Effects of decreasing metabolizable protein and rumen-undegradable protein on milk production and composition and blood metabolites of Holstein dairy cows in early lactation

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ABSTRACT

This study was conducted to evaluate the effects of decreasing dietary protein and rumen-undegradable protein (RUP) on production performance, nitrogen retention, and nutrient digestibility in high-producing Holstein cows in early lactation. Twelve multiparous Holstein lactating cows (2 lactations; 50 ± 7 d in milk; 47 kg/d of milk production) were used in a Latin square design with 4 treatments and 3 replicates (cows). Treatments 1 to 4 consisted of diets containing 18, 17.2, 16.4, and 15.6% crude protein (CP), respectively, with the 18% CP diet considered the control group. Rumen-degradable protein levels were constant across the treatments (approximately 10.9% on a dry matter basis), whereas RUP was gradually decreased. All diets were calculated to supply a postruminal Lys:Met ratio of about 3:1. Dietary CP had no significant effects on milk production or milk composition. In fact, 16.4% dietary CP compared with 18% dietary CP led to higher milk production; however, this effect was not significant. Feed intake was higher for 16.4% CP than for 18% CP (25.7 vs. 24.3 kg/d). Control cows had greater CP and RUP intakes, which resulted in higher concentrations of plasma urea nitrogen and milk urea nitrogen; cows receiving 16.4 and 15.6% CP, respectively, exhibited lower concentrations of milk urea nitrogen (15.2 and 15.1 vs. 17.3 mg/dL). The control diet had a significant effect on predicted urinary N. Higher CP digestibility was recorded for 18% CP compared with the other diets. Decreasing CP and RUP to 15.6 and 4.6% of dietary dry matter, respectively, had no negative effects on milk production or composition when the amounts of Lys and Met and the Lys:Met ratio were balanced. Furthermore, decreasing CP and RUP to 16.4 and 5.4%, respectively, increased dry matter intake.

Key words: crude protein, milk production, blood metabolite, rumen-protected methionine

INTRODUCTION

Dairy cows utilize dietary CP ($N \times 6.25$) with greater efficiency than other ruminants but still excrete about 2 to 3 times more N in manure than in milk (Broderick, 2003), which results in increased milk production costs and environmental concerns related to N pollution. However, several studies have reported no improvement in milk and protein production when dietary CP is increased from 16.1 to 16.7% to 18.4–18.9% (Cunningham et al., 1996; Broderick, 2003; Leonardi et al., 2003). It is well established that the amount of protein degraded in the rumen increases with increasing CP content of the diet. If RDP exceeds microbial needs, then large amounts of NH_3 are produced, absorbed into the blood, converted into urea in the liver, and excreted in the urine. In the manure, urinary urea can be rapidly hydrolyzed to NH_3 and lost by volatilization into the environment (Muck, 1982). Overfeeding CP also reduces profit margins because of the relatively high cost of protein supplements and the poor efficiency of N use by dairy cows fed high-protein diets (Broderick, 2003).

The main function of dietary CP is to supply dairy cows with MP in the form of absorbed AA to meet their requirements for maintenance and production (Broderick, 2003; Schwab et al., 2007). Hence, dairy cow rations should ideally be balanced for AA rather than for protein (Broderick, 2003; Schwab et al., 2007). The goal in protein nutrition should be optimizing N utilization efficiency, which means minimizing total N intake while meeting the requirements for milk protein synthesis and, thereby, reducing feeding costs and N excretion into the environment. As requirements have not been clearly established for all AA, the best alternative is to balance for MP with the right proportions for at least the 2 AA whose requirements are better known; namely, Lys and Met. To meet the requirements for MP without an excess of N, rations must also be balanced for undegradable (RUP) and degradable (RDP) rumen protein.

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Ruminally synthesized microbial CP (**MCP**), RUP, and, to a much lesser extent, endogenous CP contribute to the passage of MP to the small intestine (NRC, 2001). Metabolizable protein is defined as true protein digested postruminally and AA absorbed by the intestine. If the dietary protein is well balanced, the efficiency of dietary to milk protein conversion will be more than 30% but when the dietary CP is imbalanced, it will be decreased to 23% and most of the consumed protein changes to urea (Broderick, 2006; Schwab et al., 2007). In high-producing cows, MCP supplies a lesser portion of the required protein, and significant amounts of the dietary protein must, therefore, escape ruminal degradation to meet protein needs (Broderick, 2006). However, it has been demonstrated that overfeeding of RUP due to imbalanced AA profile reduces the efficiency of MP utilization for milk protein synthesis. The nutritive value of MP for dairy cows is determined by its profile of essential AA (Broderick, 2006).

Methionine and lysine have been most often identified as the 2 most limiting AA in lactating dairy cows (Schwab et al., 2007). This is largely because of their low concentrations in dietary protein compared with their concentrations in milk and ruminally synthesized MCP. NRC (2001) recommends concentrations of 7.2 and 2.4%, respectively, for Lys and Met in MP for maximal use of MP for milk protein production. It is difficult to achieve these concentrations in practice; hence, the practical concentrations of 6.6 and 2.2% recommended for Lys and Met in MP (Schwab et al., 2003). These can be generally achieved by using a combination of high-Lys protein supplements (e.g., blood, fish, and soybean meals) or a rumen-protected Met (**RPM**) product that not only limits RUP intake to the required levels but also maintains constant RDP and fermentable carbohydrate levels to fix the MCP produced (Schwab et al., 2007). If an RPM product is not available or cannot be used, a blend of RUP supplements will be required to achieve the desired ratio of 3:1 for Lys:Met in MP, which will then lower the concentrations of both Lys and Met in MP (Schwab et al., 2003). The objective of the present study was to investigate the effects of reduced dietary CP and RUP levels on production performance, blood metabolites, nutrient digestibility, and N utilization in high-producing Holstein cows in early lactation when RDP is kept constant and Lys:Met is balanced.

MATERIALS AND METHODS

Cows and Experimental Diets

This study was conducted using 12 high-producing multiparous Holstein cows (second parity, 50 ± 7 DIM, and milk production of 47 ± 4 kg/d). Cows were ran-

domly assigned to 1 of 4 diet sequences in a complete 4×4 Latin square design with 4 treatments and 3 squares. Before feeding the cows, all feed ingredients were analyzed for chemical composition (Table 1). The cows were then fed a balanced TMR formulated according to the NRC (2001) model (Table 2). The duration of each experimental period was 21 d, with 16 d for diet adaptation and 5 d for data collection. Cows were held in separator stalls for the duration of the experiment, had free access to water, and were weighed at the beginning (d 1) and end of each period (d 21). The daily CP requirement of large-breed cows (BW of 680 kg at 90 DIM) for 45 kg/d with minimal BW change as predicted by the NRC (2001) model was 17.9%. The cows fed a diet containing 18% CP were used as the control group. Dietary treatments were treatment 1, a TMR containing 18% CP (7% RUP based on DM), treatment 2, a TMR that contained 17.2% CP (6.2% RUP based on DM), treatment 3, a TMR containing 16.4% CP (5.4% RUP based on DM), and treatment 4, a TMR that contained 15.6% CP (4.6% RUP based on DM). The RDP level was kept constant across the diets (almost 11% on a DM basis), and a part of RUP was replaced with corn silage and alfalfa to maintain fixed levels of microbial protein in all diets. Fat powder (Palmak, Gujarat, India) was supplemented so that the diets had the same amount of NE_L (Tables 2 and 3). Decreasing dietary RUP gradually led to declining amounts of MP, especially from RUP sources. Predicted microbial protein synthesis remained invariable due to the constant level of RDP supplemented with urea (Table 3). Ruminally protected methionine (Mepron, Degussa Corp., Kennesaw, GA) was used to meet the requirements (59 g/d in MP) based on the NRC (2001) model (Table 3). All diets were formulated to contain a postruminally available Lys:Met ratio of approximately 3:1, as recommended by Clark et al. (1992) and the Mepron dairy ration evaluator (version 2.1, 1999; Degussa Huls Corp., Bannockburn, IL).

Sample Collection and Analysis

The experimental diets were fed as TMR twice daily at approximately 0800 and 1600 h. The feed offered was adjusted daily to yield 5 to 10%orts. Samples of individual feeds and orts (about 0.5 kg) were taken daily and stored at -20°C . Weekly composite samples from feeds and orts were dried at 60°C for 48 h, and the as-fed composition of the diets was adjusted every week. Weekly feed composites were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA) and analyzed for DM at 105°C (AOAC, 1980), ash (AOAC International, 2000; method 942.05), NDF (Van Soest et al., 1991), CP (AOAC International,

Table 1. Chemical composition of feed ingredients based on DM¹ (%) and for RUP and RDP based on CP (%) expressed as means

Ingredient	Composition, % of DM										
	DM	CP	RDP	RUP	NDF	ADF	Fat	Ash	NFC	Lys	Met
Alfalfa hay	93.2	13.5	73.7	26.2	55.7	38.8	3.4	8.5	18.7	0.19	0.73
Corn silage	22.5	10.4	84.5	15.4	52.0	31.0	5.2	8.0	24.3	0.14	0.22
Beet pulp	96.8	10.3	73.9	26.0	48.0	22.0	1.0	5.6	34.9	0.12	0.48
Ground barley	89.5	10.7	60.4	39.5	22.5	5.0	2.0	4.0	60.7	0.16	0.37
Ground corn	89.8	8.4	60.7	39.2	11.0	5.2	3.9	5.5	71.2	0.16	0.25
Soybean meal, mechanical pressure	92.0	47.4	62.8	37.2	18.0	9.3	3.0	6.5	25.0	0.61	2.71
Extruded soybean (full fat)	94.6	38.0	50.6	49.3	18.0	14.0	18.7	9.3	15.9	0.48	1.03
Canola meal	92.8	37.3	69.4	30.5	33.5	18.7	3.3	8.2	17.6	0.68	1.94
Cottonseed, whole with lint	94.4	19.3	70.0	29.9	52.9	37.0	19.3	3.9	4.5	0.26	0.79
Corn gluten meal	93.9	56.9	40.0	59.9	13.8	7.2	1.8	5.0	22.3	1.42	0.79
Fish meal	95.6	60.1	34.2	65.8	6.5	8.5	17.7	15.0	0.6	1.61	1.82

¹Proximate analysis (CP, NDF, ADF, ash, fat, and NFC), degradability of protein (% of CP) by Cornell Net Carbohydrate and Protein System (CNCPS) method (Sniffen et al., 1992; Licitra et al., 1996), amino acid profile by amino acids analyzer for concentrate ingredients, and near infrared spectroscopy for forage ingredients.

2000; method 990.03), and ether extract (AOAC, 1990; method 920.39) (Table 2). Nonstructural carbohydrate content (% of DM) of diets was calculated as follows: 100 – (NDF % + CP % + ether extract % + ash %). Chemical compositions of the principal dietary ingredients were analyzed (Table 1), and sufficient amounts of all ingredients were stored for gradual use. Corn silage was used freshly and sampled on a weekly basis for DM content.

Cows were milked 3 times daily and milk weights were recorded at each milking event. On d 16 to 21 of

each period, milk samples were collected and analyzed for fat, true protein, lactose, SNF, TS, and MUN content by Milkoscan (Foss Electric, Hillerød, Denmark; AOAC International, 1996).

On d 19 of each period, samples of rumen fluid from each cow were collected by stomach tube 3 to 4 h after morning feeding. The pH of the samples was measured immediately, and 20 mL of the rumen fluid was acidified with 0.4 mL of 50% (vol/vol) sulfuric acid and stored at –20°C for later analysis of NH₃ (Broderick et al., 2004).

Table 2. Feed ingredients of experimental diets¹ based on DM (%)

Ingredient	Dietary CP, % of DM			
	18	17.2	16.4	15.6
Alfalfa hay	15.3	15.8	16.3	16.6
Corn silage	19.4	20.4	21.4	22.4
Beet pulp	3.8	3.8	3.8	3.8
Ground barley	17.7	17.7	17.7	17.7
Ground corn	17.7	17.7	17.7	17.8
Soybean meal	6.2	6.2	6.2	6.2
Soybean extruded	5.5	5.5	5.5	5.5
Canola meal	1.1	1.1	1.1	1.1
Cottonseed whole	3.3	3.3	3.3	3.3
Corn gluten meal	5.2	3.8	1.9	0.2
Fish meal	1.9	1.4	0.8	0.2
Fat powder	0.4	0.7	1.5	2.3
Sodium bicarbonate	0.8	0.8	0.8	0.8
Magnesium oxide	0.2	0.2	0.2	0.2
Calcium carbonate	0.6	0.6	0.6	0.6
Dicalcium phosphate	0.2	0.2	0.2	0.2
White salt	0.3	0.3	0.3	0.3
Mineral and vitamin premix ²	0.4	0.4	0.4	0.4
Rumen-protected methionine (Mepron) ³	0.00	0.03	0.05	0.07
Urea 281% CP	0.00	0.07	0.22	0.33
Total, %	100	100	100	100

¹Treatments 1 to 4 consisted of TMR containing 18, 17.2, 16.4, and 15.6% CP, respectively.

²Composition: 195 g/kg of Ca, 21 g/kg of Mg, 2.2 g/kg of Mn, 0.3 g/kg of Zn, 0.3 g/kg of Cu, 0.12 g/kg of I, 0.1 g/kg of Co, 600,000 IU/kg of vitamin A, 200,000 IU/kg of vitamin D, and 0.2 g/kg of vitamin E, 2.5 g/kg of antioxidant.

³Degussa Corp. (Kennesaw, GA).

Table 3. Chemical composition and predicted values of MP, Lys, Met, and Lys:Met ratios in experimental diets¹

Chemical composition	Dietary CP, % of DM			
	18	17.2	16.4	15.6
NE _L , ² Mcal/kg of DM	1.7	1.7	1.7	1.7
CP, % of DM	18	17.2	16.4	15.6
RDP, % of DM	10.9	10.8	10.7	10.9
RUP, % of DM	7.1	6.3	5.5	4.7
MP, ³ g/d	2,867	2,711	2,533	2,366
MP from bacteria, ³ g/d	1,176	1,159	1,138	1,120
MP from RUP, ³ g/d	1,690	1,552	1,394	1,246
Lys, ³ g of MP	189.1	184.4	179.4	174.6
Met, ³ g of MP	59	59.2	59.3	59.2
Lys, ³ % of MP	6.6	6.8	7.1	7.4
Met, ³ % of MP	2.0	2.2	2.3	2.5
Lys:Met ³	3.2	3.1	3.0	2.9
Ether extract, % of DM	5.3	5.6	6.2	6.8
NDF, % of DM	31.8	32.3	32.7	33
ADF, % of DM	18.0	18.3	18.6	18.9
NFC, ⁴ % of DM	38.3	38.4	38.3	38.3
Ca, % of DM	0.81	0.81	0.81	0.81
P, % of DM	0.43	0.43	0.43	0.43

¹Treatments 1 to 4 consisted of TMR containing 18, 17.2, 16.4, and 15.6% CP, respectively.

²Computed by discounting dietary energy content based on observed DMI (NRC, 2001).

³Estimated using the NRC (2001) model.

⁴NFC = 100 – (% NDF – NDIN × 6.25) – % CP – % fat – % ash.

On 20 d of each period, blood samples were collected 3 to 4 h after morning feeding from the coccygeal vessels using an evacuated tube with anticoagulant (EDTA). Blood samples were centrifuged at $3,000 \times g$ for 15 min. Plasma samples were separated and stored in plastic tubes frozen at -20°C until analysis. Concentrations of plasma glucose, triglyceride, NEFA, BHBA, total protein, albumin, globulin, plasma urea nitrogen, and aspartic succinyltransferase were measured using a serum spectrophotometer (UNICCO, 2100, Zistchemi Co., Tehran, Iran).

Eating and ruminating activities of the cows were visually monitored in all treatments every 5 min over 24-h on d 21 of each period. Eating and ruminating activities were noted every 5 min and each activity was assumed to persist for the entire 5-min interval. The parameters observed included the time spent standing (resting; i.e., neither eating, nor drinking, nor ruminating), eating, and ruminating (standing and lying). Activities were calculated by dividing the total number of minutes by the mean of each variable measured.

On d 16 to 21 of each period, feces samples were taken from each cow after the morning feeding and acid-insoluble ash (AIA) was used as an internal marker to determine the apparent digestibility of nutrients. Feed, refusals, and fecal samples were collected (Elliot et al., 1981) and analyzed for AIA using a modification of the 2 N HCl procedure of Van Keulen and Young (1977). Digestibility by AIA was calculated as the ratio of AIA in feed (corrected for refusals) and feces (Van Soest, 1994).

Statistical Analysis

All data were analyzed as a 4×4 Latin square design using Proc Mixed of SAS (version 8.2, 2001; SAS Institute Inc., Cary, NC). Model sums of squares included mean, cow (within square), square, period, treatment (effect of diet), square $\times$ treatment and period $\times$ treatment interactions, as well as overall error. All the variables were considered to be fixed, except cow (within square) and overall error, which were taken to be random. The interaction term square in the square $\times$ treatment was removed from the model when $P > 0.25$. The first-order autoregressive covariance structure [AR (1)] and the MIXED model were used for data analysis:

$$Y_{ijkl} = \mu + T_i + A_j + P_k + (T \times P)_{ik} + b_1(P_{milk}) + e_{ijkl}$$

where y_{ijkl} = each observation, μ = overall mean, T_i = fixed effect of treatment, A_j = random effect of cow, P_k = fixed effect of period, $(T \times P)_{ik}$ = interaction of period and treatment, b_1 = covariate factor of initial milk production (P_{milk}), and e_{ijkl} = random error term.

RESULTS AND DISCUSSION

The treatment with dietary CP of 16.4% had a linearly significant effect ($P < 0.05$) on DMI (Table 4), whereas the other treatments had no significant effect

Table 4. Effects of decreasing CP (from RUP sources) on milk production, milk composition, and feed efficiency

Item	Dietary CP, ¹ % of DM				SEM	$P > F^2$		
	18	17.2	16.4	15.6		Diet	L	Q
DMI, kg/d	24.3 ^b	24.5 ^{ab}	25.7 ^a	25 ^{ab}	0.5	0.03	0.04	0.21
Milk production, kg/d	45.4	46.4	47.9	45.0	1.5	0.22	0.94	0.06
FCM, kg/d	39.0 ^{ab}	38.4 ^{ab}	41.8 ^a	39.3 ^{ab}	1.0	0.016	0.19	0.22
ECM, kg/d	42.7	42.2	45.0	42.7	1.1	0.07	0.42	0.26
Milk composition, %								
Fat	3.15	3.10	3.19	2.87	0.18	0.31	0.19	0.31
Protein	2.99	3.03	3.11	3.03	0.04	0.12	0.18	0.09
Lactose	4.82	4.86	4.93	4.91	0.3	0.11	0.03	0.17
Milk composition, g/d								
Fat	1,390 ^{ab}	1,320 ^b	1,510 ^a	1,420 ^{ab}	0.06	0.02	0.14	0.83
Protein	1,408	1,415	1,408	1,367	47.80	0.76	0.41	0.50
Lactose	2,220	2,270	2,280	2,200	80	0.69	0.81	0.25
MUN, mg/dL	17.3 ^a	16.1 ^{ab}	15.2 ^b	15.1 ^b	0.5	0.02	0.06	0.01

^{a,b}Least squares means within a row with different superscripts differ ($P < 0.05$).

¹Treatments 1 to 4 consisted of TMR containing 18, 17.2, 16.4, and 15.6% CP, respectively.

²Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

($P > 0.05$). Milk production and composition were not affected by the experimental diets (Table 4); however, milk production for 17.2 and 16.4% dietary CP diets increased numerically ($P > 0.05$). Dietary treatment 3 (16.4% CP) had a significant effect on ECM and milk fat content (g/d; $P < 0.05$). In addition, the lower protein diets (17.2, 16.4, and 15.6% dietary CP) did not limit milk yield, milk fat, or milk protein compared with the control group (18% dietary CP). These results were paralleled by quadratically increased CP digestibility and numerically increased N efficiency (milk N:N intake). In fact, decreased RUP with a reasonable supply of Lys and Met and constant levels of RDP, fermentable carbohydrate, and MP from rumen bacteria (Table 3) and the increased MCP portion of MP due to the balanced AA profile not only increased the efficiency of MP (Schwab et al., 2003), but also failed to have any negative effects on milk yield and FCM. These findings are consistent with those of other studies that reported no changes in production performance when dietary CP varied from 16.7 to 18.4% (Davidson et al., 2003), from 16.4 to 20.4% (Mulligan et al., 2004), from 16.4 to 18.0% (Wattiaux and Karg, 2004), and from 14.6 to 18.3% (Castillo et al., 2001). In contrast, one study reported significant differences in milk yield or composition with dietary alteration in CP from 13.1 to 17.0% (Frank and Swesson, 2002). Dietary protein up to 15% has been reported to affect milk production; however, no such effect is observed when it is increased beyond 17% (Groff and Wu, 2005), although only minimal effects have been reported on milk fat and protein concentration (Sutton, 1989).

The NRC (2001) model predicted an NE_L-limited milk yield of 42.2 kg/d for all 4 experimental diets but MP-limited milk yields of 45.1, 42, 38.3, and 34.8 kg/d

respectively, were predicted by the same model for the diets containing 18, 17.2, 16.4, and 15.6% CP. Responses in yields of milk and FCM indicated that NE_L, MP, and AA supplies were greater than those predicted by NRC (2001) for the lower CP and RPM-supplemented diets. Assuming that Met limited production, the predicted supply of digestible Met was more than adequate for 18% dietary CP and, because of the added RPM, for the 3 lower CP diets as well (Table 3). Supplying adequate AA levels probably accounted for the milk production observed in this trial. If we assume that the optimal ratio of absorbed Lys:Met is 3.0 (NRC, 2001) and that Lys is the second-limiting essential AA (Schwab et al., 1992), then the 3 lower CP diets with Lys:Met ratios of 3:1 had the most favorable AA patterns and supplies in the current trial. On the basis of the NRC (2001) model, Met supply was adequate in all treatments and Lys was higher than the amount predicted by the NRC (2001) model. With gradually decreasing RUP, the estimated supply of Lys gradually decreased (with decreasing MP) for the 17.2 and 16.4% CP diets. The estimated supply of Lys at 174.6 g/d was sufficient for the 15.6% CP diet according to the NRC (2001) model (Table 3), resulting in no decline in milk yield across the dietary treatments containing 18 to 15.6% CP (Table 4). When EAA are absorbed in the profile as required by the animal, the efficiency of EAA in using protein is maximized and the requirement for total absorbed AA is reduced (Schwab et al., 2004; Broderick et al., 2008).

The effect of dietary CP level on MUN content is shown in Table 4. The MUN values decreased quadratically ($P < 0.05$) with lower CP and were significantly affected by dietary treatment, with the lowest value for the 15.6 and 16.4% CP diets, followed by the 17.2%

Table 5. Effects of decreasing CP (from RUP sources) on feed efficiency, BCS, BW change, and eating, rumination, and chewing time

Item	Dietary CP, ¹ % of DM				SEM	<i>P</i> > <i>F</i> ²		
	18	17.2	16.4	15.6		Diet	L	Q
FER ³	1.8	1.9	1.8	1.8	0.07	0.75	0.48	0.44
FCM:DMI	1.6	1.5	1.6	1.6	0.05	0.76	0.97	0.86
ECM:DMI	1.7	1.7	1.7	1.7	0.05	0.91	0.67	0.97
Mean BCS	3.0	3.0	3.0	2.9	0.04	0.26	0.60	0.24
BCS change	0.09	−0.02	0.11	0.07	0.06	0.22	0.72	0.51
Mean BW, kg	600.0	591.7	588.4	599.5	7.88	0.37	0.83	0.09
BW change, kg	−8.9	8.1	4.5	−0.16	10.95	0.45	0.56	0.22
Time/day, min								
Rumination	455.4	475.4	492.9	459.6	21.34	0.29	0.65	0.08
Eating	360.8	366.2	365.8	347.1	12.70	0.40	0.30	0.18
Resting	529.2	489.2	454.2	520.8	42.68	0.29	0.65	0.08
TCA ⁴	816.2	841.7	858.7	806.7	25.09	0.16	0.88	0.09
Time, min/kg of DMI								
Rumination	18.9	19.5	19.2	18.7	0.99	0.86	0.38	0.50
Eating	14.9	15.1	14.3	14.0	0.63	0.10	0.08	0.39

¹Treatments 1 to 4 consisted of TMR containing 18, 17.2, 16.4, and 15.6% CP, respectively.

²Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

³FER (feed efficiency ratio) = milk production (kg/d):DMI (kg/d).

⁴Total chewing activity (sum of eating and rumination time).

CP diet, and the highest for the 18% CP diet. This observation is consistent with those reported elsewhere (Davidson et al., 2003; Groff and Wu, 2005; Promkot and Wanapat, 2005), whereas Flis and Wattiaux (2005) found that a 1% change in dietary CP level did not lead to any significant changes in MUN content. Research and field experience have indicated that Met is most often the first-limiting and Lys the second-limiting AA for milk protein production (Schwab et al., 2007). Metabolizable protein efficiency has reportedly been improved by supplying adequate amounts of Lys and Met, and maintaining a desirable Lys:Met ratio (Schwab et al.,

2007), resulting in decreased urea production and MUN levels (Wattiaux and Karg, 2004; Schwab et al., 2007).

No significant treatment effects ($P > 0.05$) were observed on mean BW, mean BCS, BW changes, or BCS changes (Table 5). These results are consistent with those reported by Davidson et al. (2003), who observed no changes when dietary CP level varied from 16.8 to 19.4%.

Plasma parameters (Table 6) did not exhibit any significant differences with either reduced dietary CP or RUP ($P > 0.05$). Plasma urea concentrations were numerically higher ($P > 0.05$) in cows consuming high-

Table 6. Effects of decreasing CP (from RUP sources) on blood metabolites and rumen parameters

Item ¹	Dietary CP, ² % of DM				SEM	<i>P</i> > <i>F</i> ³		
	18	17.2	16.4	15.6		Diet	L	Q
Glu, mg/dL	59.8	60.4	60.2	58.2	3.26	0.90	0.63	0.58
TG, mg/dL	20.5	21.2	20.3	19.9	3.10	0.97	0.78	0.80
BHBA, mEq/L	0.1	0.2	0.2	0.2	0.39	0.31	0.44	0.69
NEFA, mEq/L	0.3	0.3	0.3	0.3	0.25	0.86	0.21	0.78
AST, U/L	77.3	70.9	70.5	71.1	5.55	0.39	0.23	0.96
TP, g/dL	8.3	8.7	8.2	8.2	0.58	0.82	0.68	0.62
Alb, g/dL	3.6	3.7	3.6	3.4	0.31	0.79	0.44	0.67
Glob, g/dL	4.6	5.0	4.6	4.8	0.54	0.89	0.45	0.50
Alb:Glob ratio	0.7	0.7	0.6	0.6	0.05	0.67	0.12	0.73
PUN, mg/dL	19.5	18.6	17.1	16.5	1.41	0.15	0.02	0.86
Rumen parameter								
pH	5.8	6.0	6.0	6.1	0.29	0.43	0.22	0.76
N-NH ₃	82.3	81.0	82.2	84.5	3.44	0.74	0.39	0.82

¹Glu = glucose; TG = triglyceride; AST = aspartic succinyltransferase; TP = total protein; Alb = albumin; Glob = globulin; PUN = plasma urea N.

²Treatments 1 to 4 consisted of TMR containing 18, 17.2, 16.4, and 15.6% CP, respectively.

³Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

Table 7. Effects of decreasing CP (from RUP sources) on N retention, N efficiency, and apparent digestibility of nutrients

Item	Dietary CP, ¹ % of DM				SEM	<i>P</i> > <i>F</i> ²		
	18	17.2	16.4	15.6		Diet	L	Q
Intake of N, g/d	700.0 ^a	673.8 ^a	682.7 ^a	632.0 ^b	13.24	0.001	0.00	0.19
Intake of RUP N, g/d	276.1 ^a	246.8 ^b	226.2 ^c	188.0 ^d	6.40	0.00	0.00	0.19
Milk N, g/d	225.4	226.4	225.4	218.7	8.0	0.76	0.41	0.51
Predicted urine N, ³ g/d	339.2 ^a	310.4 ^{ab}	289.9 ^{ab}	281.3 ^b	11.60	0.043	0.02	0.47
Predicted fecal N, ⁴ g/d	137.8	136.9	136.7	133.3	22.10	0.45	0.34	0.64
Apparent N efficiency, ⁵ %	32.1	33.7	33.1	35.0	1.45	0.25	0.20	0.41
Total nutrient digestibility, %								
DM	71.5	73.5	73.5	75.2	2.64	0.58	0.40	0.32
CP	78.9 ^a	74.6 ^b	73.9 ^b	70.9 ^b	2.70	0.05	0.08	0.03
OM	73.9	76.3	76.4	77.7	2.54	0.51	0.42	0.29
Ether extract	71.8	70.6	71.0	71.1	3.11	0.87	0.48	0.69
NDF	49.5	49.9	50.5	50.0	2.86	0.76	0.36	0.75
ADF	47.9	48.6	48.6	47.0	2.34	0.89	0.69	0.23

^{a-d}Least squares means within a row with different superscripts differ (*P* < 0.05).

¹Treatments 1 to 4 consisted of TMR containing 18, 17.2, 16.4, and 15.6% CP, respectively.

²Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

³Predicted urine N output = 0.0283 × MUN (mg/dL) × BW (kg) (Wattiaux and Karg, 2004).

⁴Predicted fecal n = N intake – predicted urine N – milk N.

⁵Apparent N efficiency = 100 × milk N (g/d)/N intake (g/d).

protein diets; however, this difference was not statistically significant.

Daily N intake linearly decreased with decreasing dietary CP and RUP levels, whereas fecal N excretion did not change (Table 7). Fecal N consists primarily of indigestible microbial protein produced in the rumen as well as endogenous proteins, sloughed cells from the gastrointestinal tract, and undigested feed proteins (Mason, 1969; Davidson et al., 2003). In this experiment, RUP levels were found to vary among treatment groups and decreased linearly from 18 to 15.6% dietary CP (*P* < 0.05). Because undigestible feed protein is a minor component of the total fecal N, differences among treatments were not expected in the light of predicted values of fecal N (Davidson et al., 2003). Predicted urinary N excretion decreased linearly with lower dietary CP and RUP, the highest being recorded for 18% dietary CP and the lowest for 15.6% dietary CP (*P* < 0.05). Unlike N (or CP) intake, milk N did not differ among treatments (*P* > 0.05) so that N utilization efficiency was numerically higher for lower CP treatments (2, 3, and 4) than that of the control group (*P* > 0.05). This may be attributed to the improved AA balance, especially Lys and Met in MP, which enhanced (Table 7) protein utilization (Schwab et al., 2007).

CONCLUSIONS

In this study, DMI, FCM, and ECM were found to increase significantly by feeding 16.4% dietary CP, and CP digestibility was decreased by feeding 15.6% dietary CP. In addition, MUN was decreased with 15.6% di-

etary CP. Based on our results, the NRC (2001) model predicts values for CP, RUP, and MP greater than requirements. Reduced values of CP, RUP, and MP in the diets formulated to contain a postruminally available Lys:Met ratio of approximately 3:1 were found to have no significant effects on milk production and composition. In fact, decreasing dietary CP to 15.6%, especially when Lys and Met were balanced, increased N efficiency and decreased CP digestibility without any detrimental effect on production performance of the animal. Therefore, the current diet evaluation model (i.e., NRC, 2001) that predicts passage of MP-AA to the small intestine in their present form is not useful for predicting the effects that changes in CP, RUP, MP-Lys, and MP-Met might have on production performance.

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