Effects of Amounts and Degradability of Dietary Protein on Lactation, Nitrogen Utilization, and Excretion in Early Lactation Holstein Cows

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ABSTRACT

Five treatment diets varying in crude protein (CP) and rumen undegradable protein (RUP) were calculated to supply a postruminal lysine to methionine ratio of about 3:1. Diets were fed as a total mixed ration to 65 Holstein cows that were either primiparous ($n = 28$) or multiparous ($n = 37$) from 21 to 120 d in milk to determine effects on lactation and nitrogen utilization. Crude protein % and calculated RUP (% of CP) of diets [on a dry matter (DM) basis] were: 1) 19.4, 40 (HPMU), 2) 16.5, 34 (LPLU), 3) 16.8, 40 (LPMU), 4) 16.8, 46 (LPHU), 5) 17.2, 43 (LPHU+UREA), which is the result of adding 0.4% of the diet DM as urea to LPHU. The corn silage-based treatment diets contained an average of 24% acid detergent fiber and 1.6 Mcal/kg net energy of lactation. Milk urea nitrogen (MUN) concentrations and body weights (BW) were used to calculate predicted amounts of urinary nitrogen (N) using the relationship: urinary N (g/d) = $0.0259 \times \text{BW (kg)} \times \text{MUN (mg/dl)}$. Cows fed HPMU had greater CP and RUP intakes, which resulted in higher concentrations of plasma urea nitrogen, rumen ammonia, MUN, and predicted urinary N. Milk yield, fat yield, fat percent, protein yield, and protein percent were not significantly different among treatments. Parity primarily affected parameters that were related to body size and not measurements of N utilization. The interaction of treatment and parity was not significant for any measurements taken. In this study, cows fed LPHU had significantly lower MUN and predicted urinary N without limiting production. These results demonstrate the potential to optimize milk production while minimizing N excretion in lactating dairy cattle.

(Key words: rumen undegradable protein, urea, nitrogen excretion)

Abbreviation key: EAA = essential AA; HPMU = control or moderate CP, moderate RUP; LPLU = low CP,

low RUP; LPMU = low CP, moderate RUP; LPHU = low CP, high RUP; LPHU+UREA = low CP, high RUP, added urea; MP = metabolizable protein; MUN = milk urea nitrogen; PUN = plasma urea nitrogen.

INTRODUCTION

Research is currently being directed toward improving the efficiency of protein and nitrogen utilization by the lactating dairy cow while optimizing milk production and milk composition (NRC, 2001). Overfeeding CP increases feed costs and decreases the efficiency of nutrient utilization (Tamminga et al., 1992). As a result, overfeeding CP produces environmental waste from ammonia loss in the air and nitrate contamination of surface and ground water (Tamminga et al., 1992; 1996). In addition, some studies also have shown that overfeeding CP decreases animal fertility (Canfield et al., 1990; Rajala-Schultz et al., 2001). Therefore, lactating dairy cow rations should be formulated to optimize the CP content for both milk production and the efficiency of N utilization.

Lactating dairy cows require AA for milk production, and these requirements vary with the level of milk production and composition. Required AA are supplied primarily by combinations of microbial protein and RUP. The RDP portion of CP, which consists of both true protein and NPN, is used to supply nitrogen for microbial protein production in the rumen, whereas RUP passes intact from the rumen. Both microbial protein and RUP contribute to the metabolizable protein pool.

Amino acid requirements may be supplied by formulating diets to maximize microbial protein synthesis while supplying additional RUP of the amount and quality that will complement microbial protein. Dietary requirements for dairy cattle are based on the metabolizable protein (MP) requirement, which is defined as the true protein that is digested postruminally and its component AA that are absorbed by the intestine (NRC, 2001). Extending the definition of protein requirements to include the amounts of essential AA (EAA) required by the lactating dairy cow should result in the ability to

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develop diets that improve the efficiency of N utilization and minimize losses in feces, urine and gases.

Lysine and Met have been suggested to be the first and second limiting AA for milk production in lactating dairy cows fed corn-based rations (Schwab et al., 1992a; 1992b). Lysine and Met are present in body tissue, rumen bacteria, and milk in approximately a 3:1 ratio (Table 5-10, NRC, 2001). Schwab (1996) suggested that supplying Lys and Met as 15 and 5% of the duodenally digestible EAA profile or in approximately a 3:1 ratio should optimize Lys and Met availability for milk protein production. Many studies that have evaluated production responses of dairy cattle to RUP supplementation have not considered the Lys and Met content across treatments, but have used diets formulated for RUP content only (NRC, 2001). In these cases, it is not clear whether production responses were the effect of RUP content or AA supply.

This experiment was designed to use early lactation dairy cows fed diets formulated to supply postruminal Lys and Met in a 3:1 ratio to determine the effects of CP and RUP combinations on milk production and N loss in feces and urine.

MATERIALS AND METHODS

Diets and Cow Management

Sixty-five Holstein cows from the Piedmont Research Station in Salisbury, NC, were assigned randomly to one of five treatment groups within either primiparous or multiparous blocks. The number of primiparous and multiparous cows on each treatment was: HPMU 6, 7; LPLU 7, 6; LPMU 5, 8; LPHU 6, 7; and LPHU+UREA 4, 9. Cows were added to the study individually over a 6-mo period at the time each calved. At calving, cows were trained to Calan feeding stations (American Calan Inc., Northwood, NH). By 21 DIM, cows were adjusted to the feeding stations and consuming experimental diets fed as a TMR twice daily through 120 DIM.

The treatment diets were corn silage based and contained approximately 24% ADF and 1.6 Mcal/kg of NE_L on a DM basis (Table 1). Treatment diets were formulated according to Nutrient Requirements for Dairy Cattle (NRC, 1989) and contained CP (% of DM) and RUP (% of CP) as follows: 1) 19.4, 40 (HPMU); 2) 16.5, 34 (LPLU); 3) 16.8, 40 (LPMU); 4) 16.8, 46 (LPHU); 5) 17.2, 43 (LPHU+UREA). Urea was added to the LPHU diet at 0.4% of the diet DM to create the LPHU+UREA diet. The levels of CP and RUP in the treatments were intended to represent a typical early lactation diet for North Carolina herds (HPMU), a diet lower in CP than typically fed with low, intermediate and high levels of RUP (LPLU, LPMU, LPHU) and a low CP diet with a high level of RUP supplemented

with urea (LPHU+UREA). All diets were formulated to contain a post-rationally available Lys to Met ratio of approximately 3:1 using values reported by Clark et al. (1987) and the Mepron Dairy Ration Evaluator (Version 2.1, 1999; Degussa Hüls Corp., Bannockburn, IL).

Cows were housed in a free-stall barn and fed *ad libitum*. The daily feed allocations andorts were recorded for each cow to estimate intakes of DM, CP, and ADF.

Sample Collection and Analysis

The TMR for each of the five treatment diets were sampled weekly and composited monthly and analyzed for DM, CP, and ADF by the Constable Laboratory (North Carolina Department of Agriculture, Raleigh, NC). The ingredient and nutrient compositions of the treatment diets are reported in Table 1.

Cows were milked twice daily at 0330 and 1530 h, with milk weights recorded at each milking. Milk samples were collected weekly (a.m./p.m. composite) and analyzed for fat, CP, and MUN by the United Federation of DHIA Laboratory (Blacksburg, VA). Milk fat and CP were analyzed according to AOAC (1990) procedures. The Bentley ChemSpec 150 analyzer (Chaska, MN) was used to determine MUN concentrations using a modified Berthelot reaction based on the methods described by Chaney and Marbach (1962).

Blood samples were collected approximately 5 h post-feeding on 21, 30, 60, 90, and 120 DIM. Blood was collected via jugular venipuncture into Vacutainers containing EDTA and placed on ice for transport to the laboratory. These samples were centrifuged for 15 min at 2500 × *g*, and plasma was harvested and frozen until analysis. Plasma was analyzed for urea nitrogen (PUN) using the diacetyl monoxime method of Marsh et al. (1957) and for NEFA using WAKO reagent kits (Anonymous, Wako Chemicals USA, Inc., Richmond, VA). Plasma samples collected at 30 and 120 DIM were analyzed by the Experiment Station Chemical Laboratories at the University of Missouri (Columbia, MO) for AA using HPLC.

Ruminal fluid was collected approximately 5 h post-feeding on 21, 30, 60, 90, and 120 DIM using a stomach tube connected to a vacuum pump, placed on ice for transport, and frozen at -20°C until analysis. Ruminal fluid was thawed and centrifuged at 2500 × *g* for 10 min at 4°C, and the supernatant was removed. Ruminal fluid supernatant was analyzed for ammonia (Beecher and Whitten, 1970). To prepare ruminal fluid for VFA analysis, 1 ml of 25% metaphosphoric acid with an internal standard was added to 5 ml of supernatant and centrifuged at room temperature (approximately 22°C) for 15 min at 9300 × *g*. A 1-ml aliquot of this was

Table 1. Ingredient and nutrient composition of dietary treatments.

Item	Dietary treatments ¹				
	HPMU	LPLU	LPMU	LPHU	LPHU+UREA
Ingredient, % of DM					
Corn silage, unprocessed	34.8	30.0	31.7	34.6	34.0
Cottonseed hulls	5.2	5.1	5.2	5.2	5.2
Soybean hulls	4.4	10.7	14.0	4.6	5.7
Whole cottonseed	14.1	13.9	14.0	14.0	14.0
Ground, dry shelled corn	21.7	16.1	21.8	30.0	29.0
48% soybean meal	12.1	7.0	5.9	1.5	1.6
Animal fat	0.2	0.6	0.3	0.0	0.0
Corn gluten feed, dry	0.0	12.6	0.0	0.0	0.0
Blood meal	0.3	0.0	0.4	1.8	1.8
Meat and bone meal ²	5.2	0.0	3.8	6.2	6.2
Sodium bicarbonate	0.77	0.76	0.77	0.77	0.77
Salt	0.42	0.42	0.42	0.42	0.42
Dicalcium phosphate	0.00	1.18	0.57	0.00	0.00
Calcitic limestone	0.68	1.39	0.67	0.48	0.46
Potassium chloride	0.00	0.09	0.25	0.27	0.25
Vit-TM premix ³	0.10	0.09	0.09	0.10	0.10
Dyna-mate ⁴	0.07	0.02	0.16	0.24	0.24
Urea	0.00	0.00	0.00	0.00	0.37
Nutrients ⁵					
DM, % of diet	55.3	54.9	56.6	54.3	55.0
CP, % of DM	19.4	16.5	16.8	16.8	17.2
RUP, % of DM	7.76	5.94	6.70	7.70	7.40
ADF, % of DM	23.4	24.3	25.9	22.9	23.8
NFC, % of DM	38.3	34.2	35.7	39.6	38.3
NE _L , Mcal/kg	1.63	1.61	1.59	1.59	1.59

¹HPMU = 19.4% CP, 40% RUP (of CP); LPLU = 16.5% CP, 34% RUP (of CP); LPMU = 16.8% CP, 40% RUP (of CP); LPHU = 16.8% CP, 46% RUP (of CP); LPHU+UREA = 17.2% CP, 43% RUP (of CP).

²Meat and bone meal was of nonruminant origin.

³Vitamin-trace mineral premix. Contained 21.5% Ca; 5.5% S; 3.87% Zn; 3.87% Mn; 1.18% Cu; 9650 ppm Fe; 700 ppm I; 590 ppm Co; 250 ppm Se; 1,215,420 IU/kg Vitamin A; 304,545 IU/kg Vitamin D-3; 3,646 IU/kg Vitamin E.

⁴IMC-AGRICCO, Bannockburn, IL.

⁵DM, CP and ADF are analyzed values; RUP are calculated from NRC (1989). Nonfiber carbohydrates (NFC) and NE_L are calculated from NRC (2001).

analyzed for VFA by gas chromatography (model CP-3380; Varian, Walnut Creek, CA).

Body weights were measured once weekly before milking at 0330 h throughout the trial. Cows were scored for BCS weekly using the guidelines of Ferguson et al. (1994). Mean BW changes were calculated as the difference between beginning and final BW predicted by linear regression over the weeks of the trial.

Calculation of Daily Nitrogen Excretion Measures

A 10-d period was selected for all cows at some time between 80 and 110 DIM, during which cows received 136 g of a soybean hull based chromic oxide supplement fed as a topdressing at each feeding (Younker et al., 1998). The supplement formulated for this study varied from that developed by Younker et al. (1998) because it was not pelleted. To improve the cohesiveness of the supplement, since it was not pelleted, corn oil and molasses were added so that the supplement consisted of

4.7% Cr₂O₃, 84.7% soybean hulls, 6.8% corn oil, and 3.7% molasses.

Approximately 250-g fecal grab samples were taken on d 7 through 10 of the 10-d feeding period. Fecal samples were composited by cow and dried at 55°C for 72 h. After drying, the samples were ground through a Wiley mill fitted with a 1-mm screen (Arthur H. Thomas, Philadelphia, PA). Feces were analyzed at the Experiment Station Chemical Laboratories of the University of Missouri (Columbia, MO) to determine chromium content via atomic absorption spectroscopy (Williams et al., 1962). Daily fecal DM was calculated from fecal composite samples. Then, feces samples were analyzed for Kjeldahl N (AOAC, 1990) to determine daily fecal N excretion.

Daily fecal N excretion was determined using the chromium content of the chromic oxide supplement (Williams et al., 1962). Fecal N excretion was calculated by determining the fecal DM excretion using the following equation: fecal DM (g/d) = % chromium (of DMI) ×

Table 2. Formulated and actual content of crude protein and Lys to Met ratios in treatment diets.

Item	Dietary treatments ¹				
	HPMU	LPLU	LPMU	LPHU	LPHU+UREA
CP ² , %	18.0	15.0	15.0	15.0	16.0
CP ³ , %	18.4	15.6	15.7	15.6	16.7
CP ⁴ , %	19.4	16.5	16.8	16.8	17.2
RUP ² , % of CP	40.0	34.0	40.0	46.0	43.0
RUP ³ , % of CP	40.8	35.2	40.8	44.9	41.9
Lys to Met ratio ²	3:1	3:1	3:1	3:1	3:1
Lys to Met ratio ³	3.5:1	3.5:1	3.5:1	3.5:1	3.5:1
DIG Lys flow, g/d ⁵	183	165	164	156	169
DIG Met flow, g/d ⁵	52.2	47.2	46.3	44.5	48.1

¹HPMU = 19.4% CP, 40% RUP (of CP); LPLU = 16.5% CP, 34% RUP (of CP); LPMU = 16.8% CP, 40% RUP (of CP); LPHU = 16.8% CP, 46% RUP (of CP); LPHU+UREA = 17.2% CP, 43% RUP (of CP).

²Formulated according to Clark et al. (1987) and the Mepron® Dairy Ration Evaluator (Version 2.1, 1999; Degussa-Hüls Corp., Bannockburn, IL).

³Formulated according to *Nutrient Requirements for Dairy Cattle*, 2001.

⁴Analyzed content of dietary treatments.

⁵Predicted flow of digestible Lys and Met to the intestine (g/d) as calculated by the NRC (2001).

DMI (g/d) ÷ % chromium (of fecal DM), and then by determining the fecal N excretion using the fecal DM calculation so that: fecal N (g/d) = %N (of fecal DM) × fecal DM.

Daily urinary N excretion was calculated using the relationship between MUN, BW, and urinary N developed by Kauffman and St. Pierre (2001) so that urinary N (g/d) = 0.0259 × BW (kg) × MUN (mg/dl). Urinary N was calculated initially using the model developed by Jonker et al. (1998) [urinary N (g/d) = 12.54 × MUN (mg/dl)], but only the predicted urinary N data using the model developed by Kauffman and St. Pierre (2001) are reported. Kohn et al. (2002) indicate that the calibration standards for MUN analysis have changed since the Jonker et al. (1998) model was developed and that any samples analyzed for MUN since the spring of 1999 up to the present should use the Kauffman and St. Pierre (2001) model to predict urinary N instead of the model developed by Jonker et al. (1998). Therefore, we report urinary N using the Kauffman and St. Pierre (2001) model because MUN analysis was conducted on weekly milk samples collected from August 1999 to May 2000.

Statistical Analyses

This experiment used a factorial arrangement of treatments with dietary treatment and parity (primiparous or multiparous) as the main factors and treatment × parity as an interaction. Treatment × parity interactions were not significant for any of the data analyzed for this study. Data that included only one sample per cow were subjected to ANOVA for a 2-factor factorial using the general linear models procedure of SAS (1996). Data that included multiple measurements per

cow were analyzed by repeated measures ANOVA as recommended by Littell et al. (1998) using the MIXED procedure with a spatial power error model (SAS, 1996). Therefore, feed intakes, ruminal fluid, blood, and milk data were analyzed using the repeated measures procedure while fecal data were analyzed using standard ANOVA (SAS, 1996). In both types of analysis, least square means for treatments were compared with statistical significance declared at $P < 0.05$.

RESULTS AND DISCUSSION

Diet Formulation Comparison

Treatment diets were formulated according to the NRC (1989). Analysis indicated that all diets contained more CP than intended. This study was planned and conducted before the release of the most recent NRC in 2001. Using the NRC (2001) program, the original diet formulations (NRC, 1989) were reevaluated resulting in somewhat higher formulated CP content than the original calculations suggested, but still they were below the analyzed CP values (Table 2). Reevaluation of the RUP content of diets using the new NRC (2001) resulted in little difference in the estimate of RUP compared with the previous NRC (1989) guidelines.

In addition, the NRC (2001) allows for the calculation of postruminally digestible amino acid supply. According to this calculation, all five diets contained post-ruminal Lys and Met in a 3.5:1 ratio (Table 2), which is similar to the 3:1 ratio originally determined using data collected by Clark et al. (1987) and the Mepron Dairy Ration Evaluator (Version 2.1, 1999; Degussa-Hüls Corp., Bannockburn, IL). Because the ratio of Lys to Met was controlled, the predicted flow of digestible

Table 3. Daily intake, milk yield, milk composition, body weight and body condition score as affected by dietary treatment and parity.

Item	Dietary treatments ¹					SEM	Effect ($P \leq$)	
	HPMU	LPLU	LPMU	LPHU	LPHU+ UREA		Treatment	Parity
Intake								
DM, kg/d	23.3	22.9	23.1	23.4	24.1	0.74	0.82	0.01
CP, kg/d	4.53 ^a	3.78 ^b	3.89 ^b	3.92 ^b	4.13 ^b	0.13	0.01	0.01
RUP ² , kg/d	1.81 ^a	1.36 ^c	1.55 ^b	1.80 ^a	1.78 ^a	0.05	0.01	0.01
ADF, kg/d	5.46	5.57	5.99	5.36	5.72	0.18	0.11	0.01
NE _L ² , Mcal/d	38.1	36.8	36.7	37.1	38.2	1.2	0.83	0.01
Milk								
Yield, kg/d	35.3	33.0	33.5	33.3	35.2	1.6	0.75	0.01
CP, %	3.11	3.01	3.02	3.04	3.06	0.05	0.58	0.34
CP, kg/d	1.09	0.98	1.00	1.01	1.08	0.04	0.28	0.01
Fat, %	3.35	3.36	3.42	3.17	3.05	0.12	0.14	0.36
Fat, kg/d	1.18	1.09	1.14	1.05	1.07	0.05	0.37	0.01
4% FCM, kg/d	31.8	29.6	30.6	29.2	30.4	1.3	0.66	0.01
MUN ³ , mg/dl	21.9 ^a	16.0 ^c	17.6 ^b	14.3 ^d	17.0 ^{b,c}	0.5	0.01	0.44
FCM/DMI, kg/kg	1.35	1.31	1.27	1.30	1.28	0.05	0.81	0.01
BW, kg	546	554	545	560	562	15	0.89	0.01
BW change, kg	52	37	36	30	36	10	0.08	0.01
BCS ⁴	2.25	2.33	2.14	2.26	2.28	0.07	0.39	0.01

^{a,b,c,d}Means within a row with different superscripts differ ($P < 0.05$).

¹HPMU = 19.4% CP, 40% RUP (of CP); LPLU = 16.5% CP, 34% RUP (of CP); LPMU = 16.8% CP, 40% RUP (of CP); LPHU = 16.8% CP, 46% RUP (of CP); LPHU+UREA = 17.2% CP, 43% RUP (of CP).

²Calculated from NRC (1989).

³MUN = milk urea nitrogen.

⁴Body condition score [five-point scale where 1 = very thin to 5 = obese (Ferguson et al., 1994)].

Lys and Met (g/d) supplied to cows receiving the HPMU diet was higher since that diet contained more CP (Table 2). Most importantly, the Lys to Met ratio calculated with the NRC (2001) was the same for all treatments, supporting the intent to supply similar ratios of post-ruminal Lys and Met across dietary treatments.

Intake

There were no significant dietary treatment effects on daily DM and ADF intake (Table 3). As intended, daily CP intake was significantly higher ($P < 0.01$) for cows consuming the HPMU diet than for cows consuming all other treatments. By design, calculated RUP intake was similar for cows consuming the HPMU diet and the two high RUP diets (LPHU and LPHU+UREA). Similarly, calculated RUP intake of cows receiving LPLU, LPMU, and LPHU diets were significantly different ($P < 0.01$) with LPLU lower than LPMU and LPMU lower than LPHU. This reflects formulation for three levels of RUP (34, 40, and 46% of CP as RUP) within the low protein diets. After completion of the study, nonfibrous carbohydrate (NFC) contents of the diets were calculated with the NRC (2001) program. As a result, there were calculated differences in NFC that could have affected protein utilization in the rumen.

As expected, there was a significant parity effect on intake, with multiparous cows consuming more DM

($P < 0.01$) than primiparous cows, resulting in higher intakes of CP, RUP, and ADF ($P < 0.01$) in multiparous cows than primiparous cows (Table 3). For all intake measures, the interaction between treatment and parity was not significant.

Body Weights and Body Condition Scores

There were no significant treatment differences in mean BW, mean BCS, or BW change (Table 3). However, there was a significant parity effect ($P < 0.01$) for BW, BW change, and BCS. Again, the interaction between treatment and parity was not significant.

Milk Yield and Composition

As illustrated in Table 3, milk yield, milk CP yield, milk CP %, milk fat yield, and milk fat % were not significantly different as a result of dietary treatment. As a result, 4% FCM yield and feed efficiency, reported as FCM/DMI, were also not significantly different. Therefore, the lower protein diets did not limit the yield of milk, milk fat, or milk CP compared with the HPMU diet. The MUN concentration was significantly higher ($P < 0.01$) in HPMU-fed cows and significantly lower ($P < 0.01$) in LPHU-fed cows than in cows fed all other diets. Among groups receiving the other diets, MUN concentration in LPMU was significantly higher than

Table 4. Plasma urea nitrogen, plasma nonesterified fatty acids, ruminal ammonia and ruminal VFA as affected by dietary treatment and parity.

Item	Dietary treatments ¹					SEM	Effect ($P \leq$)	
	HPMU	LPLU	LPMU	LPHU	LPHU+UREA		Treatment	Parity
PUN ² , mg/dl	15.6 ^a	11.7 ^{b,c}	12.4 ^b	10.7 ^c	12.6 ^b	0.4	0.01	0.13
Plasma NEFA ³ , Meq/l	0.17	0.20	0.21	0.19	0.21	0.01	0.19	0.01
Rumen NH ₃ , mg/dl	12.1 ^a	8.4 ^{b,c}	9.3 ^b	7.4 ^c	9.2 ^b	0.5	0.01	0.16
Total VFA, mM	80.23 ^a	72.03 ^b	73.46 ^{a,b}	72.69 ^b	72.16 ^b	2.63	0.16	0.03
Acetate:propionate	2.33	2.50	2.53	2.46	2.32	0.08	0.19	0.33
VFA, mol/100 mol								
Acetate	58.35	59.59	60.39	60.11	59.04	0.52	0.06	0.87
Propionate	25.51	24.40	24.64	25.13	26.17	0.55	0.18	0.25
Isobutyrate	0.98	0.91	0.86	0.93	0.94	0.03	0.86	0.64
Butyrate	11.80 ^a	12.07 ^a	11.19 ^{a,b}	10.71 ^b	10.61 ^b	0.32	0.01	0.30
Isovalerate	1.77 ^a	1.54 ^{b,c}	1.52 ^c	1.67 ^{a,b,c}	1.71 ^{a,b}	0.07	0.03	0.01
Valerate	1.62 ^a	1.51 ^{a,b}	1.41 ^b	1.44 ^b	1.53 ^{a,b}	0.05	0.02	0.94

^{a,b,c}Means within a row with different superscripts differ ($P < 0.05$).

¹HPMU = 19.4% CP, 40% RUP (of CP); LPLU = 16.5% CP, 34% RUP (of CP); LPMU = 16.8% CP, 40% RUP (of CP); LPHU = 16.8% CP, 46% RUP (of CP); LPHU+UREA = 17.2% CP, 43% RUP (of CP).

²PUN = Plasma urea nitrogen.

³NEFA = Nonesterified fatty acids.

LPLU, which was significantly higher than LPHU ($P < 0.01$). Cows consuming LPHU+UREA did not have different MUN from LPLU or LPMU, but did have significantly higher MUN than LPHU, which was probably the result of the addition of urea in the LPHU+UREA diet. Cows receiving the LPLU diet were expected to have higher MUN concentrations than cows receiving the LPMU diet because the degradability of the LPLU diet was formulated to be higher. However, differences in MUN concentration can be affected by total CP intake, degradability, and fermentability of the diet.

Multiparous cows had higher yields of milk (38.3 kg/d), milk fat (1.2 kg/d), and milk CP (1.1 kg/d), than primiparous cows (29.8, 1.0, 0.9 kg/d, respectively) ($P < 0.01$). Although primiparous cows produced less milk, there was no parity effect on milk fat %, milk CP %, and MUN. In addition, there was not a treatment $\times$ parity interaction for milk yield and composition.

Ruminal Fluid Measures

Treatments did not result in significant differences in acetate-to-propionate ratios or in molar proportions of rumen acetate, propionate, or isobutyrate (Table 4). Total VFA concentration and the molar proportion of butyrate in the ruminal fluid were significantly higher ($P < 0.01$) for cows receiving the HPMU diet compared to the LPLU, LPHU, and LPHU+UREA diets. There were significant differences as a result of treatment in molar proportions of isovalerate and valerate. The branched chain AA, Leu and Ile, can be precursors to isovalerate and valerate, so protein degradability of the diet could result in changes in levels of these VFA.

Overall, it seems likely that the greater amount of degradable protein supplied by the HPMU diet resulted in higher fermentability of the diet, which is indicated by an increase in total production of VFA. Although there were differences in molar proportions of butyrate, isovalerate, and valerate as a result of treatments, there were no differences in the two primary VFA, acetate and propionate.

Rumen ammonia levels were significantly different as a result of dietary treatment (Table 4). Cows consuming the HPMU diet had higher rumen ammonia concentrations in ruminal fluid than cows consuming the other diets. Rumen ammonia concentrations in cows consuming LPLU, LPMU, and LPHU+UREA diets were not different. However, cows receiving the LPHU diet had lower rumen ammonia levels than cows receiving the HPMU, LPMU, or LPHU+UREA diet. Rumen ammonia levels indicate that there was more unutilized ammonia in the rumen of cows receiving the HPMU, LPMU, or LPHU+UREA diet than of those receiving the LPHU diet. These data suggest that diets were not deficient in RDP.

Plasma Urea Nitrogen and NEFA

There were no significant differences in plasma NEFA as a result of dietary treatment (Table 4). There were significant differences ($P < 0.01$) in plasma NEFA between primiparous (0.173 ± 0.009 Meq/L) and multiparous (0.214 ± 0.008 Meq/L) cows. Plasma NEFA are a measure of fatty acids from mobilized body tissue, which can indicate differences in energy balance. Therefore, primiparous cows did not appear to mobilize as

Table 5. Concentrations of essential AA (EAA) and nonessential AA (NEAA) in plasma of jugular vein as affected by dietary treatment and parity.

Amino Acid	Dietary treatments ¹					Effect ($P \leq$)		
	HPMU	LPLU	LPMU	LPHU	LPHU+UREA	SEM	Treatment	Parity
EAA, $\mu\text{g/ml}$								
Arg	12.42	12.04	12.16	11.45	11.84	0.62	0.85	0.52
His	6.71	6.65	7.00	7.44	7.92	0.34	0.06	0.78
Ile	13.52 ^{a,b}	13.88 ^a	12.64 ^{a,b,c}	11.30 ^c	12.04 ^{b,c}	0.60	0.02	0.27
Leu	19.22	19.97	18.45	18.64	19.11	0.91	0.79	0.48
Lys	11.47	10.87	11.38	11.13	12.23	0.61	0.61	0.13
Met	2.84	2.60	2.83	2.71	2.86	0.13	0.57	0.45
Phe	6.86	7.05	6.72	6.47	6.78	0.25	0.59	0.17
Thr	9.91	10.16	10.22	10.33	10.16	0.51	0.98	0.85
Trp	4.91	5.11	5.40	5.10	5.24	0.26	0.75	0.87
Val	27.31	26.49	25.44	25.87	26.66	1.50	0.92	0.48
Total EAA ²	115.23	114.83	112.25	110.45	114.88	4.63	0.93	0.45
NEAA, $\mu\text{g/ml}$								
Ala	22.23	21.90	23.09	21.65	21.50	1.09	0.85	0.06
Asp	1.87	2.03	1.91	2.22	1.89	0.19	0.65	0.65
Asn	2.62	2.50	2.49	2.45	2.21	0.14	0.34	0.03
Glu	24.04	23.57	23.39	23.51	22.24	1.42	0.95	0.91
Gln	13.93	13.93	13.28	13.65	13.63	0.64	0.93	0.26
Gly	17.18 ^{c,d}	16.50 ^d	19.40 ^{b,c}	20.74 ^{a,b}	20.46 ^{a,b}	0.99	0.01	0.04
Pro	10.10	9.38	9.51	9.97	9.14	0.43	0.49	0.89
Ser	8.35	7.90	8.53	8.79	8.40	0.37	0.55	0.07
Tyr	8.38 ^{a,b}	8.98 ^a	7.49 ^{b,c}	7.20 ^c	7.62 ^{b,c}	0.41	0.02	0.67
Total NEAA ³	108.73	106.70	109.07	110.19	107.09	3.39	0.95	0.94

^{a,b,c,d}Means within a row with different superscripts differ ($P < 0.05$).

¹HPMU = 19.4% CP, 40% RUP (of CP); LPLU = 16.5% CP, 34% RUP (of CP); LPMU = 16.8% CP, 40% RUP (of CP); LPHU = 16.8% CP, 46% RUP (of CP); LPHU+UREA = 17.2% CP, 43% RUP (of CP).

²Total essential AA.

³Total nonessential AA.

much body tissue to support milk production as did multiparous cows, which were producing higher yields of milk and milk components.

The concentrations of PUN were significantly higher in cows consuming the HPMU diet than in cows consuming any other diet (Table 4). Concentrations of PUN were not different in cows consuming LPLU, LPMU, and LPHU+UREA diets. However, cows receiving the LPHU diet had lower PUN levels than cows receiving the HPMU, LPMU, or LPHU+UREA diet. Levels of PUN were similar to those of MUN and rumen ammonia as a result of treatment, which is expected because rumen ammonia not incorporated into microbial protein is absorbed across the rumen wall and converted to urea in the liver for either excretion in urine, secretion in milk, or recycling to the rumen through saliva.

While MUN concentration reflects the average urea content in an a.m./p.m. composite sample of milk, PUN concentration measures the content of urea in the plasma at only the time the sample was collected. Plasma urea nitrogen and rumen ammonia vary in response to feed consumption, so that daily blood urea nitrogen concentration peaks approximately 1 to 2 h after the peak in rumen ammonia or 2 to 3 h postfeeding (Gustafsson and Palmquist, 1993). In the current study,

concentrations of PUN were numerically lower than those of MUN, although the treatment differences were similar for both (Table 3 and 4). This may be because PUN samples were collected approximately 5 h post-feeding and could represent concentrations lower than the cow's peak PUN concentration.

Plasma Amino Acids

Plasma concentrations of Lys and Met were not significantly different across dietary treatment or parity (Table 5). Of all EAA, only Ile concentration was significantly different according to treatment. Plasma Ile concentration was significantly lower in LPHU cows than in HPMU cows, which suggests that a comparative shortage of plasma Ile did not negatively affect N efficiency because cows fed LPHU had significantly lower PUN, MUN, and urinary N than cows fed HPMU. There was a significant effect on the concentrations of the nonessential amino acids (NEAA) Gly and Tyr. However, concentrations of total EAA and NEAA were not affected by treatment. Therefore, the supply of either of these NEAA should not have affected the efficiency of N utilization in the cows because shortages of individual NEAA can be met by making them from EAA or

Table 6. N balance in cows as affected by dietary treatment and parity.

Item	Dietary treatments ¹					SEM	Effect (<i>P</i> ≤)	
	HPMU	LPLU	LPMU	LPHU	LPHU+ UREA		Treatment	Parity
N intake, g/d	725 ^a	605 ^b	622 ^b	627 ^b	661 ^b	21	0.01	0.01
N output, g/d								
Feces	283	308	296	277	290	15	0.61	0.21
Urine ²	312 ^a	230 ^{b,c}	249 ^b	207 ^c	248 ^b	11	0.01	0.01
Milk ³	171	154	157	158	169	6	0.28	0.01
Balance	-41	-87	-80	-15	-46	...	...	...
N output, % of intake								
Feces	39.0	50.9	47.6	44.2	43.9	...	...	...
Urine ²	43.0	38.0	40.0	33.0	37.5	...	...	...
Milk ³	23.6	25.5	25.2	25.2	25.6	...	...	...
Balance	-5.6	-14.4	-12.8	-2.4	-7.0	...	...	...

^{a,b,c,d}Means within a row with different superscripts differ (*P* < 0.05).

¹HPMU = 19.4% CP, 40% RUP (of CP); LPLU = 16.5% CP, 34% RUP (of CP); LPMU = 16.8% CP, 40% RUP (of CP); LPHU = 16.8% CP, 46% RUP (of CP); LPHU+UREA = 17.2% CP, 43% RUP (of CP).

²Calculated according to UN = 0.0259 × BW × MUN (Kauffman and St. Pierre, 2001).

³Calculated using Milk N = Milk protein × 6.38 (NRC, 1989).

other NEAA (NRC, 2001). In addition, there were no significant differences as a result of parity for any plasma AA measured.

Plasma Lys and Met concentrations were similar across treatments, which suggests that similar ratios were absorbed at the intestine by cows from all treatments. Therefore, the differences seen in N utilization as a result of treatment do not appear to result from differences in Lys to Met ratio.

N Utilization

There were no significant differences in daily fecal N excretion by cows as a result of dietary treatment or parity (Table 6). Fecal N primarily consists of indigestible microbial protein produced in the GI tract, as well as endogenous protein, sloughed cells from the GI tract, and undigested feed protein (Mason, 1969). Because undigested feed protein is a minor component of total fecal N, treatment differences in fecal N were not expected.

Daily excretion of urinary N was significantly higher (*P* < 0.01) in cows consuming the HPMU diet than the other four diets (Table 6). Urinary N was higher for cows consuming LPHU+UREA than for cows consuming LPHU, but not LPLU or LPMU. Large differences were seen in urinary N excretion, which was 33.7% lower in cows consuming the LPLU diet than cows consuming the HPMU diet. Because the urinary N prediction equation used included BW, multiparous cows were predicted to excrete significantly more urinary N than primiparous cows.

Secretion of N in milk (g/d) was not significantly different across treatments but was significantly different

between parities (Table 6). The percentages of the contributions of fecal N, urinary N, and milk N to their sum are presented in Figure 1. The amount of N secreted in milk and excreted in feces and urine as a percentage of N intake ranged from 102.4 to 114.4% of the N intake of cows receiving each treatment (Table 6). Therefore, the sum of the calculated recoveries of total N in feces, urine, and milk was very close to the actual N intake of cows consuming each treatment diet.

CONCLUSIONS

In this study, both primiparous and multiparous cows fed the LPHU diet, containing 16.8% CP, 46% of CP as RUP, and postruminal Lys and Met in a 3:1 ratio, had

Figure 1. Nitrogen secretion in milk and excretion in feces and urine reported in grams and as a percent of the total. HPMU = 19.4% CP, 40% RUP (of CP); LPLU = 16.5% CP, 34% RUP (of CP); LPMU = 16.8% CP, 40% RUP (of CP); LPHU = 16.8% CP, 46% RUP (of CP); LPHU+UREA = 17.2% CP, 43% RUP (of CP).

significantly lower concentrations of rumen ammonia, PUN, and MUN without significant changes in milk yield or milk composition compared to all other treatments. In addition, feeding cows the LPHU diet resulted in a 33.7% decrease in urinary N excretion compared to the HPMU diet. Based on the measurements made in this study, there was no advantage to adding urea to the LPHU diet, and it increased urinary N loss.

All treatment diets were formulated to supply a similar 3:1 ratio of Lys and Met for absorption at the intestine. Similar concentrations of Lys and Met in the plasma suggest that diets were properly formulated for these AA. Differences in N utilization in this study appear to result from different amounts of RDP, RUP, and NPN in the diets, without being confounded by amino acid supply. To improve the efficiency of N utilization by the early lactation dairy cow, rations should be formulated to optimize milk production while minimizing N excretion in feces and urine through control of the postruminal AA supply and profile.

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