Effects of Parity and Supply of Rumen-Degraded and Undegraded Protein on Production and Nitrogen Balance in Holsteins

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ABSTRACT

Eight Holstein cows (4 primiparous and 4 multiparous) were used in a replicated 4×4 Latin square design to determine milk production response and N balance when diets had no NRC-predicted excess of rumen-undegradable protein (RUP) or rumen-degradable protein (RDP), 10% RUP excess, 10% RDP excess, or 10% excess of both RUP and RDP. Diets were fed as a total mixed ration with (dry matter basis) 25% alfalfa silage, 25% corn silage, 19 to 21% corn grain, and varying proportions of solvent soybean meal and expeller soybean meal as primary sources of supplemental RDP and RUP, respectively. Milk yield and dry matter intake (DMI) were recorded daily, and total collection of feces and urine was completed in the last 3 d of each 21-d period. Dietary crude protein averaged 17.5 and 18.5% for the recommended and excess RDP diets, respectively, and 17.3 and 18.4% for the recommended and excess RUP diets, respectively. When cows were fed excess RUP diets in the form of expeller soybean meal, DMI and milk production increased, but the opposite was true when the diets contained excess RDP in the form of solvent soybean meal. Milk composition was not affected by RDP, RUP, or by parity, and there were no parity $\times$ RDP interactions for any of the measurements. However, apparent digestibility of neutral detergent fiber, dry matter, and N increased in multiparous cows but not in primiparous cows because of excess RUP. The increase in the yield of milk N with excess RUP was not influenced by parity, but multiparous cows retained more of the additional N apparently absorbed, whereas primiparous cows excreted the additional apparently absorbed N in the urine. Overall, the difference in urinary N due to parity (70 g/d) was about 4 times greater than the impact of dietary treatments (17 g/d). Our results suggest that multiparous cows have either a much larger urea pool or a greater demand to restore body protein mobilized earlier in lactation compared with primiparous cows.

Reduction in urinary N excretion in commercial dairy herds could be obtained by separately balancing rations for first and later lactations.

(Key words: parity, nitrogen, N balance, environment)

Abbreviation key: ED = excess level of RDP, EU = excess level of RUP, NAR = nitrogen apparently retained, RU = recommended level of RUP, RD = recommended level of RDP, S = square.

INTRODUCTION

Due to increasing societal pressure to improve the environmental performance of livestock operations, research is currently being directed toward improving the efficiency of N use by lactating dairy cows to reduce unnecessary loss of N in urine (NRC, 2001). Close management of dietary protein is needed to maximize profits and to minimize the increased risk of environmental damage associated with excessive N excretion in urine and feces (Wu and Satter, 2000). At low N intake, the primary route of N excretion is through the feces, but as N intake increases above 400 g/d, urinary N increases exponentially, thus increasing the vulnerability of the N to ammonia emission and air pollution (Castillo et al., 2001). Recent results showed no production benefits in feeding rations above 16.7% CP in midlactation (Broderick, 2003) or above NRC recommendations of 16.5 and 16.2% CP in alfalfa silage- and corn silage-based diets the first 100 d of lactation (Wattiaux and Karg, 2004a). These reports also showed that the unutilized portion of the N in rations with more than 17.0% CP is lost primarily as urinary urea-N.

Most of this research, however, has been done with multiparous cows only (Castillo et al., 2001; Broderick et al., 2002; Wattiaux and Karg, 2004a). Few studies have examined in detail the response of primiparous and multiparous cows to RUP and RDP without partial or complete confounding between protein source and dietary level of RUP or RDP. Davidson et al. (2003) compared the production response of primiparous and multiparous cows on 5 diets that varied in protein level, source, and supply of RUP, and reported results

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by parity, but did not report any significant treatment by parity interactions. Broderick (2003) used spot sampling and marker techniques to measure urinary and fecal N in multiparous and primiparous cows in a 9×9 Latin square design, but did not report or discuss parity effects. Cunningham et al. (1996) used soy-based products only to change RUP content of the diet in primiparous and multiparous cows, but did not report N balance data. Kauffman and St-Pierre (2001) compared breed (Holstein vs. Jersey) to assess the impact of animal size on the use of N and found that there was a difference in N intake, fecal N, N apparently absorbed, urine N, and milk N due partially to higher DMI by the Holstein cows, but no differences were detected for N apparently retained (**NAR**) or the efficiency of conversion of dietary N to milk N between breeds. These researchers also reported a breed by dietary CP interaction for urine N. The nature of this interaction remains unclear and raises the question of whether primiparous and multiparous cows would respond differently to excess dietary N. Thus, the objectives of this study were to determine N balance and production response of primiparous and multiparous Holstein cows fed different levels of RUP and RDP with soy-based by-products as the sole supplemental protein source.

MATERIALS AND METHODS

Experimental Design

Four primiparous and 4 multiparous (fifth lactation) Holstein cows were used in a replicated 4×4 Latin square. Animals were selected on parity and DIM. Primiparous and multiparous cows averaged ($\pm$ SD) 58 ± 5.1 and 56 ± 10.9 DIM, respectively, at the start of the trial. Primiparous cows averaged 26 mo of age at calving. All cows were bred, but no pregnancies were confirmed during the trial. The treatments were arranged in a split-plot design with parity forming the main plots [squares (**S**)] and dietary treatments forming the subplots. The experimental periods lasted 21 d, with d 17 and 18 used for adaptation to urinary catheters, and d 19 to 21 for sampling and daily total collection of urine and feces.

Diets and Cow Management

In formulating diets using the NRC (2001) model, it was assumed that the primiparous cows were 30 mo of age, mature weight of 680 kg, current weight of 517 kg, 0 d pregnant, 90 DIM, and 34 kg/d of milk with 3.3% fat and 3.0% protein. The assumptions made for multiparous cows were 55 mo of age, 680 kg BW, 0 d pregnant, 90 DIM, and 45 kg/d milk with 3.3% fat

and 3.0% protein. Diets included approximately 25% alfalfa silage, 25% corn silage, and 50% concentrate mix that included minerals and vitamins (Table 1). The CP and NDF of the forages used in formulating the rations were analyzed by near-infrared spectroscopy (UW Soil & Forage Analysis Laboratory, Marshfield, WI). Experimental rations included recommended levels of RUP and RDP (**RU-RD**), 10% RUP excess (**EU-RD**) 10% excess RDP (**RU-ED**), or 10% excess of both RUP and RDP (**EU-ED**). Predicted RUP and RDP balances were adjusted primarily by changing the proportion of soy ingredients in the diet [solvent soybean meal 48, expellers soybean meal (SoyPLUS, Ralston, IA), and soybean hulls]. Thus, the EU-RD diet had the highest level of expellers soybean meal, and the RU-ED diet had the most solvent soybean meal (Table 1). Corn grain and dried molasses were adjusted to maintain a high level of NFC in all diets in an attempt to supply ruminal microorganisms with sufficient energy and avoid inefficient use of RDP when provided in excess. The level of corn grain and dried molasses was similar in all 4 diets. Sodium bicarbonate was added to avoid rumen acidosis.

Diets were fed as TMR once daily at 0900 h ad libitum for 10% refusals. Ingredient mix was adjusted based on weekly DM analysis of forages. Between sampling periods, cows were milked in the parlor with the rest of the research herd at approximately 0500 and 1700 h. However, from d 17 to 21, cows remained in their stalls and were milked with a portable milking unit in the stalls at approximately 0400 and 1600 h. Cows were cared for according to the guidelines of the Research Animal and Resource Committee at the University of Wisconsin-Madison and all experimental procedures were approved.

Sample Collection

Orts were measured daily and feed offered was adjusted to allow for 5 to 10% Orts. Samples of Orts were collected daily on d 19 to 21 of each period and frozen at -20°C for later analysis. Milk weights were recorded daily throughout the trial. Duplicate milk samples were collected from a.m. and p.m. milkings of the 3 sampling days. One sample was sent to AgSource, (Menomonie, WI) for analysis with the CombiFoss 5000 (Foss Electric, Hillerød, Denmark). The Milko-Scan 4000 was used to determine milk fat and protein (AOAC, 1990), and milk urea N using the differential pH method as a standard. The Fossomatic 5000 was used to count somatic cells by flow cytometry (AOAC, 1999). The other sample was frozen at -20°C for later analysis of protein. Total fecal production was measured on d 19 to 21. Feces were shoveled into contain-

Table 1. Ingredients and nutrient composition of experimental diets.

Item	Treatments ¹			
	RU-RD	EU-RD	RU-ED	EU-ED
Ingredients, % of DM				
Alfalfa silage	25.40	25.10	25.90	25.41
Corn silage	25.40	25.10	25.90	25.41
Corn grain, dried, ground	20.40	20.15	19.31	21.41
Molasses, dried, beet sugar	4.18	4.18	4.06	4.39
Soybean meal, solvent (48% CP)	6.60	6.05	11.75	11.31
Soybean meal, expellers ²	5.06	7.49	1.22	4.12
Soybean, hulls	10.09	9.32	9.07	5.13
Limestone ⁴	0.00	0.09	0.21	0.15
Salt + Se ⁴	0.65	0.42	0.41	0.58
Vitamin mix ^{3,4}	1.07	0.94	1.07	1.09
Dicalcium phosphate ⁴	0.54	0.56	0.54	0.30
Sodium bicarbonate ⁴	0.65	0.56	0.54	0.69
Nutrient composition, % of DM				
CP	17.0	18.0	17.6	18.7
NDF	26.0	26.9	25.4	23.9
Forage, NDF	17.2	17.0	17.5	17.2
ADF	18.6	19.0	19.0	17.0
NFC	45.4	43.8	45.4	46.0
Starch	32.2	27.9	31.1	31.4
NDICP ⁵	1.29	1.47	1.06	1.20
ADICP ⁶	1.30	0.78	1.07	1.80
Fatty acids	2.72	2.65	2.41	2.54
Ash	9.21	9.13	9.29	9.02
Ca	0.60	0.60	0.70	0.60
P	0.40	0.40	0.40	0.40

¹RU = Recommended level of RUP, EU = excess level of RUP, RD = recommended level of RDP, ED = excess level of RDP.

²SoyPLUS, Ralston, IA.

³Vitamin mix contained 1,500,000 IU vitamin A; 500,000 IU vitamin D; and 5000 IU vitamin E.

⁴The mineral and vitamin premix was adjusted according to the supply of other dietary ingredients to meet dietary requirements rather than using a "flat" percentage across all diets.

⁵NDICP = Neutral detergent insoluble CP.

⁶ADICP = Acid detergent insoluble CP.

ers and weighed twice a day. Duplicate samples were taken at each sampling time. One sample was dried at 60°C for 36 h and the other was frozen at -20°C for later analysis. Urine collection was conducted on d 19 to 21 using indwelling catheters (75-mL balloon lubricious catheter, C. R. Bard, Inc., Covington, GA). Five hundred milliliters and 400 mL of 50% H₂SO₄ were added to collection carboys for multiparous and primiparous cows, respectively. The volume of 50% H₂SO₄ added to the carboys was determined by measuring pH after 12 and 24 h of collection on d 17 and 18 of period 1 to ensure a pH below 3.0. Urine volume was measured twice daily at milking time and samples were frozen at -20°C for later analysis. Two hundred milliliters of rumen fluid was collected from 5 different locations in the rumen with a metal filter probe through ruminal cannula every 4 h for a 24-h period starting 4 h after feeding on d 18. One-milliliter subsamples were mixed with 0.02 mL of 50% trichloroacetic acid and frozen at 20°C for later analysis of ammonia (Chaney and Marbach, 1962). Body weight was

calculated as the average of measurements performed in the p.m. of d 18 and the a.m. of d 21.

Sample Analysis

Feed, milk, urine, and fecal samples. Orts samples, alfalfa silage (n = 9), corn silage (n = 8), concentrate mix (n = 4 for each mix), and fecal samples were dried at 60°C and ground to pass a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Orts and fecal samples were composited for each cow by period based on the fresh weight of Orts or feces produced from each collection day. Dry matter content was determined by drying at 100°C for 24 h; ash content was determined at 500°C for 16 h. Organic matter was calculated as 100 - ash. Crude protein was determined by microKjeldahl (AOAC, 1990). For alfalfa silage, corn silage, concentrate mix, and fecal samples, NDF was determined according to Van Soest et al. (1991) using α -amylase (# FAA; Ankom Technology, Fairport, NY) and sodium sulfite. Calculation of NDF concentra-

tion included a correction for ash content according to Mertens (1999) and adapted for Ankom²⁰⁰ Fiber Analyzer (Ankom Technology). Samples of alfalfa silage, corn silage, and concentrate mix were also analyzed for ADF using the method described by Goering and Van Soest (1970) and adapted for the Ankom²⁰⁰ Fiber Analyzer (Ankom Technology). In addition, N bound to neutral and acid detergent residues were determined by microKjeldahl (AOAC, 1990) and expressed as neutral detergent insoluble CP and acid detergent insoluble CP. Fatty acids were determined following the procedure described by Sukhija and Palmquist (1988) and represented the sum of C₁₄ to C₁₈. The NFC fraction was calculated as 100 – [(NDF – neutral detergent insoluble CP) + ether extract + CP + ash], where ether extract was calculated as fatty acids plus one (p. 14; NRC, 2001). Starch was determined by gelatinization procedure using α -amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO), amyloglucosidase (Sigma A3514; Sigma Chemical Co.), and sodium acetate buffer adapted from Holm et al. (1986) followed by a colorimetric assay for glucose as outlined by Karkalas (1985). Urine and milk samples were composited based on production for each cow each period. Total milk N was determined by macroKjeldahl (AOAC, 1990). Urine N was determined by microKjeldahl (AOAC, 1990).

Rumen samples. The pH of ruminal fluid was determined within 1 min of collection with an Horiba compact pH meter (B-213; Spectrum Technologies, Inc., Plainfield, IL). Rumen ammonia was determined using the preserved samples as outlined by Bal et al. (2000). Daily averages for ruminal pH and ammonia concentration were calculated as the mean of the 6 equally spaced observations.

Forage RUP and RDP determination. Alfalfa silage (n = 9) and corn silage (n = 8) samples were dried at 60°C and ground to pass a 2-mm screen (Wiley mill, Arthur H. Thomas). Composite samples [5 ± 0.07 g ($\pm$ SD)] of these 2 forages were placed in 9 × 18 cm Dacron bags (Balson-Erlanger, New York, NY) with nominal pore size of 52 ± 5 μ m. Incubation times of 0, 2, 4, 8, 16, 24, 48, and 72 h were used (NRC, 2001). Two cows were fed a diet of 25% alfalfa silage, 25% corn silage, and 50% concentrate (DM basis). Bags were soaked, inserted, and washed according to Coblenz et al. (1997; 1998). After rinsing, the bags were dried at 60°C for 72 h, and DM and N were determined as previously described. Kinetic parameters (A, B, and C fractions and Kd, the rate of degradation of the B fraction) were determined using a nonlinear least square method (Proc NLIN, SAS Institute, 1998) assuming a first-order kinetic model as outlined in Wattiaux et al. (1994).

Statistical Analyses

Milk production, percentage and yield of milk components, DMI, nutrient intake, rumen measurements, and total collection data were analyzed using Proc Mixed of SAS (SAS Institute, 1998) according to the following model:

$$Y_{ijklm} = \mu + S_i + c_{ij} + P_k + RUP_l + RDP_m + RUP \times RDP_{lm} + S \times RUP_{il} + S \times RDP_{im} + E_{ijklm}$$

where μ = overall mean; S_i = fixed effect of the *i*th square (parity), *i* = 1, 2; c_{ij} = random effect of the *j*th cow within the *i*th square, *j* = 1 to 4 ~ N (0, σ_c^2); P_k = fixed effect of the *k*th period, *k* = 1 to 4; RUP_l = fixed effect if the *l*th RUP level, *l* = 1, 2 (EU diets) vs. (RU diets); RDP_m = fixed effect of *m*th RDP level, *m* = 1, 2 (ED diets) vs. (RD diets); $RUP \times RDP_{lm}$ = interaction of the *l*th RUP level with the *m*th RDP level, (RU-RD and EU-ED diets) vs. (RU-ED and EU-RD diets); $S \times RUP_{il}$ = interaction of the *i*th square with the *l*th level of RUP; $S \times RDP_{im}$ = interaction of the *i*th square with the *m*th RDP level; and E_{ijklm} = error term ~ N (0, σ_e^2).

Statistical significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.1$. Main effects are reported in the tables, but interactions are reported and discussed in the text.

RESULTS

Feed and Diet Composition

Nutrient composition of forages and grain mixes are reported in Table 2. The CP content of the alfalfa silage was low in relation to its concentration of NDF and ADF. The CP value for the alfalfa silage in this study was closer to that of a mature alfalfa silage with NDF >46% (see Table 15-1 in NRC, 2001). In contrast, the corn silage had a normal CP content but was low in NDF and ADF. The values reported here were only marginally different from the pretrial measurements. Estimates of the soluble (A fraction), degradable (B fraction), and indigestible (C fraction) percentage of CP in the alfalfa silage and corn silage were 70 ± 0.0 and 69 ± 0.5 , 24 ± 0.7 and 13 ± 3.1 , and 9 ± 0.2 and 18 ± 0.0 ($\pm$ SD), respectively. The rate of degradation of the B fraction (h^{-1}) for the CP in alfalfa silage and corn silage was 0.15 ± 0.06 and 0.05 ± 0.01 ($\pm$ SD), respectively. The calculated RUP and RDP as a percentage of DM was 2.7 (15% of CP) and 15.2 (85% of CP) for the alfalfa silage and 2.1 (24% of CP) and 6.6 (76% of CP) for the corn silage, respectively.

Table 2. Nutrient composition of forages and grain mixes.

Item	Forages ¹		Grain mixes ²			
	AS	CS	RU-RD	EU-RD	RU-ED	EU-ED
DM, % as-fed	34.4 (0.05)	31.4 (0.02)	87.1	88.2	87.6	87.4
			% of DM			
CP	17.9 (0.58)	8.7 (0.62)	20.8	22.7	22.2	24.3
RUP ³	2.7	2.1	...	...	...	...
RDP ³	15.2	6.6	...	...	...	...
NDF	33.7 (2.18)	33.8 (2.50)	17.9	19.9	16.3	13.7
ADF	30.4 (2.21)	20.3 (1.80)	11.5	12.6	12.1	8.3
NFC	32.6	51.0	49.2	45.6	49.4	50.4
Ash	14.3 (0.14)	4.9 (0.05)	8.8	8.6	8.9	8.4
NDICP ⁴	1.3 (0.04)	0.7 (0.14)	1.6	1.9	1.1	1.4
ADICP ⁵	1.0 (0.39)	0.6 (0.22)	1.8	0.8	1.3	2.8
Fatty acids	1.8 (2.93)	1.3 (1.85)	3.9	3.8	3.3	3.6
Starch	2.6 (1.01)	38.6 (6.43)	44.1	35.4	42.4	42.5
Ca	...	...	0.48	0.50	0.59	0.46
P	...	...	0.49	0.50	0.50	0.49

¹AS = Alfalfa silage; CS = corn silage; values in parentheses indicate standard deviation.

²RU = Recommended level of RUP, EU = excess level of RUP, RD = recommended level of RDP, ED = excess level of RDP.

³Measured by in situ ruminal incubation.

⁴NDICP = Neutral detergent insoluble CP.

⁵ADICP = Acid detergent insoluble CP.

The CP of the grain mixes increased with the addition of RDP or RUP to the diet, with the EU-ED diet having the highest CP (Table 2). The NDF in the grain mixes was a reflection of their content in soybean hulls and expellers soybean meal, with the latter having half the content of NDF of the former (NRC, 2001).

Differences in the nutrient composition of the rations (Table 1) were due primarily to differences in the chemical analysis of the grain mixes because the percentage of alfalfa and corn silage was similar in all diets. Given similar percentages of corn silage and corn grain in the diets, starch content was expected to be constant. The lower than expected value for the EU-RD diet is difficult to explain, but may be related to error in preparation of certain batches of the grain mix for that particular diet. Overall, the concentration of NFC, fatty acids, and ash was relatively constant among diets.

Body Weight, Intake, Digestibility, and Rumen Measurements

Body weight. In this trial, multiparous cows weighed 182 kg more than the primiparous cows (Table 3). Average BW did not differ across dietary treatment, but there was an $S \times RDP$ interaction ($P = 0.04$). This interaction was due primarily to a differential response, with multiparous cows gaining 3.8 kg but primiparous cows losing 10.5 kg when fed the ED diets. However, observation of individual cow data indicated that this effect was due primarily to 1 of the 4 primipa-

rous cows that may have been under stress during the first period. This cow was recovering from a difficult calving, but had improved performance in the 3 subsequent periods.

DM and nutrient intake. There were no $S \times RDP$ or $S \times RUP$ interactions for DMI and nutrient intake, but DMI differed between parity groups, with the primiparous cows eating 8.7 kg/d less than the multiparous cows. Intake of OM, NDF, starch, and NFC followed the same pattern as for DMI (Table 3).

Dry matter intake of cows on EU diets was 2.1 kg/d higher compared with the RU diets. In contrast, DMI was 1.2 kg/d lower in cows fed the ED diets compared with those fed the RD diets. Intake of OM and NDF showed the same pattern as for DMI. Starch intake was influenced by a $RUP \times RDP$ interaction ($P = 0.02$). Intake of starch was 0.65 kg/d higher when cows consumed the RU-RD or the EU-ED diets (7.4 kg/d) compared with the RU-ED or the EU-RD diets (6.7 kg/d). This interaction reflected in part the average lower starch concentrations of the EU-RD and RU-ED diets compared with the RU-RD and EU-ED diets (Table 1), and the numerically lower DMI on the RU-ED diet compared with the other diets. Intake of NFC was 0.82 kg/d higher on the EU diets than the RU diets. The higher DMI on the EU diets was the primary cause of this effect as NFC concentration was similar across diets.

Nutrient digestibility. Digestibility of DM, N, and NDF tended to be influenced by an $S \times RUP$ interaction ($P = 0.10, 0.07$, and 0.06 , respectively). When fed the

Table 3. Least squares means DMI, nutrient intakes, and rumen conditions for primiparous and multiparous Holstein cows fed diets at or above RUP and RDP recommendations.

	Treatments ¹				SEM	Parity		SEM	P value		
	RU-RD	EU-RD	RU-ED	EU-ED		Primiparous	Multiparous		RUP ²	RDP ³	Parity
BW, kg	578	581	575	577	11.6	487	669	16.0	0.42	0.26	<0.001
Nutrient intake											
DMI, kg/d	22.7	24.4	21.1	23.6	1.13	18.6	27.3	1.40	0.005	0.08	0.005
OM, kg/d	20.7	22.3	19.3	21.5	1.21	16.9	24.9	1.54	0.008	0.10	0.02
NDF, kg/d	5.9	6.6	5.4	5.6	0.29	4.8	7.0	0.29	0.01	<0.001	0.005
Starch, kg/d	7.3	6.8	6.6	7.4	0.35	5.7	8.3	0.43	0.42	0.76	0.005
NFC, kg/d	10.3	10.7	9.6	10.8	0.51	8.4	12.3	0.63	0.01	0.37	0.005
Digestibility, %											
DM	65.1	65.2	65.1	66.9	1.18	65.5	65.7	1.01	0.41	0.43	0.85
N	66.5	67.4	67.9	70.5	1.06	67.6	68.6	0.96	0.09	0.03	0.51
NDF	43.9	45.6	44.7	42.6	2.05	44.5	43.9	1.45	0.92	0.57	0.79
Rumen measurements											
Rumen NH ₃ , mg/dL	11.6	11.8	12.3	13.1	1.35	11.9	12.6	1.31	0.72	0.44	0.63
Rumen pH	6.3	6.3	6.3	6.4	0.05	6.4	6.3	0.05	0.52	0.34	0.12

¹RU = Recommended level of RUP, EU = excess level of RUP, RD = recommended level of RDP, ED = excess level of RDP.

²Effect of increasing RUP = (EU diets) vs. (RU diets).

³Effect of increasing RDP = (ED diets) vs. (RD diets).

EU diets, DM digestibility increased by 2.8 percentage units in the multiparous cows (64.3 vs. 67.2%), but decreased by 1.0 percentage units in the primiparous cows (65.9 vs. 64.9%). For N digestibility, there was an increase of 3.5 percentage units (66.8 vs. 70.3%) in multiparous cows, but no change (67.6 vs. 67.6%) in the primiparous cows. Furthermore, the digestibility of NDF increased 4.0 percentage units (41.9 vs. 45.9%) in multiparous cows, but decreased 4.3 percentage units (46.6 vs. 42.3%) for the primiparous cows, when fed EU diets compared with RU diets. There was an RDP effect on apparent N digestibility, which was 2.3 percentage units higher (66.9 vs. 69.2%) for ED diets compared with RD diets. In contrast, there was an opposite trend with a tendency for higher N digestibility when cows were fed RU diets compared with EU diets (68.9 vs. 67.2%, respectively).

Rumen measurements. There were no interactions or significant effects of RUP, RDP, or parity on rumen pH in this study. Although differences in the 24-h average for rumen ammonia concentration were only numerical, they reflected dietary CP and in particular, the anticipated RDP supply. There was only a minimal increase in the rumen ammonia concentration for the RU-RD diet compared with the EU-RD diet (Table 3) despite the 1-percentage-unit increase in dietary CP. However, there was a substantial increase in rumen ammonia when comparing the RU-RD to the RU-ED diet (Table 3). Despite a higher dietary CP, ruminal ammonia concentration in the EU-ED diet was expected to be similar to that of the RU-ED diet. In this case however, ruminal ammonia concentration was 0.8 mg/dL higher in the EU-ED diet suggesting a greater

than anticipated supply of RDP most likely due to higher DMI on the EU-ED diet.

Milk Production and Composition

Overall average milk production in this trial was 38.8 ± 8.2 kg/d ($\pm$ SD), but milk production was 13.8 kg/d higher in multiparous cows than in primiparous cows (Table 4). This difference resulted in fat and true protein yield differences, but there were no differences in the percentage of fat and true protein in the milk between parities.

The absence of $S \times$ RUP or $S \times$ RDP interactions indicated that milk production response to feeding excess RUP or RDP was not influenced by parity. However, milk production was 1.6 kg/d lower on the ED compared with RD diets. In contrast, milk production increased by 1.5 kg/d when cows were fed the EU diets compared with RU diets. Milk component yields and percentages were not affected by level of RUP or RDP (Table 4). However, there was a RUP $\times$ RDP interaction for milk fat percentage and yield ($P = 0.02$ and 0.02). Milk fat was 0.32 percentage units higher (3.59 vs. 3.27%) and 0.13 kg/d higher (1.38 vs. 1.25 kg/d) when cows were fed the diets with an excess of either RUP or RDP compared with the diets with recommended RUP and RDP or in excess of both. Urea in the milk was not affected by parity, RUP, or RDP, but it increased numerically with dietary CP.

N Balance

Daily intake, digestion, and excretion of N. In this trial, overall N intake, milk N, fecal N, urinary

Table 4. Least squares means milk production and composition for primiparous and multiparous Holstein cows fed diets at or above RUP and RDP recommendations.

	Treatment ¹				SEM	Parity			P value		
	RU-RD	EU-RD	RU-ED	EU-ED		Primiparous	Multiparous	SEM	RUP ²	RDP ³	Parity
Milk, kg/d	38.8	40.6	37.4	38.6	1.51	31.9	45.7	1.91	0.06	0.05	0.002
Milk true protein, %	2.59	2.66	2.67	2.61	0.09	2.69	2.58	0.12	0.96	0.76	0.53
Milk protein, kg/d	1.00	1.07	0.99	0.99	0.05	0.85	1.17	0.06	0.32	0.19	0.01
Milk fat percentage	3.31	3.52	3.66	3.23	0.17	3.65	3.21	0.19	0.39	0.81	0.15
Milk fat, kg/d	1.28	1.42	1.34	1.22	0.07	1.16	1.47	0.07	0.88	0.27	0.04
MUN, mg/dL	11.9	12.5	12.6	13.4	0.62	12.4	12.8	0.66	0.17	0.12	0.67

¹RU = Recommended level of RUP, EU = excess level of RUP, RD = recommended level of RDP, ED = excess level of RDP.

²Effect of increasing RUP = (EU diets) vs. (RU diets).

³Effect of increasing RDP = (ED diets) vs. (RD diets).

N, and NAR were ($\pm$ SD) 658 ± 160 , 179 ± 42 , 210 ± 57 , 208 ± 46 , and 61.4 ± 67 g/d, respectively. As a reflection of higher DMI, multiparous cows consumed 247 g/d more N than the primiparous cows (Table 5). In contrast, RUP and RDP had a distinctly lesser impact on N intake, which was 103 g/d higher in the EU diets compared with the RU diets and numerically 9 g/d lower on the ED diets than the RD diets (Table 5). The former difference was a result of both higher dietary CP and greater DMI on the EU diets, whereas the latter difference was due in part to the negative effect of the ED diets on DMI (Table 3). The N apparently digested, calculated as N intake $\times$ N digestibility, was

176 g/d higher for the multiparous than for the primiparous cows. This difference was more than twice the largest difference due to RUP and RDP, which averaged 83 g/d when cows were fed the EU compared with the RU diets. This dietary effect was due both to the higher DMI and to the tendency for higher N digestibility when cows were fed the EU diets (Table 3).

Fecal N was 72 g/d higher in multiparous cows than in primiparous cows. In addition, fecal N excretion was increased by 21 g/d when cows were fed the EU diets compared with the RU diets. In contrast, the ED diets lowered fecal N excretion by 22 g/d. Milk N excretion was 66 ± 15 g/d higher in multiparous cows compared

Table 5. Least squares means N balance for primiparous and multiparous Holstein cows fed diets at or above RUP and RDP recommendations.

	Treatment ¹				SEM	Parity			P value		
	RU-RD	EU-RD	RU-ED	EU-ED		Primiparous	Multiparous	SEM	RUP ²	RDP ³	Parity
N intake, g/d	618	707	596	712	32	535	782	40	<0.001	0.62	0.005
NAD, ⁴ g/d	409	476	406	504	21	360	536	23	<0.001	0.46	0.002
Fecal N, g/d	209	232	190	208	14	174	246	18	0.006	0.004	0.03
Total milk N, g/d	177	189	169	181	9.4	146	212	11	0.007	0.23	0.004
Urine N, g/d	194	213	202	223	9.9	173	243	11	0.02	0.24	0.003
NAR, ⁵ g/d	38	73	34	100	18	42	81	15	0.008	0.50	0.12
N, % of intake											
Fecal	33.5	32.6	32.0	29.5	0.01	32.4	31.5	0.009	0.08	0.03	0.51
Milk	28.7	27.3	29.9	25.5	0.02	28.1	27.6	0.02	0.04	0.80	0.86
Urine	31.3	30.7	35.0	31.6	0.01	32.9	31.4	0.009	0.16	0.11	0.29
NAR ⁵	6.4	9.4	3.1	13.4	2.03	6.6	9.6	2.03	0.03	0.91	0.33
N, % of apparently digested											
Milk	43.3	40.4	44.0	36.9	0.03	41.6	40.3	0.03	0.03	0.42	0.74
Urine	47.3	45.7	51.9	45.2	0.02	49.0	46.0	0.02	0.10	0.40	0.24
NAR ⁵	9.5	13.9	4.1	18.8	0.04	9.4	13.7	0.03	0.03	0.95	0.39
Nitrogen ratios											
UN:FN ⁶	0.94	0.95	1.10	1.09	0.05	1.03	1.01	0.05	0.97	0.002	0.87
Milk N:manure N ⁷	0.45	0.44	0.44	0.43	0.03	0.43	0.44	0.03	0.49	0.74	0.84

¹RU = Recommended level of RUP, EU = excess level of RUP, RD = recommended level of RDP, ED = excess level of RDP.

²Effect of increasing RUP = (EU diets) vs. (RU diets).

³Effect of increasing RDP = (ED diets) vs. (RD diets).

⁴NAD = N apparently digested (N intake g/d $\times$ N digestibility %).

⁵NAR = Apparently retained N calculated as N intake – milk N – urine N – fecal N.

⁶UN:FN = Ratio of urine N to fecal N.

⁷Milk N: manure N = ratio of N in milk to N in urine and feces.

with primiparous cows (Table 5). Excretion of N in milk was not altered when cows were fed the ED diets, but was 12 g/d higher on EU compared with RU diets (185 vs. 173 g/d). This positive response to dietary RUP was due primarily to higher milk production rather than a change in milk composition (Table 4). Urine N excretion tended to be influenced by an $S \times RUP$ interaction ($P = 0.10$). There was a larger increase in the urine N excretion on the EU diets compared with the RU diets for the primiparous cows than for the multiparous cows [33 (190 vs. 157) vs. 7 (247 vs. 240 g/d), respectively]. Overall, the multiparous cows excreted 70 g/d more N in urine than the primiparous cows. This higher urinary N excretion was associated with both a higher amount of N apparently digested and a larger volume of urine excreted (32.2 vs. 20.7 L/d for the multiparous and primiparous cows, respectively, $P = 0.003$). Notwithstanding the interaction just described, overall urine N excretion was 20 g/d higher when cows were fed the EU diets compared with RU diets (218 vs. 198 g/d, respectively). Urine volume also increased from 25.7 to 27.3 L/d ($P = 0.04$) for the EU compared with the RU diets. In contrast, there was no difference between the ED and RD diets for both urine N excretion (204 vs. 213) and urine volume (26.9 and 26.0 L/d, respectively, $P = 0.20$). This result followed the absence of an effect of RDP on the amounts of N apparently absorbed and excreted in the milk.

The NAR was influenced by the $S \times RUP$ interaction ($P = 0.03$). When fed the EU diets, there was an increase in NAR for both the primiparous and the multiparous cows, but the increase was much larger for the multiparous cows (91 vs. 10 g of N/d). Overall, NAR by the cows was 51 g/d higher when fed the EU diets compared with the RU diets.

Feces, milk, urine, and NAR as a percentage of N intake. In this trial, the percentage of consumed N found in milk, feces, urine, and apparently retained averaged 27.8 ± 5.4 , 31.9 ± 4.1 , 32.2 ± 5.4 , and 8.0 ± 9.8 , ($\pm SD$) respectively. Although there were no differences in any of these measurements between primiparous and multiparous cows, there was a tendency for an $S \times RUP$ interaction ($P = 0.07$) for the percentage of intake N excreted in the feces. For multiparous cows, there was a decrease of 3.5 percentage units of intake N excreted in feces (33.2 vs. 29.7%), but no change for the primiparous cows (32.3 vs. 32.4%) when fed EU compared with RU diets. The level of RUP or RDP influenced the percentage of intake N excreted in milk, feces, and NAR, but not that excreted in urine (Table 5). As reflected by the N digestibility results presented above, there was a tendency for a lower percentage of intake N to be excreted in the feces when cows were fed the EU diets compared with the RU diets (31.1 vs.

32.8%, respectively). Furthermore, there was a decrease in the percentage of N intake excreted in the feces when fed the ED compared with the RD diets (30.8 vs. 33.1%, respectively). The percentage of N intake excreted in milk was 2.9 percentage units higher when cows were fed RU compared with EU diets (29.3 vs. 26.4%, respectively). Thus, the efficiency of N use to synthesize milk N was higher when cows were fed the RU diets. The percentage of intake N apparently retained was not influenced by parity but was 6.6 percentage units higher when cows were fed the EU diets compared with the RU diets (11.4 vs. 4.8%). These results indicated that although there was a lower efficiency of conversion of intake N into milk N on the EU diets, there might have been a higher efficiency in conversion of N into body protein or an alternative N pool within the body, rather than additional urinary N excretion.

Milk, urine, and NAR as a percentage of N apparently digested. Parity did not influence milk N, urinary N, and NAR expressed as a percentage of N apparently digested. However, there was 5.0 percentage units less of the apparently digested N in milk when cows were fed the EU diets compared with the RU diets. There was a tendency for an $S \times RUP$ interaction ($P = 0.08$) indicating that urinary N excretion expressed as a percentage of N apparently digested decreased when multiparous cows were fed the EU diets but not for primiparous cows. When fed the EU diets, urinary N expressed as a percentage of apparently digested N decreased by 8.5 percentage units in multiparous cows (50.3 vs. 41.8% for RU and EU diets, respectively) but increased by 0.2 percentage units for the primiparous cows (48.9 vs. 49.1% for RU and EU diets, respectively). There were no $S \times RDP$ or $S \times RUP$ interactions, but 9.6 percentage units more of the apparently digested N was apparently retained when cows were fed the EU diets compared with the RU diets.

N ratios. Parity did not influence the urinary N to fecal N or the milk N to manure N ratios (Table 5). However, the urinary N to fecal N ratio increased by 0.15 units when cows were fed the ED diets compared with the RD diets. This is not because of higher urinary N but rather because of lower fecal N excretion.

DISCUSSION

Dietary Balances of RUP and RDP

The formulated diets had RDP or RUP balance of either 0 or 10% excess calculated as $[100 \times (\text{supplied} - \text{required})/\text{required}]$. To further assess the RUP and RDP differences among experimental diets, forage digestion kinetic parameters and actual cow perfor-

mance (BW, DMI, milk yield, and percentages of protein and fat) were introduced in the NRC (2001) model a posteriori. The average excess RDP in the RD and ED diets was then 4 and 14%, respectively, but the average excess RUP in the RU and EU diets was 6 and 32%, respectively. The supply of RUP was greater in the EU diet relative to the RU diet because of the higher DMI (Table 3) rather than a change in requirements for milk protein production (Table 4).

RUP and RDP Effects

As in this trial, Wu and Satter (2000) and Broderick et al. (2002) reported higher DMI when diets with higher levels of expeller soybean meal were fed as a source of additional RUP compared with diets with solvent soybean meal as the primary source of supplemental protein. Our results indicated that this effect was true regardless of parity. Milk production responses to EU diets averaged 1.5 kg/d in this trial, 2.8 kg/d in Broderick et al. (2002), and 2.7 kg/d in Cunningham et al. (1996). The latter study agreed with our findings that this milk production response occurred regardless of parity. Other studies however, reported no difference in milk production with additional expeller soybean meal (Wu and Satter, 2000), formaldehyde-treated soybean meal (Castillo et al., 2001), or animal by-products as the main sources of RUP (Wattiaux et al., 1994; Davidson et al., 2003). In this trial, the ED diets decreased DMI and fecal N but increased apparent digestibility of N leading to no changes in N intake or N apparently absorbed. These effects were true for both primiparous and multiparous cows. Remarkably, milk yield was depressed by 1.7 kg/d on the average in both parities when fed the ED diets. This result is consistent with a recent report, which suggested that excess RDP might be detrimental to milk production, particularly in the early part of the lactation (Wattiaux and Karg, 2004b).

Parity Effects

In agreement with Knowlton et al. (2001), multiparous cows in this trial consumed more N, apparently absorbed more N, and excreted more N in milk, urine, and feces. Furthermore, our results agreed with those of Davidson et al. (2003) indicating differences in yields of milk, fat, and protein, but no difference in milk fat and protein percentages due to parity. In addition, parity did not influence the efficiency of conversion of dietary N into milk N nor the decreased efficiency associated with the EU diets whether milk N was expressed as a percentage of N intake or N apparently absorbed (Table 5).

RUP and RDP by Parity Interactions

In this trial, there were no parity $\times$ RDP interactions, but there were a number of parity $\times$ RUP interactions. The tendency for greater positive responses in apparent digestibility of DM, N, and NDF when fed the EU diets in multiparous relative to primiparous cows was most likely associated with changes in ruminal kinetics despite similar increases in DMI in both parity groups. Unfortunately, this contention could not be verified because no recent studies have reported ruminal passage kinetics and whole tract digestibility responses to increasing DMI in primiparous and multiparous cows.

Parity also influenced the metabolic fate of the additional N apparently absorbed in response to the EU diets. Only 6% of the additional 109 g/d of N apparently absorbed was found in the urine of multiparous cows, but 59% of the additional 56 g/d of N apparently absorbed was found in the urine of primiparous cows. When comparing soybean meal to blood meal, Knowlton et al. (2001) did not detect a dietary CP $\times$ parity interaction for urinary N, but Kauffman and St-Pierre (2001) reported a breed effect (Holstein vs. Jersey). Our results also showed that 17% of the additional N was apparently absorbed in primiparous cows but 84% was apparently retained in multiparous cows. Across all dietary treatments, NAR was 39 g/d higher in multiparous than in primiparous cows (Table 5). Factors contributing to this difference may include a greater need to restore body protein reserves mobilized early in lactation (Botts et al., 1979) or a higher urea-N pool in multiparous cows compared with primiparous cows. The latter contention was supported by higher serum urea N in multiparous than in primiparous cows (data not shown) and the fact that plasma volume increased exponentially with cow BW (Turner and Herman, 1931). Additional contributing factors included experimental error (differential losses of N from the urine collection carboys) and the additional N required for scurf, hair, and hoof growth (not measured), but not a differential fetal growth because none of the cows were confirmed pregnant during this trial.

Although the parity difference in NAR was likely biologically meaningful, cautious interpretation is warranted. As is typical, NAR was calculated as N intake minus the sum of milk, urinary, and fecal N. This value (also referred to as N balance) accumulates the error associated with each of the 4 measurements included in its calculation. As in this trial, many studies with dairy cattle (Castillo et al., 2001; Kauffman and St-Pierre, 2001; Ruppert et al., 2003) have reported positive N balances with no significant change in BW. Sometimes unrealistically high positive bal-

ances have been reported. In a meta-analysis of 35 trials with dairy cows, Spanghero and Kowalski (1997) found that the mean and median N balance were 39 and 24 g/d, respectively. This phenomenon has been observed in sheep (MacRae et al., 1993) and in pigs placed in respiration chambers (Rasch and Benevenga, 2004). The latter authors reported less than 100% recovery of ^{15}N fed to weaned piglets, indicating that there is a nongaseous N-containing excretory product or metabolite that may be escaping detection when total collection is performed.

CONCLUSIONS

Dry matter intake and milk production increased with increasing RUP (additional expeller soybean meal) in the diets. In contrast, DMI and milk production decreased when the proportion of solvent soybean meal was increased as a way to increase RDP above NRC-predicted requirements. Parity did not influence these responses, the percentage of milk fat and protein, or the efficiency of conversion of dietary N to milk N. However, the greater increase in NDF digestibility and whole tract apparent digestibility of DM and N in multiparous cows compared with primiparous cows in response to the same magnitude of increase in DMI suggested differences in the kinetics of digestion across parity. The responses to feeding RUP above the recommended level resulted in a greater increase in urine N in primiparous than in multiparous cows, but a much greater amount of NAR in multiparous than in primiparous cows. Grouping first-parity cows separately from multiparous cows may contribute more to reducing urinary N excretion than precision feeding of heterogeneous groups of cows in commercial herds.

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