Effects of Low Rumen-Degradable Protein or Abomasal Fructan Infusion on Diet Digestibility and Urinary Nitrogen Excretion in Lactating Dairy Cows

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ABSTRACT

Post-ileal carbohydrate fermentation in dairy cows converts blood urea nitrogen (BUN) into fecal microbial protein. This should reduce urinary N, increase fecal N, and reduce manure NH₃ volatilization. However, if intestinal BUN recycling competes with ruminal BUN recycling, hindgut fermentation may reduce NH₃ for rumen microbial protein synthesis. Eight lactating Holstein cows were used in a replicated 4 × 4 Latin square design with 14-d periods. Treatments were arranged as a 2 × 2 factorial. Diets contained either adequate rumen-degradable protein (RDP; high RDP) or were 28% below predicted RDP requirements (low RDP). Cows received abomasal infusions of either 10 L/d of saline or 10 L/d of saline containing 1 kg/d of inulin. We hypothesized that reducing ruminal NH₃, either by restricting RDP intake or by diverting BUN to feces with inulin, would reduce rumen microbial protein synthesis, as would be evidenced by significant main effects of treatments on rumen NH₃, milk production, and urinary purine derivative excretion. Furthermore, we thought it likely that effects of inulin might be greater when rumen NH₃ was already low, as would be indicated by significant interactions between inulin infusion and dietary RDP level on rumen NH₃, milk production, and urinary purine derivative excretion. Rumen NH₃ was reduced by the low-RDP diet, but urinary purine derivative excretion and milk production were unaffected. However, the low-RDP diet reduced apparent total tract digestibility of OM and starch and reduced *in situ* rumen NDF digestibility. Abomasal inulin reduced the BUN concentration but did not affect milk yield or rumen NH₃, suggesting that RDP requirements are not affected by hindgut fermentation. Inulin shifted 23 g/d of N from urine to feces. However, based on fecal purine excretion, we estimated that only 8 g/d of the increased fecal N was due to increased fecal microbial output. Inulin reduced true digestibility of dietary pro-

tein or increased nonmicrobial as well as microbial endogenous losses. This latter effect may be an artifact of our experimental model that delivers easily fermented, soluble fiber to the small intestine. Normal dietary alterations to similarly increase large intestinal fermentation would probably arise from larger quantities of less rapidly digested carbohydrates. Increasing hindgut fermentation in practical diets should reduce manure NH₃ volatilization without impairing rumen fermentation, but the reduction is likely to be small.

Key words: fecal nitrogen, fructan, urea, urinary nitrogen

INTRODUCTION

Ammonia emissions from dairy manure reduce air quality (James et al., 1999). Urinary N is rapidly converted to NH₃ during manure collection and storage, whereas fecal N is converted to NH₃ at a much slower rate (Varel et al., 1999). As a consequence, the rate of NH₃ volatilization from urine-rich manure is greater than the rate of NH₃ volatilization from feces-rich manure. Developing nutritional strategies to shift some N excretion from the urine to the feces should reduce NH₃ volatilization from dairy manure.

Diets that increase large intestinal carbohydrate fermentation appear to shift some N from urine to feces. Supplying fermentable substrates to the large intestine increased bacterial conversion of BUN into fecal microbial protein in sheep (Ørskov et al., 1970; Thornton et al., 1970; Mason et al., 1981), and abomasal infusions of 1 kg/d of pectin were shown to reduce urinary N by approximately 10% in lactating dairy cows (Gressley and Armentano, 2005). However, rumen ammonia and urinary excretion of purine derivatives tended to decrease with pectin infusion (Gressley and Armentano, 2005). These results suggest that fermentation in the large intestine may have reduced urea recycling to the rumen, consequently reducing rumen microbial protein production.

Additionally, pectin is a viscous fiber and studies in monogastrics have shown that feeding viscous fibers increases fecal losses of feed and endogenous protein

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compared with feeding nonviscous fibers (Larsen et al., 1993; Souffrant, 2001). The negative effects of pectin on protein nutrition observed by Gressley and Armentano (2005) may have been due both to direct effects of pectin on reducing N recycling to the rumen and to indirect effects of pectin because of its viscosity, decreasing protein digestibility.

An experiment was conducted to determine whether increasing the fermentable substrate supply to the large intestine of dairy cows would increase the requirement for RDP. Cows were fed a diet balanced to meet protein requirements or a diet balanced to contain 28% less RDP than required (NRC, 2001). Additionally, cows were infused abomasally with either 0 or 1 kg/d of inulin, a source of fructans, which are a type of soluble fiber. Unlike pectin, inulin is a nonviscous fiber and the effects of inulin on protein nutrition should be due only to direct effects on microbial activity of the large intestine. We hypothesized that if hindgut fermentation decreases N recycling to the rumen, then rumen ammonia and urinary purine derivative excretion should be decreased with abomasal inulin, and that these effects should be more pronounced on the low-RDP diet.

MATERIALS AND METHODS

Animals and Treatments

Eight multiparous lactating Holstein cows were fed the same diet (high RDP; Table 1) for a 2-wk preexperimental period to determine ad libitum intake. Cows were fed to obtain approximately 10% feed refusals. Each cow's ad libitum DMI was calculated as DMI during the final 4 d of the preexperimental period after correcting for 100°C DM content of the refusals. Following the preexperimental period, cows were blocked by milk production and assigned to two 4 × 4 Latin squares with 14-d periods. Each cow was fed at 90% of her predicted ad libitum DMI for the remainder of the experiment. Cows ranged from 76 to 187 DIM at the start of period 1. At least 1 mo prior to the start of period 1, cows were fitted with 10-cm rumen cannulas (Bar Diamond, Parma, ID). Cows were milked twice daily at 0330 and 1530 h, and milk weight was recorded at each milking. Bovine somatotropin (500 mg of Posilac, Monsanto Company, St. Louis, MO) was injected every 2 wk. The Animal Care and Use Committee for the College of Agriculture and Life Sciences at the University of Wisconsin-Madison approved all animal procedures.

Treatments were arranged as a 2 × 2 factorial, with 2 dietary treatments and 2 abomasal infusion treatments. Cows were fed either a ration balanced according to NRC (2001) to provide adequate nutrients to support 36 kg/d of milk with 3.5% fat (high RDP) or

Table 1. Ingredient composition of the experimental diets and analyzed nutrient contents of the experimental diets and inulin

Item	Diet		Inulin ¹
	High RDP	Low RDP	
Ingredient	(% of DM)		
Corn silage	60.0	60.0	
Cracked corn	20.7	21.7	
Cottonseed	6.0	6.0	
Soybean meal	4.7	4.7	
Corn gluten meal	3.1	3.1	
Blood meal	1.0	1.0	
Menhaden fish meal	1.0	1.0	
Urea	1.0	0.0	
Sodium bicarbonate	0.8	0.8	
Trace mineral salt ²	0.7	0.7	
Calcium carbonate	0.5	0.5	
Vitamin mix ³	0.3	0.3	
Potassium chloride	0.2	0.2	
Nutrient	(% of DM)		
NDF	26.2	26.2	0.0
NDF from forage	21.5	21.5	0.0
ADF	16.3	16.3	0.0
Fatty acids ⁴	3.69	3.75	0.0
CP	16.1	13.5	0.0
RDP ⁵	10.1	7.4	0.0
RUP ⁵	6.0	6.1	0.0
Starch	36.2	36.3	0.1
Ash	5.7	5.7	0.0
NFC ⁶	47.3	49.8	99.0
NDSOM ⁷	68.0	68.1	99.9
NE _L ⁸	1.63	1.63	—
DM, %	47.7	47.7	97.6

¹Chicory inulin (Raftiline; Orafit Active Food Ingredients, Oreye, Belgium). Nutrient composition is reported as analyzed except fatty acids, which are reported according to the manufacturer.

²Contained 0.55% Mn, 0.55% Zn, 0.35% Fe, 0.14% Cu, 0.008% I, 0.006% Se, and 0.002% Co.

³Contained 3,304 IU/g of DM of vitamin A, 1,101 IU/g of DM of vitamin D, and 11 IU/g of DM of vitamin E.

⁴Represented the sum of C₈ to C₂₂.

⁵RDP and RUP calculated from NRC (2001).

⁶NFC = 100 – (FA + 1 + NDF + CP + ash).

⁷NDSOM = neutral detergent-soluble OM. NDSOM = 100 – NDF – ash.

⁸Predicted by NRC (2001).

a ration formulated to contain 72% of RDP supplied by the adequate diet (low RDP). The high-RDP diet contained 1% urea, and urea was replaced with cracked corn grain to create the low-RDP diet (Table 1). Abomasal infusion treatments were 10 L/d of saline (9 g of NaCl/L of tap water) containing either 0 or 1 kg/d of chicory inulin (Raftiline, Orafit Active Food Ingredients, Oreye, Belgium). Treatment combinations were 1) the low-RDP diet with abomasal saline infusion, 2) the low-RDP diet with 1 kg/d of abomasal inulin, 3) the high-RDP diet with abomasal saline infusion, and 4) the high-RDP diet with 1 kg/d of abomasal inulin. Abomasal infusions were performed from d 4 to 14 of each period (11 total days of infusion) and were continuous through-

out the day except for about 1.5 h during milking. Infusion lines were inserted through the ruminal cannula, through the omasum, and into the abomasum. Plastisol (Bar Diamond) discs (12 cm in diameter and 9 mm in height) were used to anchor the infusion line in the abomasum, and placement of infusion lines in the abomasum was monitored daily. Infusion solutions were prepared using an electric mixer (Talboys laboratory stirrer, model #107, Troemner LLC, Thorofare, NJ). Variable-speed peristaltic pumps (Mec-o-matic VSP-20, W. W. Grainger, Inc., Lincolnshire, IL) were used to deliver the infusion mixtures at a rate of 10 ± 0.5 L/d.

Sampling

Feed samples were taken weekly, dried for 48 h in a 60°C forced-air oven, and composited by period. Samples of any refusals were taken during the last 3 d of each period. To calculate DMI, samples of offered and refused feed were dried for 48 h at 100°C, then weighed and discarded. During abomasal infusions (d 4 to 14 of each period), a bolus containing La_2O_3 dissolved in HCl and carried on soyhulls was pulse-dosed into the rumen twice daily at 0300 and 1500 h. A total of 1.46 g of La/d was supplied to predict fecal output and nutrient digestibility.

Rumen fluid samples were taken on d 12 of each period, beginning just prior to the 0800-h feeding and at every 2 h thereafter for a total of 12 samples per period. Samples were comprised of equal volumes of rumen fluid taken from the caudal ventral sac, cranial ventral sac, caudo-dorsal blind sac, and dorsal sac of the rumen using a stainless-steel filter probe (Gressley and Armentano, 2005). Rumen fluid pH was measured (twin pH-meter, model B-213, Spectrum Technologies Inc., Plainfield, IL). One milliliter of rumen fluid was added to each of two 1.5-mL microfuge tubes containing 0.02 mL of 50% H_2SO_4 . Tubes were frozen at -20°C for later VFA analysis. The same process was used to preserve rumen fluid for NH_3 analysis, except that the tubes contained 0.02 mL of 50% TCA as the preservative.

In situ rumen measurements were also performed on d 12. Quadruplicate Dacron polyester bags (9×15 cm, 52 ± 5 μm pore size) containing 5 g of soyhulls were incubated in each cow for 12 h to determine disappearance of NDF. Bags were placed in a nylon laundry bag, soaked in warm water for 5 min prior to incubation, and inserted in the ventral sac of the rumen. After incubation, bags were rinsed in cold water and washed in a commercial washing machine for 2 rinse cycles of 15 min each. Disappearance of DM and NDF after 12 h was corrected for DM and NDF losses in bags that

were soaked and laundered without ruminal incubation.

Fecal, urine, and blood samples were taken 3 times each on d 13 and 14 of each period. Samples were taken every 8 h, beginning at 0830 h on d 13 and at 1230 h on d 14. Feces were sampled rectally and midstream urine samples were taken following manual vulval stimulation. One cow was unresponsive to manual stimulation used to collect urine samples, and urine data therefore represent results from 7 of the 8 cows. A portion (approximately 100 g) of each fecal grab sample was dried for 48 h at 60°C and composited by cow within period for later component analysis. Composites contained equal dry weights of feces from each sampling point. A second portion of feces (approximately 20 g) was immediately frozen at -20°C for later N analysis. Prior to N analysis, samples were thawed and composited by cow within period, with an equal weight from each sampling time. Composite fecal samples were homogenized for 2 min in a Waring blender. Urine samples were acidified with 50% H_2SO_4 (0.02 mL/1 mL of urine) and frozen at -20°C for later N analysis. An additional acidified sample was diluted 1:5 with distilled water for later purine derivative and creatinine analysis. Prior to N analysis, urine samples were composited by cow within period with an equal volume for each sampling time. Blood samples (10 mL) were taken from the coccygeal vein or artery into evacuated tubes (BD Vacutainer clot activator, Becton Dickinson Co., Franklin Lakes, NJ). Blood was centrifuged at $2,000 \times g$ for 30 min and serum aliquots were refrigerated (4°C) for a maximum of 48 h until urea analysis.

Cows were weighed at 1100 h on d 7 and 8 of each period. Duplicate milk samples from both a.m. and p.m. milkings were taken during the final 3 d of each period. One set of duplicate samples was analyzed for fat, true protein, and MUN by infrared analysis (Foss 605 for fat and protein, and Foss 6000 for MUN, AgSource Milk Analysis Laboratory, Menomonie, WI). The second set of samples was refrigerated until the end of each sampling period, composited by cow within period according to milk production, and frozen at -20°C until N analysis.

Sample Analyses

Dried feed and fecal samples were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Dry matter was determined by drying overnight in a 100°C forced-air oven. Organic matter was determined by oven combustion at 550°C for 12 h. Neutral detergent-soluble organic matter (**NDSOM**) was calculated as $100\% - \text{NDF} - \text{ash}$. Nitrogen was analyzed by Kjeldahl (AOAC, 2000; section 4.2.09, method

984.13). Neutral detergent fiber was determined using α -amylase (Sigma no. A3306, Sigma Chemical Co., St. Louis, MO) with sodium sulfite and was corrected for ash concentration according to Van Soest et al. (1991), adapted for the Ankom²⁰⁰ fiber analyzer (Ankom Technology, Macedon, NY). Acid detergent fiber was determined on separate samples using the method of Goering and Van Soest (1970), adapted for the Ankom²⁰⁰. Feed fatty acids were analyzed according to Sukhija and Palmquist (1988) and represented the sum of C₈ to C₂₂. Starch was determined according to Bal et al. (2000), except that glucose hydrolyzed from starch was analyzed according to Karkalas (1985). Starch content was corrected for free glucose measured in samples not treated with amylase and amyloglucosidase.

Rumen fluid samples were analyzed for VFA concentrations by gas chromatography (Clarus 500, Perkin-Elmer, Norwalk, CT) using a GP 10% SP-1200/1% H₃PO₄ on 80/100 Chromasorb WAW packed glass column (Supelco, Bellefonte, PA). Rumen NH₃ was analyzed as described by Bal et al. (2000). The concentration of La in fecal samples was determined by direct current plasma emission spectroscopy (Spectra Metrics, Inc., subsidiary of Beckman Instruments, Inc., Andover, MA) according to Combs and Satter (1992). Samples were corrected for background La concentration found in a pooled fecal sample taken from cows on the final day of the preexperimental feeding period. Fecal output was predicted based on fecal La concentration as

$$\text{fecal DM (kg/d)} = \text{La dosed (mg/d)} / \text{La in feces (mg/kg of fecal DM)}.$$

Digestibility calculations included infused inulin as a component of intake.

The fructan content of inulin and fecal samples was determined using a commercial kit (fructan assay kit, Megazyme International, Bray, Co. Wicklow, Ireland). Fecal purines were analyzed according to Pereira and Armentano (2000); however, an equimolar ratio of guanine and adenine was used for the standard to better reflect rumen microbial purine composition.

Urinary creatinine was measured using a colorimetric assay (Teco no. C515-480, Teco Diagnostics, Anaheim, CA). Daily urine output (L/d) was estimated from urinary creatinine and BW as $28.1 \text{ (mg/kg)} \times \text{BW (kg)} \times [1/\text{urinary creatinine (mg/L)}]$ (Gressley and Armentano, 2005). The concentration of allantoin was determined by the methods of Chen and Gomes (1992), except that 1 M HCl was used instead of 0.5 M HCl to maintain a pH below 3. The uric acid concentration was determined colorimetrically (Teco no. U580-240, Teco Diagnostics). Blood urea N was also determined using a commercial kit (Teco no. B551-132, Teco Diagnostics).

After the experiment ended and data were analyzed, a significant decrease in milk fat yield was observed for the inulin infusion. Milk sample composites were made for milk N determination as described above. However, only a portion of the composite samples was required for N analysis. The remaining frozen milk samples were used to determine milk fatty acid composition. Milk fatty acids were analyzed according to Leonardi et al. (2003), except that the gas chromatograph was a PerkinElmer Clarus 500.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (SAS Institute, 1999). Most variables had only one value per cow per period and were analyzed using the model

$$Y = \mu + R + I + R \times I + S + R \times S + I \times S + R \times I \times S + P + S \times P + C + e$$

where μ is the overall mean; R is the fixed effect of dietary RDP level; I is the fixed effect of abomasal infusion treatment; S is the fixed effect of square; P is the fixed effect of period; C is the random effect of cow within square; and e is the random residual error.

Repeated measurements of BUN and rumen VFA, NH₃, and pH were analyzed as

$$Y = \mu + R + I + R \times I + S + R \times S + I \times S + R \times I \times S + P + S \times P + C + R \times I \times P \times C + H + R \times H + I \times H + R \times I \times H + e,$$

where H is the fixed effect of hour after the initial 0800-h feeding and other terms are as described above. All interactions were considered fixed effects except $R \times I \times P \times C$, which was considered random. The autoregressive covariate structure was used and the subject was $R \times I \times P \times C$. Significance was declared at $P \leq 0.05$ and a trend was declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

Nitrogen Balance

Inulin increased fecal N by 23 g/d ($P < 0.001$) and decreased urinary N by 24 g/d ($P = 0.02$; Table 2). The effects on urinary and fecal N were nearly identical to those observed when cows were infused abomasally with pectin (Gressley and Armentano, 2005), indicating that different rapidly fermentable substrates led to similar shifts in N excretion. The effects of inulin infusion on N excretion were similar to those observed in monogastrics (Younes et al., 1997; Propst et al., 2003).

Table 2. Effect of dietary RDP and abomasal inulin infusion on nitrogen intake and excretion

Item	Treatment ¹				SED ²	Contrast ³		
	LP	LPI	HP	HPI		RDP	I	RDP × I
	(g of N/d)					(P-value)		
Intake	432.7	439.4	518.6	508.1	10.3	0.001	0.79	0.26
Milk	147.1	150.7	151.8	146.6	3.4	0.90	0.73	0.09
Feces ⁴	149.3	166.9	143.1	172.4	7.6	0.95	0.001	0.30
Urine ⁵	140.5	124.2	245.8	213.4	11.8	0.001	0.02	0.36
Balance ⁶	-7.3	-1.7	-31.4	-31.2	13.5	0.02	0.77	0.78
	(%)							
Fecal N/intake N	34.6	38.1	27.8	33.9	1.8	0.001	0.003	0.34
Apparent N digestibility	65.4	61.9	72.2	66.1	1.8	0.001	0.003	0.34
Fecal N/fecal DM	2.02	2.04	2.11	2.26	0.05	0.001	0.03	0.08
Urine N/intake N	33.4	28.4	48.2	42.7	2.2	0.001	0.009	0.89
Urine N/(fecal N + urine N)	48.9	42.8	62.8	55.1	2.3	0.001	0.002	0.64
Urine N, g/L	7.2	6.4	10.8	9.9	0.6	0.001	0.07	0.86

¹Treatments: LP = low-RDP diet with abomasal saline infusion; LPI = low-RDP diet with 1 kg/d of abomasal inulin; HP = high-RDP diet with abomasal saline infusion; HPI = high-RDP diet with 1 kg/d of abomasal inulin.

²SED = standard error of the difference among treatment means.

³RDP = main effect of RDP level; I = main effect of abomasal inulin infusion; RDP × I = RDP × inulin interaction.

⁴Fecal output predicted using La as a marker.

⁵Urinary output predicted from creatinine concentration assuming excretion of 28.1 mg/kg of BW.

⁶N balance = intake N – milk N – urine N – fecal N.

Increasing dietary RDP by adding urea increased N intake ($P < 0.001$) and urinary N excretion ($P < 0.001$) without affecting outputs of milk N or fecal N. These results are in accordance with those of others who have found that increasing dietary protein primarily increases urinary N, causing an increase in the fraction of excreted N coming from urine (Wright et al., 1998; Broderick, 2003).

Although cows were limit fed, there were feed refusals, averaging 1.8% for higher producing cows fed the high-RDP diet, 3.4% for higher producing cows fed the low-RDP diet, 10.0% for lower producing cows fed the high-RDP diet, and 5.6% for lower producing cows fed the low-RDP diet. This led to a tendency for a square by diet interaction on DMI ($P = 0.07$), and a significant interaction on N intake ($P = 0.02$). Increasing dietary RDP increased N intake from 462 to 559 g/d for the high-producing cows but only from 410 to 468 g/d for the low-producing cows. There were additional interactions between square and diet on N balance measures that may have been consequences of this interaction. Increasing dietary RDP increased milk N from 152 to 163 g/d for the higher producing square but decreased milk N from 145 to 135 g/d for the lower producing square ($P = 0.001$). Increasing RDP increased fecal N from 161 to 173 g/d in the higher producing cows but decreased fecal N from 156 to 143 g/d in the lower producing cows ($P = 0.04$). Increasing RDP numerically reduced DMI from 19.0 to 18.2 kg/d for low-producing cows, which

may have led to decreased milk N from reduced energy availability and decreased fecal N from endogenous and undigested feed sources. Finally, the significant effect of dietary RDP on calculated N balance ($P = 0.02$) was due exclusively to effects observed in higher producing cows. The calculated N balance decreased in those cows from +3 to -45 g/d with increasing RDP, whereas the N balance changed only from -13 to -17 g/d for the lower producing cows ($P = 0.04$).

The restricted feeding protocol did not lead to complete consumption of the diet, as described above. The diets were mixed at 0700 h and fed in 3 equal portions at 0800, 1600, and 2100 h. Cows were observed to consume feed rapidly at 0800 and 1600 h but were more reluctant to consume the feed provided at 2100 h. Additionally, ambient temperatures increased to well above average for period 3 (April of 2005), and refusals averaged 10% during period 3 compared with 4, 2, and 5% for periods 1, 2, and 4, respectively, suggesting that feed heating may have increased refusals. Finally, it is possible that urea reduced palatability of the diet, but this reduced intakes only for cows in the low-producing group.

Based on studies using ileally cannulated lactating dairy cows (Knowlton et al., 1998; Younker et al., 1998; Callison et al., 2001), an average of 1 kg/d of OM is digested in the large intestine. Therefore, the inulin infusion in the present experiment should have approximately doubled the amount of large intestinal fermentation, and it resulted in a 24 g/d decrease in urinary

N excretion. In the current study, decreasing dietary RDP by 2.7 percentage units caused a 97 g/d decrease in urinary N excretion. This suggests that increasing intestinally fermentable carbohydrate by 1 kg/d and decreasing dietary RDP by 0.7 percentage units should cause a roughly equivalent drop in urinary N.

Intake and Digestion

Feed offered was restricted to 90% of ad libitum intake, and although there were some feed refusals, oral DMI was not affected by treatment (Table 3). When cows were abomasally infused with 1 kg/d of inulin, oral plus infused DM ($P = 0.009$), OM ($P = 0.006$), and NDSOM ($P < 0.001$) all increased predictably. Fecal excretion of inulin was low compared with infused amounts, indicating that about 99% of infused inulin was digested, presumably in the hindgut. This is in agreement with data from ruminants and monogastrics (Biggs and Hancock, 1998; Roberfroid and Delzenne, 1998).

Apparent digestibility of DM and OM tended to be reduced with abomasal inulin ($P = 0.10$), and digestibilities of starch ($P = 0.01$) and NDSOM ($P = 0.007$) were significantly reduced. Abomasal infusion of pectin was similarly found to decrease starch digestibility (Gressley and Armentano, 2005). Because inulin was completely digested, the inulin infusions appear to have decreased ruminal or postruminal digestibility of dietary nutrients, including starch. Reduced digestibility may have been due to the infused fiber interfering with small intestinal nutrient digestion, the infused fiber competing with other nutrients for large intestinal digestion, or perhaps fiber infusion increasing carbohydrate storage in large intestinal microbes.

Decreasing RDP reduced apparent digestibility of OM ($P = 0.05$), starch ($P = 0.008$), NDSOM ($P = 0.008$), and fatty acids ($P = 0.007$) and tended to reduce DM digestibility ($P = 0.06$). These results suggest that the reduced-RDP diet was deficient in N to support ruminal or postruminal carbohydrate and fat digestion compared with the high-RDP diet. Others have similarly found that a predicted protein deficiency reduced diet digestibility in dairy heifers (Marini and Van Amburgh, 2005) and in lactating dairy cows (Cameron et al., 1991; Ruiz et al., 2002).

Fecal Purines

Fecal DM output significantly increased by 0.80 kg/d for the inulin infusion ($P = 0.002$; Table 4). Fecal purines were used to predict how much of the increase in fecal DM output with inulin infusion was due to increased fecal microbial DM excretion and how much

was due to reduced true digestibility of dietary DM. Inulin increased the fecal purine output by 0.41 and 2.34 g/d for cows receiving the low- and high-RDP diets, respectively. The purine content of ruminal microbes averages 1.2% of microbial DM, ranging from 0.7 to 2.1% (Obispo and Dehority, 1999; Rodríguez et al., 2000; Carro and Miller, 2002; Reynal et al., 2003; Vicente et al., 2004). Assuming that fecal bacteria have the same mean (and range) in purine content, the average fecal purine excretion measured in these cows was used to estimate the increase in fecal microbial DM output attributable to the inulin infusion. Inulin increased the fecal microbial DM output by 34 g/d (range 20 to 59 g/d) for cows fed the low-RDP diet and 195 g/d (111 to 334) for cows fed the high-RDP diet. These calculations indicate that increased fecal microbial output accounted for only 12% (range 7 to 20%) and 31% (range 18 to 53%) of the decrease in DM digestibility with inulin infusion for cows fed the low- and high-RDP diets, respectively. The majority of the increase in fecal DM output with inulin infusion was apparently due to inulin reducing true digestibility of dietary components, including starch.

Similar calculations were performed to predict the extent of the increase in fecal N with inulin infusion that was due to increased fecal microbial N. Based on a summary of 7 reports of rumen bacteria purine and N contents (Gressley and Armentano, 2005), the average increase in fecal bacteria N with inulin infusion was 13 g/d (range 8 to 22 g/d) for the high-RDP diet but only 2 g/d (range 1 to 4 g/d) for the low-RDP diet. This suggests that increased fecal microbial N accounted for only 11% of the 18 g/d increase in fecal N with inulin infusion for the low-RDP diet, but accounted for 44% of the 29 g/d increase in fecal N with inulin infusion for the high-RDP diet. Because infused inulin was >99% degraded for both diets, the efficiency of conversion of inulin to fecal microbial N was apparently reduced with the low-RDP diet. Perhaps urea recycling to the large intestine was reduced with the low-RDP diet, and lower NH_3 concentrations available to intestinal microbes may have contributed to the reduced efficiency. When cows were infused abomasally with pectin, fecal microbial N (as calculated from fecal purines) was responsible for approximately 77% of the observed increase in fecal N (Gressley and Armentano, 2005). The authors suggested that the remaining 23% was because the high viscosity of pectin reduced the digestibility of dietary or endogenous proteins. In the current experiment, bacterial N was responsible for even less of the increase in fecal N with infused inulin, a nonviscous fiber source. Both abomasal pectin and inulin appear to have negatively influenced protein nutrition by increasing fecal

Table 3. Effect of dietary RDP and abomasal inulin infusion on intake and digestion

Item	Treatment ¹				SED ²	Contrast ³		
	LP	LPI	HP	HPI		RDP	I	RDP × I
Oral DMI ⁴	(kg/d)					(P-value)		
	20.0	20.3	20.2	19.8	0.4	0.53	0.88	0.25
Infused	(Inulin, g/d)							
Fecal output	0	924	0	924	—	—	—	—
	14.1	21.2	11.8	16.9	2.1	0.05	0.002	0.51
	(Oral plus infused, ⁵ kg/d)							
DM	20.0	21.3	20.2	20.8	0.4	0.53	0.009	0.25
OM	18.9	20.2	19.0	19.6	0.4	0.51	0.006	0.25
Starch	7.26	7.39	7.30	7.15	0.15	0.38	0.96	0.22
NDF	5.26	5.34	5.30	5.19	0.11	0.50	0.85	0.25
NDSOM ⁶	13.6	14.8	13.7	14.4	0.3	0.51	0.001	0.25
Fatty acids	0.75	0.76	0.74	0.73	0.02	0.12	0.97	0.27
	(Apparent digestibility, ⁷ %)							
DM	62.9	61.7	66.1	63.3	1.6	0.06	0.10	0.50
OM	64.1	62.8	67.5	64.6	1.6	0.05	0.10	0.50
Starch	94.3	92.0	95.8	94.3	0.9	0.008	0.01	0.53
NDF	23.0	21.0	29.1	21.9	3.8	0.21	0.11	0.34
NDSOM	80.0	77.9	82.2	79.9	1.0	0.008	0.007	0.88
Fatty acids	65.5	66.1	70.4	67.9	1.4	0.007	0.38	0.16
	(Apparently digested, ⁷ kg/d)							
DM	12.6	13.2	13.4	13.1	0.5	0.35	0.72	0.31
OM	12.1	12.7	12.9	12.7	0.5	0.31	0.63	0.31
Starch	6.84	6.80	7.00	6.75	0.17	0.68	0.24	0.40
NDF	1.23	1.13	1.56	1.14	0.21	0.29	0.10	0.31
NDSOM	10.9	11.5	11.3	11.5	0.3	0.37	0.06	0.35
Fatty acids	0.491	0.505	0.525	0.494	0.010	0.40	0.53	0.12

¹Treatments: LP = low-RDP diet with abomasal saline infusion; LPI = low-RDP diet with 1 kg/d of abomasal inulin; HP = high-RDP diet with abomasal saline infusion; HPI = high-RDP diet with 1 kg/d of abomasal inulin.

²SED = standard error of the difference among treatment means.

³RDP = main effect of RDP level; I = main effect of abomasal inulin infusion; RDP × I = RDP × inulin interaction.

⁴Intake of the basal ration only, not including inulin infused.

⁵Intake of the basal ration plus inulin infused.

⁶NDSOM = neutral detergent-soluble organic matter = DM – NDF – ash.

⁷Digestibility calculations include infused inulin. Fecal outputs predicted utilizing La as a marker.

endogenous protein output, decreasing endogenous protein resorption, or reducing true protein digestibility.

Fecal microbial DM and microbial N outputs were calculated from fecal purines as outlined above. Isolation of cecal or large intestinal microbes in the current requirement would have required additional cannulation surgeries. To our knowledge, there are no published measurements of purine content of microbes isolated from the cecum, colon, or feces of ruminant animals. Therefore, we assumed that the purine content of ruminal microbes is similar to that of microbes produced in the large intestine. Because the reported purine content of ruminal microbes varies, we estimated fecal microbial DM and microbial N outputs based on the mean and range of published data. The rumen microbial purine:N (g/mmol) content in 7 published studies ranged from 0.74 to 1.85, with an average of 1.25 (Gressley

and Armentano, 2005). A microbe-rich fraction isolated from dog feces had a purine:N ratio of 1.52 (Karr-Lilienthal et al., 2004), demonstrating that fecal and ruminal microbes have similar purine:N ratios. Only a fraction of the increase in fecal N and DM outputs with the inulin infusion appeared to be due to increased fecal microbial output. However, the precise magnitude of the increase in fecal microbial output with inulin infusion could not be determined from our data.

Ruminal Characteristics

There were no significant effects of treatments on rumen pH or on rumen VFA, except that increasing RDP reduced the molar percentage of isovalerate ($P = 0.05$; Table 5). There was also a significant interaction of diet by hour after feeding for isovalerate ($P < 0.001$)

Table 4. Effect of dietary RDP and abomasal inulin infusion on fecal characteristics

Item	Treatment ¹				SED ²	Contrast ³		
	LP	LPI	HP	HPI		RDP	I	RDP × I
	(P-value)							
Fecal DM, ⁴ kg/d	7.39	8.16	6.81	7.64	0.28	0.02	0.002	0.89
Fecal DM, %	15.41	15.51	15.11	14.79	0.30	0.03	0.62	0.33
Purines, g/d	10.28	10.69	9.07	11.41	0.41	0.40	0.001	0.005
Purines, mmol/kg of DM	9.67	9.16	9.30	10.40	0.31	0.06	0.18	0.003

¹Treatments: LP = low-RDP diet with abomasal saline infusion; LPI = low-RDP diet with 1 kg/d of abomasal inulin; HP = high-RDP diet with abomasal saline infusion; HPI = high-RDP diet with 1 kg/d of abomasal inulin.

²SED = standard error of the difference among treatment means.

³RDP = main effect of RDP level; I = main effect of abomasal inulin infusion; RDP × I = RDP × inulin interaction.

⁴Fecal DM output determined using La as a marker.

because of the low-RDP diet having a lower molar percentage than the high-RDP diet 0 and 2 h after feeding but a higher percentage for the remainder of the day (data not shown). Across treatments, the rumen VFA concentration averaged 83 mM. These values are low compared with an average of 102 mM found in experiments using cows with similar DMI and milk production (Kalscheur et al., 1997; Kennelly et al., 1999; Ruiz et al., 2001). The predominant carbohydrate sources in their dietary concentrates consisted of ground corn, cornmeal, and an equal mix of rolled barley and rolled

corn, respectively. The present study used cracked corn grain as opposed to ground or rolled corn in an effort to reduce large intestinal fermentability of the corn grain. However, the cracked corn grain also may have caused ruminal carbohydrate digestion to be low for this experiment compared with other experiments.

Rumen NH₃ data were not normally distributed (data not shown). Forty-three percent of the data points were between 0 and 2 mM, 23% were 2 to 4 mM, 13% were 4 to 6 mM, 7% were 6 to 8 mM, and the remaining 14% ranged from 8 to 23 mM. Rumen NH₃ values were log

Table 5. Effect of dietary RDP and abomasal inulin infusion on urinary purine derivatives and ruminal characteristics

Item	Treatment ¹				SED ²	Contrast ³		
	LP	LPI	HP	HPI		RDP	I	RDP × I
	(P-value)							
pH	6.74	6.71	6.75	6.75	0.03	0.33	0.47	0.60
VFA, mM	82.7	82.8	82.8	81.8	2.3	0.82	0.79	0.73
NH ₃ , ⁴ mM	1.68	1.27	3.42	3.95	1.12	0.001	0.43	0.03
Urinary purine derivatives, ⁵ mmol/d	450	487	501	481	18	0.11	0.56	0.05
In situ NDF digestibility, ⁶ %	24.6	25.3	27.6	27.3	1.3	0.02	0.85	0.57
	(mol/100 mol)							
Acetate	62.7	63.0	63.2	63.4	0.6	0.27	0.58	0.91
Propionate	23.9	23.4	23.5	23.2	0.6	0.46	0.36	0.82
Butyrate	8.07	8.32	8.20	8.39	0.34	0.69	0.39	0.89
Isobutyrate	1.38	1.33	1.29	1.31	0.04	0.12	0.76	0.27
Valerate	1.55	1.53	1.52	1.50	0.06	0.53	0.59	0.99
Isovalerate	2.43	2.45	2.30	2.23	0.12	0.05	0.75	0.57

¹Treatments: LP = low-RDP diet with abomasal saline infusion; LPI = low-RDP diet with 1 kg/d of abomasal inulin; HP = high-RDP diet with abomasal saline infusion; HPI = high-RDP diet with 1 kg/d of abomasal inulin.

²SED = standard error of the difference among treatment means.

³RDP = main effect of RDP level; I = main effect of abomasal inulin infusion; RDP × I = RDP × inulin interaction.

⁴Log₁₀(NH₃) used for statistical analysis. Reported values are geometric means calculated as 10 taken to the least squares means. Arithmetic means were 2.56, 2.06, 5.39, and 5.94 mM for the low-RDP diet with saline, low-RDP diet with inulin, high-RDP diet with saline, and high-RDP diet with inulin, respectively.

⁵Urinary excretion of allantoin plus uric acid.

⁶Determined as disappearance of NDF from soyhulls after a 12-h rumen incubation.

Figure 1. Rumen NH₃ and BUN. Treatments were an NRC (2001)-adequate RDP diet with abomasal saline (HP, ◇), NRC-adequate RDP diet with 1 kg/d of abomasal inulin (HPI, ◆), 28% less RDP than predicted requirements with abomasal saline (LP, □), and 28% less RDP with 1 kg/d of abomasal inulin (LPI, ■). Rumen ammonia data were log transformed prior to statistical analysis, and results presented are geometric means. Main effects of diet ($P < 0.001$), period ($P < 0.001$), and hour after initial feeding ($P < 0.001$) and interactions of diet $\times$ infusion ($P = 0.02$) and diet $\times$ hour after initial feeding ($P < 0.001$) were significant for rumen ammonia, and pooled SED = 1.38 mM. Main effects of diet ($P < 0.001$), infusion ($P = 0.03$), and hour ($P = 0.001$) and the interaction of diet $\times$ hour ($P = 0.02$) were significant for BUN, and pooled SED = 0.85 mg/dL.

transformed prior to statistical analyses, and data reported in Table 5 and Figure 1 are geometric means back-transformed from the log data. Arithmetic means were 2.56, 2.06, 5.39, and 5.94 mM for the low-RDP diet with abomasal saline infusion, the low-RDP diet with 1 kg/d of abomasal inulin, the high-RDP diet with abomasal saline infusion, and the high-RDP diet with 1 kg/d of abomasal inulin, respectively.

Abomasal pectin infusion tended to decrease rumen NH₃ and urinary purine derivative excretion in cows fed adequate protein diets (Gressley and Armentano, 2005). In the current experiment we hypothesized that inulin would reduce rumen NH₃ and urinary purine derivatives, and that this decrease would be most se-

vere with the low-RDP diet. However, there were no main effects of inulin on rumen NH₃ or urinary purine derivatives. The interaction between diet and inulin on rumen NH₃ indicated that inulin decreased rumen NH₃ with the low-RDP diet, but inulin actually increased rumen NH₃ with the high-RDP diet. Overall, urea recycling to the rumen was apparently unaffected by increasing intestinal fermentation. The abomasal inulin infusion was designed to approximately double the amount of hindgut fermentation compared with that of cows that did not receive inulin. The infused amount was selected to approximate a maximum increase in hindgut fermentation that could be expected by practical dietary manipulations. These results suggest that manipulating dairy diets to increase hindgut fermentation and reduce manure ammonia volatilization should not reduce N recycling to the rumen for microbial protein production.

As expected, decreasing the RDP reduced rumen NH₃ ($P < 0.001$), by 1.74 mM for saline-infused cows, and by 2.68 mM for inulin-infused cows. There was a significant diet $\times$ hour interaction after feeding for rumen NH₃ ($P = 0.03$) because of wider daily fluctuations for cows fed the high-RDP diet compared with the low-RDP diet (Figure 1). The geometric mean NH₃ concentration for the high-RDP diet was 3.7 mM, similar to the requirement of 3.6 mM found by Satter and Slyter (1974). Therefore, the high-RDP diet was successful at providing adequate, but not excessive, NH₃. Geometric mean rumen NH₃ for the low-RDP diet was 1.5 mM, which is substantially below the 3.6 mM recommendation. Despite this, there was no main effect of dietary RDP on urinary purine derivative excretion or on the yield of milk or milk protein, suggesting that ruminal NH₃ was adequate to support rumen microbial CP production even with the low-RDP diet. Satter and Slyter (1974) indicated that the rumen NH₃ required for maximum microbial CP production may be closer to 1.4 mM, and our data would support this view. However, the additional RDP for the high-RDP diet was supplied by urea. If the RDP source contained peptides and proteins, the microbial responses may have been different.

Although there was no effect of diet on urinary purine derivatives, the low-RDP diet may not have provided adequate NH₃ to support ruminal digestion, as evidenced by decreased *in situ* rumen NDF digestibility ($P = 0.02$). Similarly, increasing dietary urea in protein-deficient diets increased *in situ* NDF digestibility in prepartum (Dorshorst and Grummer, 2002) and postpartum (Ruiz et al., 2002) cows. In addition, the low-RDP diet reduced total tract apparent digestibility of OM, starch, and NDSOM, and part of the decrease may have been due to low rumen NH₃ reducing ruminal digestibility. In his review of *in vivo* and *in vitro* studies,

Table 6. Effect of dietary RDP and abomasal inulin infusion on milk production and composition and blood urea N

Item	Treatment ¹				SED ²	Contrast ³		
	LP	LPI	HP	HPI		RDP	I	RDP × I
						(P-value)		
Milk, kg/d	29.7	31.3	30.4	29.3	0.7	0.25	0.63	0.02
Fat, %	3.73	3.43	3.60	3.56	0.09	0.93	0.02	0.06
Fat, kg/d	1.10	1.08	1.10	1.04	0.03	0.29	0.05	0.45
Protein, %	3.24	3.18	3.22	3.23	0.04	0.54	0.40	0.26
Protein, kg/d	0.96	0.99	0.97	0.94	0.02	0.25	0.95	0.05
MUN, mg/dL	10.9	9.1	16.5	15.9	0.4	0.001	0.001	0.06
MUN, g/d	3.3	2.9	5.0	4.8	0.2	0.001	0.01	0.52
BUN, mg/dL	6.6	5.7	13.1	12.2	0.6	0.001	0.05	0.98

¹Treatments: LP = low-RDP diet with abomasal saline infusion; LPI = low-RDP diet with 1 kg/d of abomasal inulin; HP = high-RDP diet with abomasal saline infusion; HPI = high-RDP diet with 1 kg/d of abomasal inulin.

²SED = standard error of the difference among treatment means.

³RDP = main effect of RDP level; I = main effect of abomasal inulin infusion; RDP × I = RDP × inulin interaction.

Hoover (1986) found that the requirement for rumen NH_3 averaged 2.4 mM for microbial growth, but it was 5.7 mM for nutrient digestion. The low-RDP diet apparently contained adequate RDP to support rumen microbial protein production but was deficient in RDP to support ruminal carbohydrate digestion.

Milk Production and Composition

Decreasing RDP and infusing inulin each significantly decreased MUN concentration, MUN output, and BUN concentration (Table 6; Figure 1). The increased BUN and MUN with increasing RDP reflect the increased rumen NH_3 . However, the decreased BUN and MUN with inulin infusion were independent of effects on rumen NH_3 . Reductions in BUN attributable to increased fecal microbial protein production were observed when rats were fed up to 15% inulin (Younes et al., 1997) or when sheep were given up to 90 g/d of cecal glucose infusions (Thornton et al., 1970).

Despite RDP effects on nutrient digestibility, there were no significant main effects of RDP on milk production or composition. Rumen-degradable protein was predicted to be 28% below requirements for the low-RDP diet; however, the predicted RDP deficiency did not reduce milk yield. When urea was used to adjust diets from well below predicted protein requirements to up to 14% CP, milk yield significantly increased in several studies (Polan et al., 1976; Wohlt and Clark, 1978; Ruiz et al., 2002). However, in the current experiment, urea was used to increase dietary CP from 13.5 to 16.1%, and no effects on production were observed. This agrees with other studies that have found that addition of RDP to diets containing at least 13% CP

does not increase milk production (Holter et al., 1982; Armentano et al., 1993).

Milk fat yield significantly decreased with abomasal inulin ($P = 0.05$). The same effect was also observed when pectin was infused abomasally (Gressley and Armentano, 2005), indicating that abomasal fiber infusion appears to elicit a decrease in milk fat. The milk fatty acid composition is reported in Table 7. Milk fat depression in cows is typically associated with a decrease in the secretion of short-chain fatty acids (C_4 to C_{14}) into milk due at least in part to the biological activity of specific conjugated linoleic acids absorbed from the rumen (Bauman and Griinari, 2003). As a consequence, milk proportions of short-chain fatty acids decrease while conjugated linoleic acids and long-chain fatty acids ($\geq \text{C}_{18}$) increase. However, the inulin infusion significantly increased the proportion of short-chain fatty acids ($P = 0.02$ for C_4 to C_{13} , and $P = 0.03$ for $< \text{C}_{16}$) and tended to decrease the proportion of 18:2 ($P = 0.06$). Also, there was no indication of an increase in either *trans*-10 18:1 or *trans*-10, *cis*-12 18:2 attributable to inulin infusion. The reduction in milk fat yield with inulin infusion was apparently due to a decrease in the secretion of 18-carbon fatty acids. Milk fatty acids with 18 or more carbons typically arise from circulating lipids that are derived from dietary and microbial fatty acids (Bauman and Griinari, 2003). However, inulin did not affect fatty acid digestibility (Table 2), indicating that the reduction in milk fat was apparently not due to a decrease in fat digestibility.

The model used in this experiment, as well as in the experiments of Gressley and Armentano (2005), was developed to quantify effects of hindgut fermentation. However, this model exposed the small intestine to solu-

Table 7. Effect of dietary RDP and abomasal inulin on milk fatty acids

Item	Treatment ¹				SED ²	Contrast ³		
	LP	LPI	HP	HPI		RDP	I	RDP × I
	(g/100 g of fatty acids)							
C ₄ to C ₁₃ ⁴	11.8	12.4	11.8	12.3	0.3	0.86	0.02	0.86
14:0	10.5	10.8	10.6	10.6	0.2	0.80	0.16	0.23
14:1	0.90	0.93	0.91	0.93	0.04	0.90	0.44	0.89
16:0	29.4	29.6	29.5	29.7	0.4	0.75	0.52	0.96
16:1	1.38	1.35	1.37	1.22	0.16	0.56	0.48	0.61
C18:0	13.0	12.8	12.9	12.7	0.4	0.71	0.51	0.90
C ₁₅ + C ₁₇ + C ₁₉ ⁵	1.92	1.88	1.86	1.91	0.06	0.81	0.96	0.35
18:1	25.5	24.9	25.8	25.6	0.5	0.36	0.32	0.53
Trans-4	0.012	0.021	0.015	0.008	0.010	0.53	0.93	0.34
Trans-5	0.008	0.000	0.002	0.002	0.004	0.44	0.17	0.21
Trans-6 to trans-8 ⁶	0.20	0.20	0.20	0.21	0.01	0.09	0.68	0.53
Trans-9	0.17	0.17	0.17	0.17	0.01	0.63	0.77	0.26
Trans-10	0.28	0.26	0.27	0.26	0.01	0.50	0.12	0.97
Trans-11	0.68	0.69	0.77	0.79	0.04	0.004	0.75	0.85
Trans-12	0.33	0.32	0.31	0.34	0.02	0.94	0.45	0.15
Cis-9 ⁷	22.0	21.5	22.2	22.1	0.5	0.37	0.39	0.59
Cis-11	0.76	0.70	0.74	0.73	0.03	0.68	0.15	0.35
Cis-12	0.30	0.31	0.27	0.26	0.03	0.09	0.84	0.69
Cis-13	0.054	0.065	0.048	0.039	0.012	0.07	0.92	0.23
Cis-14 + trans-16	0.22	0.20	0.21	0.20	0.01	0.65	0.46	0.52
Cis-15	0.063	0.045	0.051	0.041	0.015	0.45	0.22	0.72
CLA ⁸ cis-9, trans-11	0.36	0.37	0.43	0.42	0.02	0.007	0.92	0.59
CLA trans-10, cis-12	0.013	0.017	0.019	0.013	0.003	0.62	0.54	0.05
18:2	3.36	3.10	3.12	2.94	0.15	0.08	0.06	0.70
18:3	0.192	0.192	0.182	0.184	0.011	0.64	0.64	0.45
Others ⁹	0.405	0.406	0.452	0.397	0.067	0.69	0.58	0.56
Unidentified peaks	1.63	1.62	1.77	1.66	0.14	0.39	0.57	0.63
<C ₁₆ ¹⁰	23.1	24.1	23.3	23.8	0.5	0.85	0.03	0.54
>C ₁₆ ¹¹	42.0	40.9	41.8	41.3	0.8	0.91	0.18	0.62

¹Treatments: LP = low-RDP diet with abomasal saline infusion; LPI = low-RDP diet with 1 kg/d of abomasal inulin; HP = high-RDP diet with abomasal saline infusion; HPI = high-RDP diet with 1 kg/d of abomasal inulin.

²SED = standard error of the difference among treatment means.

³RDP = main effect of RDP level; I = main effect of abomasal inulin infusion; RDP × I = RDP × inulin interaction.

⁴C₄ to C₁₃ = sum of 4:0, 5:0, 6:0, 7:0, 8:0, 10:0, 12:0, and 13:0.

⁵C₁₅ + C₁₇ + C₁₉ = sum of 15:0, 15:1, 17:0, 17:1, and 19:0.

⁶Includes 18:1 trans-6, -7, and -8.

⁷The cis-9 peak includes all cis <11, trans-13, trans-14, and trans-15 18:1 isomers.

⁸CLA = conjugated linoleic acid.

⁹Others = sum of 20:0, 20:1, 20:2n-6, 20:3n-6, 20:3n-3, 20:4n-6, 20:5n-3, 21:0, 22:0, 22:1n-9; 22:2, 22:6n-3, 23:0, 24:0, and 24:1.

¹⁰<C₁₆ = sum of C₄ to C₁₃, 14:0, and 14:1.

¹¹>C₁₆ = sum of 18:0, 18:1, 18:2, 18:3, and "others."

ble and highly fermentable fiber. In typical production situations, a cow's small intestine would never be exposed to similar amounts of highly digestible fiber. Some of the unexpected effects of the inulin infusion on digestion and milk fat may have been artifacts of the model.

Predicted Protein Requirements

During the 2-wk preexperimental period, cows were fed the high-RDP ration ad libitum. Milk production

averaged 37.9 kg/d and DMI averaged 23.5 kg/d, about 8% less than the 25.6 kg/d estimated by NRC (2001). Using these values and the milk composition determined for cows fed the high-RDP diet and infused with saline (Table 6), the predicted RDP balance (NRC, 2001) was -48 g/d, the RUP balance was -64 g/d, the MP balance was -54 g/d, NE_L-allowable milk was 38.0 kg/d, and MP-allowable milk was 36.8 kg/d. Following this period, each cow was restricted to 90% of her predicted ad libitum intake, and feed offered averaged 21.2 kg/d. At the restricted feeding level, the predicted MP bal-

Table 8. Predicted protein requirements and milk production (NRC, 2001)

NRC prediction ¹	Treatment ²			
	LP	LPI	HP	HPI
	(g/d)			
RDP required	2,073	2,101	2,079	2,043
RDP supply	1,490	1,509	2,030	1,994
RDP balance	-593	-592	-49	-48
RUP required	1,531	1,588	1,177	1,131
RUP supply	1,210	1,231	1,213	1,185
RUP balance	-322	-356	36	54
MP balance	-272	-301	30	46
	(kg/d)			
MP-allowable milk ³	24.1	25.0	31.0	30.3
NE _L -allowable milk	31.1	33.0	31.8	31.2

¹Values predicted using actual milk production, milk composition, BW, and basal ration intake for each treatment (NRC, 2001). Default values for nutrient contents of basal ration ingredients were modified to reflect analyzed contents of CP, NDF, and ADF. Abomasal inulin infusion was not included when predicting requirements.

²Treatments: LP = low-RDP diet with abomasal saline infusion; LPI = low-RDP diet with 1 kg/d of abomasal inulin; HP = high-RDP diet with abomasal saline infusion; HPI = high-RDP diet with 1 kg/d of abomasal inulin.

ance was reduced to -248 g/d, NE_L-allowable milk was 33.6 kg/d, and MP-allowable milk was 32.7 kg/d. The high-RDP diet was therefore predicted to be equally limiting in both energy and protein. When using the same intake and production values, the low-RDP diet should have been severely limiting in protein, with -577 g/d of MP, 33.9 kg/d of NE_L-allowable milk, and 25.9 kg/d of MP-allowable milk.

Table 8 reports NRC-predicted protein requirements and supplies (NRC, 2001) using treatment means for intake, milk production, and milk composition from Tables 3 and 6. The predictions include only nutrients supplied by the rations, and abomasal inulin was not considered in the predictions. For both high-RDP treatments, RDP supply was just below requirements and RUP supply was just above requirements, resulting in an estimated MP balance that averaged +38 g/d. Energy-allowable milk was predicted to be 31.5 kg/d and MP-allowable milk was 30.7 kg/d, and these were similar to actual production, which averaged 29.9 kg/d. For the low-RDP treatments, the predicted RDP supply was 72% of requirements, with the RDP balance averaging -593 g/d. Although RUP supply was not different from that of the high-RDP treatments, the NRC (2001) guidelines increased the RUP requirement because of the predicted RDP deficiency, resulting in a negative RUP balance and a calculated MP balance of -287 g/d. Average milk production for the low-RDP treatments was 30.5 kg/d, 24% more than the NRC-predicted MP-allowable milk. Cows were able to maintain milk production despite this predicted deficiency in RDP. Periods were

only 2 wk long, and it is possible that cows could have been drawing on body N, but this explanation is not attractive because cows on the low-RDP diet were in less of a negative N balance than cows on the high-RDP diet.

The NRC (2001) model appears to have overestimated RDP requirements for these cows. As described above, rumen VFA were low compared with other published data, and ruminal carbohydrate digestibility may have been lower than predicted by the NRC (2001). Additionally, the NRC (2001) does not differentiate between RDP provided as NH₃, peptides, and AA, and microbial requirements for NH₃ may be lower than requirements for peptides and AA.

CONCLUSIONS

Abomasal inulin infusion increased fecal N and decreased urinary N, MUN, and BUN as expected, in agreement with similar experiments using pectin. We had hypothesized that these changes would be due to large intestinal bacteria utilizing energy from inulin to convert BUN into fecal microbial protein. As determined by fecal purine excretion, increased fecal microbial N excretion was responsible for only 11 to 44% of the increase in fecal N with abomasal inulin, suggesting that inulin reduced true digestibility of dietary protein or increased nonmicrobial endogenous protein losses. Inulin did not affect ruminal NH₃ or urinary purine derivative excretion, which indicates that recycling of urea to the rumen and production of rumen microbial CP were relatively unaffected by hindgut fermentation. Increasing hindgut fermentation appears to shift some N excretion from the urine to the feces, and this should increase the environmental stability of manure N. However, the reduction in manure ammonia losses is likely to be small. Inulin infusion was associated with negative responses such as reduced diet digestibility and milk fat yield, but those responses may have been an artifact of the model.

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