) (¹⁵NH₄)₂SO₄ (Cambridge Isotope Laboratories, Inc., Andover, MA) dissolved in distilled water at final concentration of 30.769 mg/ml and infused into the rumen through the rumen cannula via a peristaltic pump (Technicon Instruments Corp., Tarrytown, NY). Due to uneven pump flow, the amount of solution infused per period varied between cows; in Period 1 cows received on average 3688 ± 45 ml and in Period 2 – 3838 ± 45 ml. Infusion was initiated at 0600 h on day 15 and ceased at 0600 h on day 18 of each period. At 0600 h on d 1 of the infusion, 160 ml ¹⁵N-solution were primed into the rumen of each cow.

Total tract apparent digestibility of nutrients was determined using acid-insoluble ash (**AIA**; Van Keulen

and Young, 1977) as an internal digestibility marker. Concentration of AIA in dietary DM was (mean $\pm$ SE) 1.34 ± 0.104 and $1.60 \pm 0.130\%$, RFSS and RFNDF, respectively. Nutrient digestibility was calculated as described by Schneider and Flatt (1975). Flow of DM and nutrients at the duodenum was determined using the double-marker method of Faichney (1975). Li/Co-EDTA and AIA were utilized as liquid and solid phase markers, respectively. Li/Co-EDTA (Uden et al., 1980) was applied to the diets at a daily dose equivalent to 4.2 g of Co/cow from d 5 through the last day of each period. Microbial protein flow to the duodenum was determined with purines as a microbial marker, assuming all purines present in duodenal digesta were of microbial origin. True digestibility of DM, OM, and NAN in the rumen was calculated as: total duodenal flow of DM, OM, or NAN – duodenal flow of microbial DM, OM, or NAN. Cr-EDTA (Uden et al., 1980) was used as a ruminal liquid passage rate marker and was pulse-dosed (equivalent of 2.5 g Cr) into the rumen of the cows at 0600 h on d 15 of each period.

Samples and Analyses

Feeds. Diets and individual feed samples were dried at 70°C to constant weight in an air-forced oven and analyzed for ash, total N, and CP insoluble in NDF solution (Hristov et al., 2001), NDF and ADF (Ankom 200 Fiber Analyzer, Ankom Technology), lignin (sulfuric acid lignin, Daisy^{II} Incubator, Ankom Technology), starch (McCleary et al., 1994; total starch analysis kit from Megazyme International Ireland Ltd., Wicklow, Ireland), and ether extract (AOAC official method 920.39, AOAC International, 1999). A heat-stable amylase (α -amylase, EC No 232-560-9, Sigma Chemical Co., St. Louis, MO) was used in the NDF analysis; sodium sulfite was not used in the analysis. Molasses composition was obtained from the manufacturer (Westway Trading Corp., Minneapolis, MN). Concentration of NSC in individual feed ingredients and the diets was calculated as: $NSC = 100 - [CP + (NDF - NDFCP) + ash + ether extract]$ (CPM Dairy). Concentration of nonstarch polysaccharides (**NSP**) was calculated as: $NSP = NSC - starch$ (Van Soest et al., 1991). Available NDF (**ANDF**) was calculated as: $ANDF = NDF - (lignin \times 2.4)$ (CPM Dairy). Intake of ruminally fermentable ANDF, starch, and NSP was calculated as: DMI from a dietary ingredient $\times$ concentration of ANDF, starch, or NSP in the dietary ingredient $\times [kd/(kd + kp)]$, where kd and kp were rate of degradation and rate of passage, respectively. Rate of degradation (kd) was taken from CPM Dairy and kp was calculated based on DMI, BW, and forage content of the diet (for forage ingredients) and forage kp (for concentrate ingredients) (CPM

Dairy). Rates of degradation of carbohydrate fraction B2 (cellulose and hemicellulose) were applied to ANDF and of fraction B1 (starch, pectins, and β -glucans) - to starch and NSP. Carbohydrate fraction A rates were used for molasses NSP; for simplicity, the difference in degradation rates between soluble sugars and other NSP components of the other dietary ingredients was considered to have a minor effect on NSP rate of fermentation. This type of approach assumes homogeneity of the starch or NSP fractions among individual feeds. For example, while in corn grain (RFNDF) fraction B1 will be predominantly starch, fraction B1 of barley grain or beet pulp will contain large quantities of soluble fiber (β -glucans or pectins, respectively; Van Soest et al., 1991). Total dietary intake of ANDF, starch, or NSP was the sum of the intakes with the individual ingredients. Total fermentable carbohydrates (**TFC**) intake was calculated as: $TFC \text{ intake} = \text{fermentable ANDF intake} + \text{fermentable starch intake} + \text{fermentable NSP intake}$.

Rumen. Rumen samples were taken before (0 h, background) and 1, 3, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, 57, 61, 65, 69, 72, 75, 78, 84, 90, 96, 102, 108, and 114 h after initiation of the $(^{15}\text{NH}_4)_2\text{SO}_4$ infusion. Samples were taken from four locations in the rumen: ventral sac, reticulum, and two from the feed mat in the dorsal rumen (approximately 250 g each). The four samples were composited and squeezed through two layers of cheesecloth. The filtrate was stored on ice and analyzed for pH, ammonia, VFA, reducing sugars (**RS**), and polysaccharide-degrading (**PDA**; carboxymethylcellulase, amylase, and xylanase) and deaminative enzyme activities as described elsewhere (Hristov et al., 2000a). Proteolytic activity in ruminal fluid was determined using ^{15}N -labeled casein as a substrate according to Hristov et al. (2002). Ammonia samples were also analyzed for ^{15}N enrichment (Hristov et al., 2001). Bacterial pellets were isolated through differential centrifugation (an initial centrifugation at $400 \times g$ for 5 min and a subsequent centrifugation of the low-speed supernatant at $20,000 \times g$ for 15 min at 4°C), freeze-dried, and analyzed for OM, N and ^{15}N enrichment (Hristov et al., 2001) and purines (Makkar and Becker, 1999). Samples taken at 0, 1, 3, 5, 9, 13, and 17 h were analyzed for Cr concentration (Iris ICP atomic emission spectrophotometer, Thermo Jarrell Ash Corp., Franklin, MA).

Duodenum. Duodenal samples (300 ml per sampling) were taken at 0900, 1500, and 2100 h (d 4), and at 0300, 0600, 1200, 1800, and 0000 h [d 5 and 6 of the $(^{15}\text{NH}_4)_2\text{SO}_4$ infusion]. Samples were stored frozen at -40°C . After thawing, digesta samples were composited on a weight basis (per cow and period). The composited samples were separated into fluid and solid phases by

filtering through a 100 μ m fabric (Sefar America Inc., Depew, NY). Both phases were freeze-dried, ground through 1 mm sieve, and analyzed for OM, Co (Iris ICP atomic emission spectrophotometer), AIA, N and NAN (Firkins et al., 1992), purines, NDF, and ADF.

Feces and Urine. Grab fecal samples (200 g per sampling) were collected from the rectum at the same sampling times used for duodenal digesta. Samples were composited per cow and period and oven-dried at 70°C to constant weight. Samples were ground through 1 mm sieve and analyzed for OM, N, C (model 1500 C/N analyzer, Carlo Erba Instruments, Milan, Italy), AIA, NDF, and ADF. Urine was collected during the last three days of each period. Catheters and sample preparation are described elsewhere (Hristov et al., 2000b). Urine samples were analyzed for N and allantoin (Hristov et al., 2000b).

Milk. Total milk output was measured and milk was analyzed for fat, protein, and milk urea nitrogen (MUN) (Treasure Valley Milk Testing Lab, Nampa, ID) during the last 7 d of each period. After initiation of the ($^{15}\text{NH}_4$) $_2\text{SO}_4$ infusion, cows were milked at: 0 (background), 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 125, 135, 145, and 155 h. At each milking, milk weights were recorded and two milk samples were taken: one for analyses of milk fat, protein, and MUN and another for analysis of ^{15}N -enrichment of milk protein. Milk protein was precipitated with 65% (w/v) solution of TCA at 5% (w/v) final concentration. Protein pellets were sedimented by centrifuging at 20,000 $\times g$ for 15 min at 4°C, freeze-dried, and analyzed for N and ^{15}N -enrichment.

Calculations and Statistical Analyses

Milk and ruminal bacteria ^{15}N -enrichment (APE) curves were plotted vs time and fitted to the multicompartmental model of Dhanoa et al. (1985), restricted to two compartments and to a five-parameter modified Gaussian model (SigmaPlot 5.0, SPSS Inc., Chicago, IL), respectively. Criteria for best fit were: R^2 (the coefficient of determination), the ANOVA P value for the regression (describing the association between the dependent and independent variables), the Predicted Residual Error Sum of Squares (PRESS, an indicator of how well a regression model predicts new data), the Normality test (indicating the normality of distribution of source population around the regression), and the residuals distribution around the 0 line. Ammonia concentration in the rumen of the cows varied greatly during sampling and ^{15}N infusion (Figure 1). As a result, regression models were not created for ^{15}N enrichment of ruminal ammonia N and consequently, proportions of bacterial and milk protein N originating from ammo-

nia N were not calculated. Areas under the predicted milk protein and bacterial ^{15}N curves (AUC; ^{15}N atom % excess in milk protein N or bacterial N $\times$ h) were computed using the trapezoidal rule (AREA.XFM transform, SigmaPlot 5.0). Proportions of milk protein N originating from ruminal bacterial N were derived based on the respective AUC (Nolan and Leng, 1974).

Intake, rumen fermentation, duodenal nutrient flows, digestibility, and urine data were analyzed using ANOVA assuming a crossover design. The model used was:

$$Y_{ijkl} = \mu + G_i + C(G)_{ij} + P_k + D_l + e_{ijkl} \quad (1)$$

where μ is the overall mean, G , C , P , and D are group, cow, period, and diet, and e is an error term under the usual assumptions for ANOVA.

Rumen fermentation data were averaged per period and cow.

The cumulative amounts of ^{15}N excreted in milk protein (as percentage of the ^{15}N infused in the rumen of each individual cow) were fitted to a logistic model:

$$y = m/(1 + \exp(-k(x-L))) \quad (2)$$

where: y , cumulative ^{15}N excreted in milk protein as percentage of the ^{15}N infused in the rumen at time x ; m , theoretical maximum for y ; k , rate related parameter; L , time to reach 50% of m (maximum ^{15}N recovery in milk protein).

This model was fitted to each treatment and each model was assessed for adequate fit. The estimated models were compared using the dummy variable regression technique (Bates and Watts, 1988).

All data were analyzed using SAS (SAS, 1996).

RESULTS

As estimated by CPM Dairy, the two diets contained (DM basis): $\text{NE}_L = 1.66$ and 1.67 Mcal/kg; metabolizable protein = 10.6 and 10.4%; protein fractions A + B1 (NPN and soluble true protein) = 33 and 33% of CP; protein fractions B2 + B3 (N soluble in neutral and acid detergents) = 60 and 59% of CP; and protein fraction C (N insoluble in acid detergent) = 7 and 8% of CP (RFSS and RFNDF, respectively). Diets contained similar concentrations of CP (Table 1). Diet RFSS had 23% lower NDF content and 51% higher starch content than diet RFNDF. Concentration of ANDF was by 33% higher and NSC was 16% lower in RFNDF than in RFSS. The diets contained similar proportions of NSP. The estimated intake of ruminally fermentable ANDF was 37% higher ($P < 0.05$) and that of ruminally fermentable starch was 48% lower ($P < 0.001$) on RFNDF compared

Figure 1. Ammonia concentration in ruminal fluid during intraruminal ^{15}N infusion (means $\pm$ SE). Closed symbols, RFSS; open symbols, RFNDF.

to RFSS (Table 2). Thus, the intake of ruminally fermentable fiber was significantly higher in the cows fed RFNDF whereas the intake of ruminally fermentable starch was higher in the cows fed the RFSS diet. Cows fed RFSS had higher (33%; $P < 0.05$) intakes of NSC and tended to have higher ($P < 0.1$) total fermentable CHO intake than the cows fed RFNDF.

Ruminal pH and concentration of RS in ruminal fluid were not different ($P > 0.05$) between the two diets (Table 3). Ammonia concentration was lowered ($P < 0.05$) by RFNDF compared to RFSS. Polysaccharide-degrading and deaminative or proteolytic activities in the rumen were not affected ($P > 0.05$) by treatment. There were no differences ($P > 0.05$) in total or individual VFA concentrations or acetate to propionate ratio between the diets. Fractional outflow rate of the fluid phase of the rumen contents did not differ ($P > 0.05$) between diets. Microbial nitrogen (MN) flow to the small intestine was similar ($P > 0.05$) between the diets. Similarly, daily excretion of urinary allantoin or the efficiency of microbial N synthesis in the rumen were not different ($P > 0.05$) between the diets.

There were no differences ($P > 0.05$) in ruminal apparent or true digestibility and total tract apparent digestibility of DM, OM, CP, and the fiber fractions between

the diets (Table 4); except apparent CP digestibility in the reticulo-rumen was higher ($P < 0.05$) for RFSS compared to RFNDF. True ruminal DM digestibility tended to be higher ($P < 0.1$) on RFSS than on RFNDF. Fecal ammonia-N concentration was similar ($P > 0.05$) between the two diets and fecal C:N ratio tended to be higher ($P < 0.1$) for RFSS than for RFNDF.

Milk yield, FCM yield, milk fat and protein percentages and yields, and proportion of N intake excreted in milk did not differ ($P > 0.05$) between the two diets (Table 5). Average MUN content of milk was lower ($P < 0.05$) for RFNDF than for RFSS.

Nitrogen intake tended to be higher ($P < 0.1$) on diet RFSS compared to diet RFNDF (Table 6). There were no differences ($P > 0.05$) in N excreted with urine or feces between the two diets. From 35 (RFNDF) to 37% (RFSS) of the ingested N was excreted with the urine and from 23 (RFSS) to 32% (RFNDF) with the feces. Numerically, larger proportion of the total N lost to the cows was excreted with the urine when RFSS was fed and with the feces when RFNDF was fed.

Cows received similar ($P > 0.05$) doses of ^{15}N and ^{15}N -enrichment of ruminal ammonia N was not different ($P > 0.05$) between the two diets (Table 7). Bacterial N from RFSS cows had higher ($P < 0.01$) ^{15}N -enrichment

Table 2. Dry matter and nutrient intakes (kg/d, LS means and associated SE).

	Diet ¹		SE	<i>P</i>
	RFSS	RFNDF		
DM	24.2	21.5	0.72	NS
OM	21.7	19.2	0.63	†
N	0.754	0.658	0.0216	†
NDF	6.3	7.3	0.30	NS
ADF	3.3	3.8	0.16	NS
Available NDF (ANDF) ²	5.3	6.3	0.26	NS
Fermentable ³ ANDF	1.9	2.6	0.10	*
NSC ⁴	11.6	8.7	0.24	*
Starch	5.8	3.8	0.09	**
Fermentable starch	4.8	2.5	0.03	***
NSP ⁵	5.1	4.9	0.17	NS
Fermentable NSP	3.3	3.4	0.12	NS
Total FCHO ⁶	10.0	8.5	0.22	†

¹RFSS, ruminally fermentable nonstructural carbohydrates; RFNDF, ruminally fermentable fiber.

²Available NDF was calculated as: NDF – (Lignin × 2.4).

³For calculating fermentability of ANDF, starch and NSP see Materials and Methods.

⁴NSC (nonstructural carbohydrates) was calculated as: 100 – CP – (NDF–NDFCP) – Ash – Ether extract.

⁵NSP (nonstarch polysaccharides) was calculated as: NSC – starch.

⁶Total FCHO (total fermentable carbohydrates intake) was found as: Fermentable ANDF intake + Fermentable starch intake + Fermentable NSP intake.

†*P* < 0.1

**P* < 0.05

***P* < 0.01

****P* < 0.001

NS = nonsignificant (*P* > 0.05).

than bacterial N from RFNDF cows. The area under the ruminal bacterial ¹⁵N curve was larger (*P* < 0.01) for RFSS than for RFNDF. The area under the milk protein ¹⁵N curve was larger (*P* < 0.05) for RFNDF than for RFSS. The estimated proportion of milk protein N originating from ruminal bacterial N was 50% higher (*P* < 0.01) when cows received the RFNDF diet vs the RFSS diet. Between 12.7 (RFSS) and 14.4% (RFNDF) of ¹⁵N infused intraruminally was recovered in milk protein N after 155 h of milking. The proportion was higher (*P* < 0.01) for RFNDF than for RFSS. Time to reach 50% of maximum ¹⁵N recovery and rate of ¹⁵N excretion in milk protein were not different (*P* > 0.05) between the two diets. The overall excretion lines (Figure 2) were different at *P* < 0.05.

DISCUSSION

The two diets tested in this trial had similar metabolizable protein and NE_L concentrations but contrastingly different proportions of structural vs. nonstructural carbohydrates. Fiber in RFNDF had higher estimated ruminal fermentability than RFSS fiber,

Table 3. Ruminal fermentation parameters (LS means and associated SE).

Parameter	Diet ¹			
	RFSS	RFNDF	SE	<i>P</i>
pH	6.01	6.02	0.045	NS
Ammonia, mM	11.4	9.8	0.24	*
RS ² , mM	2.57	2.17	0.152	NS
Volatile Fatty Acids, mM				
Acetate	77.4	75.5	1.48	NS
Propionate	26.5	25.9	0.13	†
<i>iso</i> -Butyrate	1.56	1.48	0.074	NS
Butyrate	19.3	17.0	0.48	†
<i>iso</i> -Valerate	2.09	2.00	0.147	NS
Valerate	2.60	2.68	0.144	NS
Total VFA	129.5	124.6	1.76	NS
Acetate/Propionate	2.99	2.95	0.031	NS
Polysaccharide-degrading activities ³				
CMCase	49.4	44.3	2.10	NS
Xylanase	189.8	184.9	22.94	NS
Amylase	86.0	72.6	4.96	NS
Deaminative activity ⁴	4.4	3.9	0.63	NS
Proteolytic activity ⁵	47.6	49.8	1.20	NS
Liquid phase FOR ⁶ , %/h	15.0	18.0	0.01	NS
MN ⁷ flow, g/d	340	321	12.8	NS
MN, g/kg OMTDR ⁸	25	30	2.1	NS
Urinary allantoin, g/d	81.7	83.3	1.68	NS

¹RFSS, ruminally fermentable nonstructural carbohydrates; RFNDF, ruminally fermentable fiber.

²RS, reducing sugars.

³Expressed as nmol of RS as glucose released per ml of ruminal fluid per minute; CMCase, carboxymethylcellulase.

⁴Expressed as μmol of ammonia released from 1 ml ruminal fluid per hour.

⁵Expressed as μg of N from ¹⁵N-casein released from 1 ml of ruminal fluid per min.

⁶FOR, fractional outflow rate.

⁷MN, microbial nitrogen.

⁸OMTDR, organic matter truly digested in the rumen.

†*P* < 0.1.

**P* < 0.05.

NS = nonsignificant (*P* > 0.05).

although determined ruminal digestibility was only numerically different between the diets. Beet pulp and particularly brewer's grains have high concentration of ruminally fermentable NDF (Tamminga et al., 1990). The difference in NSC content was primarily from starch; NSP content was similar between the diets. Estimated starch fermentability in RFSS was also higher than fermentability of starch in RFNDF. As a result, diet RFSS provided considerably higher intakes of ruminally fermentable starch. Although, the differences in NSP content and fermentable NSP intake were not significant, the diets differed in the composition of the NSP fraction. Constituents of NSP in RFSS would include free sugars (from molasses) and β-glucans (from barley) and NSP in RFNDF would originate primarily from pectins (beet pulp, Bohn et al., 1998).

Thus, the main dietary factors that could have affected the results from the present trial were: the higher

Table 4. Apparent and true ruminal and apparent total tract digestibility (%) of nutrients (LS means and associated SE).

	Diet ¹		SE	P
	RFSS	RFNDF		
Rumen				
DM	40.5	32.0	2.34	NS
(DM, true)	58.6	51.9	1.71	† ²
OM	44.6	37.2	2.09	NS
(OM, true)	61.8	56.4	1.57	NS
CP	33.7	26.7	1.11	*
(CP, true)	78.7	75.5	1.78	NS
NDF	36.9	40.9	7.10	NS
ADF	24.5	37.3	7.10	NS
Total tract				
DM	74.9	69.9	2.81	NS
OM	76.1	71.6	2.85	NS
CP	76.8	68.1	2.77	NS
NDF	50.0	55.8	5.03	NS
ADF	39.9	49.3	5.50	NS
Fecal ammonia-N, mg/g DM	0.48	0.42	0.019	NS
Fecal C:N ratio	16.1	14.2	0.34	†

¹RFSS, ruminally fermentable nonstructural carbohydrates; RFNDF, ruminally fermentable fiber.

²P = 0.1086

†P < 0.1.

*P < 0.05.

NS = nonsignificant (P > 0.05).

starch content and higher starch degradation rate of barley vs. corn and presence of free sugars from molasses in RFSS and the provision of greater amount of ruminally fermentable fiber with RFNDF. Comparing diets with different rates of starch degradability in the rumen (barley vs. corn), Casper et al. (1990) found increased propionate production and decreased ammonia concentration with the barley diet, although in a latter study, the effects were related to the level of ruminally degradable protein (Casper et al., 1999). Similarly, a trend for increased propionate with diet RFSS was ob-

Table 5. Milk yield and composition (LS means and associated SE).

	Diet ¹		SE	P
	RFSS	RFNDF		
Milk yield, kg/d	26.5	28.9	0.79	NS
4% FCM yield, kg/d	25.8	27.8	0.60	NS
Milk fat, %	3.80	3.82	0.052	NS
Yield, kg/d	1.01	1.08	0.020	NS
Milk protein ² , %	3.35	3.37	0.072	NS
Yield, kg/d	0.89	0.96	0.039	NS
As % of N intake ³	18.4	23.1	1.58	NS
Milk urea N, mg/dl	18.2	15.6	0.43	*

¹RFSS, ruminally fermentable nonstructural carbohydrates; RFNDF, ruminally fermentable fiber.

²Crude protein.

³Found as: (milk protein yield ÷ 6.38) ÷ N intake.

*P < 0.05.

NS = nonsignificant (P > 0.05).

Table 6. Nitrogen losses (LS means and associated SE).

	Diet ¹		SE	P
	RFSS	RFNDF		
N intake, g/d	754	658	21.6	†
Total N excreted, g/d	455	442	25.7	NS
Urine N	279	233	17.8	NS
As % of total excreted	61.5	52.7	2.61	NS
As % of intake	37.1	35.3	1.86	NS
Fecal N	176	208	14.9	NS
As % of total excreted	38.5	47.3	2.61	NS
As % of intake	23.2	31.9	2.77	NS

¹RFSS, ruminally fermentable nonstructural carbohydrates; RFNDF, ruminally fermentable fiber.

†P < 0.1.

NS = nonsignificant (P > 0.05).

served in the present experiment. Leiva et al. (2000) indicated no difference in ruminal VFA and lactate concentrations, N balance or recovery, and milk yield between diets containing soluble fiber (citrus pulp) and starch (corn). In the present study, ruminal ammonia concentration was lower with the RFNDF diet. The lower (−13%) intake of total dietary N, respectively ruminally degradable N, in RFNDF compared to RFSS was most likely the main reason for the reduced concentration of ruminal ammonia with the former diet. Structural carbohydrate-fermenting ruminal bacteria derive their N exclusively from ammonia (Russell et al., 1992); it is possible that enhanced growth of fiber-degrading populations with RFNDF contributed to the reduction in ammonia concentration with this diet. Addition of readily available carbohydrates to the diet can produce variable effects on ruminal fermentation; sugar and starch supplementation of a forage diet increased dietary OM and NDF digestibility but had a negative effect on ruminal fermentation as total VFA concentration was reduced and ammonia concentration was increased (Heldt et al., 1999b). In another study, addition of 5% sugars to a fresh ryegrass/corn grain-based diet did not result in any significant effects on ruminal fermentation or milk yield and composition (McCormick et al., 2001). In this latter experiment, blood plasma urea levels were increased with the sugar addition. Diets, in which brewer's grains replaced part of the forage, produced higher propionate concentrations in the rumen but had no effect on total VFA concentration; when brewer's grains replaced dietary concentrate, ruminal ammonia levels were reduced compared to the control diet (Younker et al., 1998). Pereira and Armen-tano (2000), however, found no effect of the addition of nonforage NDF from wheat middlings and brewer's grains on ruminal VFA and ammonia concentrations. Rumen function and chewing activity were not affected

Table 7. ^{15}N transformations (LS means and associated SE).

	Diet ¹		SE	P
	RFSS	RFNDF		
^{15}N dosed, mg	5104	5029	38.4	NS
Ruminal ammonia- ^{15}N , APE ²	0.441	0.556	0.1434	NS
Bacterial- ^{15}N , APE	0.241	0.189	0.0015	**
AUC ³ , bacterial N	27.5	21.3	0.36	**
AUC, milk protein N	7.99	9.36	0.151	*
Milk protein N from ruminal bacterial N ⁴ , %	29.4	44.0	0.69	**
Regression analysis, cumulative milk ^{15}N excretion (estimate $\pm$ approx. SE)				
Maximum ^{15}N excreted, % of infused	12.7 $\pm$ 0.36	14.4 $\pm$ 0.59		**
Time to 50% of maximum ⁵ , h	54.7 $\pm$ 1.02	55.2 $\pm$ 1.53		NS
Rate related parameter	0.045 $\pm$ 0.0017	0.043 $\pm$ 0.0023		NS
Comparison of excretion lines				*

¹RFSS, ruminally fermentable nonstructural carbohydrates; RFNDF, ruminally fermentable fiber.

²APE, atom percent excess.

³Area under the ^{15}N curve, APE $\times$ h.

⁴Calculated as: $(\text{AUC}_{\text{milk protein}} \div \text{AUC}_{\text{rumen bacteria}}) \times 100$.

⁵Time to reach 50% of maximum ^{15}N recovery in milk protein, i.e. 50% of 12.7 (RFSS) or 14.4% (RFNDF) recovery.

* $P < 0.05$.

** $P < 0.01$.

NS = nonsignificant ($P > 0.05$).

when forage NDF in the diet was replaced with nonforage fiber sources (Slater et al., 2000).

Both CPM Dairy and NRC 2001 (NRC, 2001) models predicted higher microbial protein flow at the duode-

num with RFSS than with RFNDF (2233 vs 1985 and 2009 vs. 1781 g/d, CPM Dairy and NRC 2001, RFSS and RFNDF, respectively) reflecting carbohydrate composition (CPM Dairy) or total TDN content of the diets

Figure 2. Cumulative excretion curves of ^{15}N in milk protein as proportion of ^{15}N infused intraruminally (predicted values; error bars represent 95% confidence intervals on the predicted values). Closed symbols, RFSS; open symbols, RFNDF.

(NRC, 2001). Only a numerical difference in the microbial protein flow between the two diets was found in the present experiment. Most published work indicates microbial protein synthesis in the rumen is energy-dependent rather than nitrogen-dependent. Microbial protein produced in the rumen was found to always be higher when the diet contained higher proportion of nonstructural carbohydrates in DM (Hoover and Stokes, 1991). Despite some reports where increased dietary starch degradability had no effect on microbial protein synthesis (Shabi et al., 1998), diets containing larger amounts of ruminally fermentable starch, usually produce higher microbial protein yields. Hall and Herejk (2001) fed bermudagrass NDF alone or in combination with sucrose, pectin or starch to mixed ruminal populations *in vitro*. The authors reported that pectin and sugar-supplemented diets produced lower maximal TCA-precipitable protein (assumed to be microbial protein) than the starch diet.

Both, brewer's grain and sugar beet pulp contain higher levels of ruminally fermentable NDF per kg of DM than barley (149 and 109%, respectively; Tamminga et al., 1990) and total tract NDF digestibility is also high compared to common forages such as alfalfa silage (Sievert and Shaver, 1993). Inclusion of brewer's grain and sugar beet pulp in the present study, however, only numerically increased ruminal and total tract digestibility of fiber fractions, particularly ADF digestibility. Similarly, Younker et al. (1998) did not find any significant effects of brewer's grains on site of NDF digestion, ruminal or total tract NDF digestibility, bacterial N flow to the small intestine, or milk yield and composition. In a trial with growing steers, the effect of supplemental carbohydrates on OM and NDF digestibility was largely dependent on ruminally available N in the diet (Heldt et al., 1999a). Although starch, glucose, or fiber (oat) supplementation generally increased digestion, reduced ammonia, and increased ruminal concentration of VFA, a number of two- and three-way interactions rendered the results difficult to interpret. Pereira and Armentano (2000) investigated the effects of two levels of forage NDF and addition of nonforage NDF (wheat middlings and brewer's grains) on rumen fermentation and nutrient digestion. The experimental design resulted in three dietary levels of starch and free glucose. The nonforage fiber diet had lowered digestibility of DM and OM but NDF digestion was not affected. The authors concluded that addition of ruminally degradable fiber did not affect total tract NDF digestion (Pereira and Armentano, 2000).

Transfer of ammonia N into milk protein was measured directly; total excretion of ammonia N tracer in milk protein was determined gravimetrically. Expressed as proportion of ^{15}N infused into the rumen,

more ^{15}N was recovered in milk protein with RFNDF than with RFSS. In agreement, the ^{15}N -AUC in milk protein was larger for RFNDF than for RFSS. The specific ^{15}N -enrichment of bacterial N and the area under the ^{15}N -bacterial N curve were larger with RFSS than with RFNDF indicating a more intensive incorporation of ^{15}N tracer by RFSS bacteria but also a potentially smaller overall bacterial N pool with this diet compared to RFNDF. These two effects were not separated in this trial. Since total milk protein yield was not different between the diets (in fact, it was numerically higher for RFNDF), the larger amount of ^{15}N recovered in milk protein from RFNDF cows could be most likely explained with a larger total flow of bacterial N to the small intestine, which in turn was used for milk protein synthesis by the cows. This hypothesis, however, was not supported by the similar microbial protein flows or urinary allantoin excretion between the two diets. Methodological problems with the procedures used in this experiment may have prevented detection of existing differences in microbial N flow from the rumen between the diets. Reports have indicated a considerable recovery of feed purines in duodenal digesta; from 23% of the total purine bases in digesta (Perez et al., 1997) to 17 to 26% in duodenal solids purine bases (Hristov et al., 2000c). It is possible that, due to fractionation of bacterial/microbial purines between the reticulo-rumen and the duodenum, MN flow to the small intestine was not accurately estimated in the present trial. Studies with another widely used bacterial marker, diaminopimelic acid (DAPA), indicated a significant dissociation of cell-contained DAPA before reaching the point of sampling—the duodenum (Denholm and Ling, 1989). Due to disproportional metabolism, the ratio of microbial purine bases to NAN in digesta phases could be significantly different than the ratio in ruminal bacterial cells. As a result, a significant discrepancy between the amount of NAN from microbial origin (carrying the ^{15}N label) and the amount of microbial purines reaching the duodenum may occur. Due to liberation of free intracellular NAN, this phenomenon is more likely to be observed in the fluid phase of digesta. In such a situation, there will be a significant discrepancy between the measurements of bacterial/microbial flow to the duodenum using ^{15}N vs purines as microbial markers. The data of Perez et al. (1997) showed that the microbial contribution to the duodenal NAN, estimated using purine bases as microbial marker, was 97, 74, 88, and 83% (liquid-associated bacteria composition; four diets) of the microbial contribution derived using ^{15}N as a marker. Estimates based on our previous data (Hristov et al., 2000c), indicated that purine-estimated microbial contribution to NAN in fluid duodenal digesta was 0.36 and 0.55 (barley

Figure 3. Milk urea N concentration during intraruminal ^{15}N infusion (means $\pm$ SE). Closed symbols, RFNDF; open symbols, RFSS.

and corn-based diets, respectively) of the ^{15}N -derived estimates. In the solid phase of digesta, however, the two methods gave similar estimates: ratios of 0.89 and 1.02, respectively. We believe a significant portion of the inconsistency between the ^{15}N - and duodenal flow-derived data for microbial N incorporation into milk protein observed in the present study can be attributed to the inability of the duodenal techniques to fully account for the ^{15}N -NAN of microbial origin reaching the small intestine and eventually used for milk protein synthesis.

Concentration of MUN reflects blood urea concentration and is in close relation to urinary N excretion in dairy cows (Jonker et al., 1998). The lowered MUN content when RFNDF was fed suggests more efficient utilization of dietary protein/ruminal ammonia with this diet compared to RFSS. Concentration of MUN varied greatly between cows and sampling times (Figure 3). This large variation once again questions the usefulness of spot- or individual cow-sampling for MUN analysis as an indicator of the N status of the cow (Hof et al., 1997; Trevaskis and Fulkerson, 1999).

Ammonia N can leave the rumen through microbial uptake or absorption (BBSRC, 1998); comparatively smaller amounts of ammonia leave the rumen with the

fluid digesta phase (Beever, 1996). Microbial utilization of ammonia in the rumen is a complex process and can vary greatly, primarily depending on dietary factors. Proportions of ammonia-derived bacterial N in the rumen can be from 46 to 80% (Leng and Nolan, 1984; Siddons et al., 1985; Hristov and Broderick, 1996). In spite of this high affinity, microbial utilization of ruminal ammonia can be low and variable. Siddons et al. (1985) reported 32 and 66% of the irreversible loss of ruminal ammonia in sheep was through incorporation into microbial N (grass silage and dried grass, respectively). Thus, depending on the diet and type of animal, a significant proportion of the reduction in ruminal ammonia is due to absorption. As reported in sheep, absorbed ammonia is detoxified to urea in the liver and to a lesser extent used for synthesis of glutamine; less than 4% of the net ^{15}N (as NH_3) transfer across the liver was as glutamine (Lobley et al., 1995). Therefore, absorption and incorporation into microbial protein are responsible for ammonia N clearance from the rumen. A variation of the technique used in this study to determine transfer of ruminal ammonia N into milk protein was utilized in goats (Petri and Pfeffer, 1987 and Petri et al., 1988). The proportions of milk protein derived from rumen bacterial N found in these studies (from

32 to 49%) were similar to the values estimated for lactating dairy cows in the present experiment. From the data of Petri et al. (1988) it can be calculated that, in the lactating goat, from 10.3 (phosphorus deficient diet) to 12.9–14.0% of the irreversible loss of ruminal ammonia N was recovered in milk protein via microbial protein. These estimates are similar to the 12.7 and 14.4% maximum recovery of ^{15}N found in the present experiment.

CONCLUSIONS

Diets differing in concentration of ruminally available starch and sugars and fiber produced similar level and pattern of fermentation acids in the rumen. The diet higher in ruminally available fiber resulted in lower crude protein intake and lower ruminal ammonia and urea nitrogen concentration in milk. Diet composition did not affect microbial protein synthesis in the rumen, partitioning of N losses between urine and feces, ruminal or total tract digestion of nutrients, or milk yield and fat and protein content. The diet high in ruminally available starch and sugars enhanced ^{15}N -ammonia capture by ruminal bacteria but overall transfer of ^{15}N -ammonia into milk protein was higher when cows were fed the ruminally fermentable fiber diet.

ACKNOWLEDGMENTS

This study was partially supported by funds from the Idaho Agriculture Experiment Station and by a grant from the United Dairywomen of Idaho. The authors would like to thank W. Price for assistance with statistical evaluation of the results and the staff of the Department of Animal and Veterinary Sci. Experimental Dairy for their conscientious care of the experimental cows.

REFERENCES

- AOAC International. 1999. AOAC Official Method 920.39 Fat (Crude) or Ether Extract in Animal Feed. In *Official Methods of Analysis of AOAC International*. 16th Edition (P. Cunniff, Ed.), AOAC International, Gaithersburg, MD.
- Bates, D. M. and D. G. Watts. 1988. *Nonlinear Regression Analysis and Its Applications*. John Wiley & Sons, NY, pp. 365.
- BBSRC (Biotechnology and Biological Sciences Research Council), Technical Committee on Responses to Nutrients, Report no. 11. 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. *Nutrition Abstracts and Reviews*. Series B, *Livestock Feeds and Feeding*, vol. 68, No. 11, pp. 937–989.
- Beever, D. E. 1996. Rumen function. In *Quantitative Aspects of Ruminant Digestion and Metabolism* (J. M. Forbes and J. France, eds.). CAB International, Wallingford, U.K. pp. 515.
- Bohn, K., M. A. Clarke, K. Buchholz, K.-M. Bliesener, R. Buczyk, K. Thielecke, and D. Miehe. 1998. Composition of sugarbeet and sugarcane and chemical behavior of constituents in processing. In *Sugar Technology. Beet and Cane Sugar Manufacture* (P. W. van der Poel, H. Schiweck, and T. Schwartz, eds.). Verlag Dr. Albert Bartens KG–Berlin, p. 116.
- Casper, D. P., H. A. Maiga, M. J. Brouk, and D. J. Schingoethe. 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. *J. Dairy Sci.* 82:1779–1790.
- Casper, D. P., D. J. Schingoethe, and W. A. Eisenbeisz. 1990. Response of early lactation dairy cows fed diets varying in source of non-structural carbohydrate and crude protein. *J. Dairy Sci.* 73:1039–1050.
- Denholm, A. M. and J. R. Ling. 1989. In vivo metabolism of 2,2'-diaminopimelic acid and its accuracy as a marker of bacterial biomass in duodenal digesta of sheep. *Asian-Australian J. Anim. Sci.* 2:315–316.
- Dhana, M. S., R. C. Siddons, J. France, and D. L. Gale. 1985. A multicompartamental model to describe marker excretion patterns in ruminant feces. *Br. J. Nutr.* 53:663–671.
- Faichney, G. J. 1975. The use of markers to partition digestion within the gastrointestinal tract of ruminants. In *Digestion and Metabolism in the Ruminant*. Proceedings of the IV International Symposium on Ruminant Physiology, pp. 277–291 (I. W. McDonald and A. C. I. Warner, eds.). Sydney: The University of New England Publishing Unit.
- Feng, P., W. H. Hoover, T. K. Miller, and R. Blauwiel. 1993. Interactions of fiber and nonstructural carbohydrates on lactation and ruminal function. *J. Dairy Sci.* 76:1324–1333.
- Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. *J. Dairy Sci.* 80:1426–1437.
- Firkins, J. L., W. P. Weiss, and E. J. Piwonka. 1992. Quantification of intraruminal recycling of microbial nitrogen using nitrogen-15. *J. Anim. Sci.* 70:3223–3233.
- Hall, M. B. and C. Herejk. 2001. Differences in yields of microbial crude protein from in vitro fermentation of carbohydrates. *J. Dairy Sci.* 84:2486–2493.
- Heldt, J. S., R. C. Cochran, C. P. Mathis, B. C. Woods, K. C. Olson, E. C. Titgemeyer, T. G. Nagaraja, E. S. Vanzant, and D. E. Johnson. 1999a. Effects of level and source of carbohydrate and level of degradable protein on intake and digestion of low-quality tall-grass-prairie hay by beef steers. *J. Anim. Sci.* 77:2846–2854.
- Heldt, J. S., R. C. Cochran, G. L. Stokka, C. G. Farmer, C. P. Mathis, E. C. Titgemeyer, and T. G. Nagaraja. 1999b. Effects of different supplemental sugars and starch fed in combination with degradable intake protein in low-quality forage use by beef steers. *J. Anim. Sci.* 77:2793–2802.
- Hof, G., M. D. Vervoorn, P. J. Lenaers, and S. Tamminga. 1997. Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. *J. Dairy Sci.* 80:3333–3340.
- Hoover, W. H. and S. R. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. *J. Dairy Sci.* 74:3630–3644.
- Hristov, A. N., and G. A. Broderick. 1996. Ruminal microbial protein synthesis in cows fed alfalfa silage, alfalfa hay or corn silage and fitted with only ruminal cannulae. *J. Dairy Sci.* 79:1627–1637.
- Hristov, A. N., P. Huhtanen, L. M. Rode, T. A. McAllister, and S. N. Acharya. 2001. Ruminal metabolism of nitrogen from ^{15}N -labeled alfalfa preserve as hay or silage. *J. Dairy Sci.* 84:2738–2750.
- Hristov, A. N., M. Ivan, L. M. Rode, and T. A. McAllister. 2000a. Fermentation characteristics and ciliate populations in the rumen of cattle fed medium or high-concentrate barley-based diets. *J. Anim. Sci.* 79:515–524.
- Hristov, A. N., T. A. McAllister, and K.-J. Cheng. 1997. Effect of carbohydrate level and ammonia availability on utilization of α -amino nitrogen by mixed ruminal microorganisms in vitro. *Proceedings, Western Section, American Society of Animal Science*, vol. 48:186–189.
- Hristov, A. N., T. A. McAllister, Z. Xu, and C. J. Newbold. 2002. Proteolytic activity in ruminal fluid from cattle fed two levels of barley grain: a comparison of three methods of determination. *J. Sci. Food Agric.* 82:1886–1893.
- Hristov, A. N., T. A. McAllister and K.-J. Cheng. 2000b. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: effects on nutrient digestion in cattle fed a barley grain diet. *J. Anim. Sci.* 78:477–487.

- Hristov, A. N., T. A. McAllister, and G. A. Broderick. 2000c. Proportion of purines from bacterial origin in ruminal contents and duodenal digesta of heifers fed barley- or corn-based diets. *Reprod. Nutr. Dev.* 40:204.
- Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using mild urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *J. Dairy Sci.* 81:2681–2692.
- Lebzien, Von P., D. Giesecke, S. Wiesmayr, and K. Rohr. 1993. Messung der mikrobiellen proteinsynthese im pansen von khen mittels ^{15}N -bestimmung im duodenalchymus und allantoinausscheidung in der milch. *J. Anim. Physiol. Anim. Nutr.* 70:82–88.
- Leiva, E., M. B. Hall, and H. H. Van Horn. 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral-detergent soluble carbohydrates. *J. Dairy Sci.* 83:2866–2875.
- Leng, R. A. and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. *J. Dairy Sci.* 67:1072–1089.
- Lobley, G. E., A. Connell, M. A. Lomax, D. S. Brown, E. Milne, A. G. Calder, and D. A. H. Farningham. 1995. Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism. *Br. J. Nutr.* 73:667–685.
- MacRae, J. C., F. R. C. Backwell, B. J. Bequette and G. E. Lobley. 1995. Protein metabolism in specific organs. In *Proceedings of the 7th EAAP Symposium on Protein Metabolism and Nutrition*. EAAP Publication No. 81, p. 297. A. F. Nunes, A. V. Portugal, J. P. Costa and J. R. Robeiro, eds. J. Vale de Santarem, Portugal: Estacao Zootechnica Nacional.
- Makkar, H. P. S. and K. Becker. 1999. Purine quantification in digesta from ruminants by spectrophotometric and HPLC methods. *Br. J. Nutr.* 81:107–112.
- McCleary, B. V., V. Solah, and T. S. Gibson. 1994. Quantitative measurement of total starch in cereal flours and products. *J. Cereal Sci.* 20:51–58.
- McCormick, M. E., D. D. Redfearn, J. D. Ward, and D. C. Blouin. 2001. Effect of protein source and soluble carbohydrate addition on rumen fermentation and lactation performance of Holstein cows. *J. Dairy Sci.* 84:1686–1697.
- Nocek, J. E. and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.* 71:2070–2107.
- Nolan, J. V. and R. A. Leng. 1974. Isotope techniques for studying the dynamics of nitrogen metabolism in ruminants. *Proc. Nutr. Soc.* 33:1–8.
- NRC. 2001. National Research Council. Nutrient requirements of dairy cattle. Seventh Revised Edition. National Academy Press, Washington, D.C.
- Pereira, M. N. and L. E. Armentano. 2000. Partial replacement of forage with nonforage fiber sources in lactating cow diets. II. Digestion and rumen function. *J. Dairy Sci.* 83:2876–2887.
- Perez, J. F., J. Balcells, J. A. Guada, and C. Castrillo. 1997. Contribution of dietary nitrogen and purine bases to the duodenal digesta—comparison of duodenal and polyester-bag measurements. *Anim. Sci.* 65:237–245.
- Petri, A. and E. Pfeffer. 1987. Changes of ^{15}N enrichment in N of rumen ammonia, rumen bacteria and milk protein during and following continuous intraruminal infusion of $^{15}\text{NH}_4\text{Cl}$ to goats. *J. Anim. Physiol. Anim. Nutr.* 57:75–82.
- Petri, A., H. Mischen, G. Breves, O. Richter, and E. Pfeffer. 1988. Response of lactating goats to low phosphorus intake 2. Nitrogen transfer from the rumen ammonia to rumen microbes and proportion of milk protein derived from microbial amino acids. *J. Agric. Sci. (Camb.)* 111:265–271.
- Rohr, K. 1986. Physiological aspects in the rumen in relation to the level of production in ruminants. *Arch. Anim. Nutr.* 36:182–192.
- Russell, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *J. Dairy Sci.* 66:763–775.
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551–3561.
- SAS Institute Inc. 1996. SAS/STAT® Software: Changes and Enhancements through Release 6.11. Cary, NC: SAS Institute Inc., 1104 pp.
- Schneider, B. H. and W. P. Flatt. 1975. The Evaluation of Feeds through Digestibility Experiments. University of Georgia Press, Athens, GA.
- Shabi, Z., A. Arieli, I. Bruckental, Y. Aharoni, S. Zamwel, A. Bor, and H. Tagari. 1998. Effects of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dairy cows. *J. Dairy Sci.* 81:1991–2000.
- Siddons, R. C., J. V. Nolan, D. E. Beever, and J. C. MacRae. 1985. Nitrogen digestion and metabolism in sheep consuming diets containing contrasting forms and levels of N. *Br. J. Nutr.* 54:175–187.
- Sievert, S. J. and R. D. Shaver. 1993. Carbohydrate and *Aspergillus oryzae* effects on intake, digestion, and milk production by dairy cows. *J. Dairy Sci.* 76:245–254.
- Slater, A. L., M. L. Eastridge, J. L. Firkins, and L. J. Biding. 2000. Effects of starch source and level of forage neutral detergent fiber on performance by dairy cows. *J. Dairy Sci.* 83:313–321.
- Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562–3577.
- Strobel, H. J. and J. B. Russell. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. *J. Dairy Sci.* 69:2941–2947.
- Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. *J. Dairy Sci.* 75:345–357.
- Tamminga, S., A. M. Van Vuuren, C. J. Van der Koelen, R. S. Ketelaar, and P. L. Van der Togt. 1990. Ruminal behavior of structural carbohydrates, nonstructural carbohydrates and crude protein from concentrate ingredients in dairy cows. *Neth. J. Agric. Sci.* 38:513–526.
- Trevaskis, L. M. and W. J. Fulkerson. 1999. The relationship between various animal and management factors and milk urea, and its association with reproductive performance of dairy cows grazing pasture. *Livest. Prod. Sci.* 57:255–265.
- Uden, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium, and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31:625–632.
- Van Keulen, J. and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44:282–287.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarter polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Younker, R. S., S. D. Winland, J. L. Firkins, and B. L. Hull. 1998. Effects of replacing forage fiber or nonfiber carbohydrates with dried brewer's grains. *J. Dairy Sci.* 81:2645–2656.