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Responses of Dairy Cows to Supplemental Highly Digestible Rumen Undegradable Protein and Rumen-protected Forms of Methionine*

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ABSTRACT : Metabolizable protein (MP) supply and amino acid balance in the intestine were manipulated through selection of highly digestible rumen-undegradable protein (RUP) sources and protected methionine (Met) supplementation. Four ruminally-cannulated, multiparous Holstein cows averaging 193±13 days in milk were used in a 4×4 Latin square design to assess N utilization and milk production responses to changes in RUP level, post-ruminal RUP digestibility and protected Met supplementation. Treatments were A) 14.0% crude protein (CP), 8.0% rumen degradable protein (RDP) and 6.0% RUP of low intestinal digestibility (HiRUP-LoDRUP); B) 14.1% CP, 8.1% RDP and 6.0% RUP of high intestinal digestibility (HiRUP-HiDRUP); C) 13.1% CP, 7.9% RDP and 5.2% RUP of high intestinal digestibility (LoRUP-HiDRUP), and D) 13.1% CP, 7.9% RDP and 5.2% RUP of high intestinal digestibility plus rumen escape sources of Met (LoRUP-HiDRUP+Met). Experimental diets were formulated to have similar concentrations of RDP, net energy of lactation (NE_L), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium, phosphorus and ether extract using the NRC model (2001). Results showed that dry matter intake (DMI), production of milk fat and protein were similar among treatments. Milk production was similar for diet HiRUP-LoDRUP, HiRUP-HiDRUP and LoRUP-HiDRUP+Met, and significantly higher than diet LoRUP-HiDRUP. Milk fat and protein percentage were higher for cows receiving HiDRUP treatments, with the greatest increases in the diet LoRUP-HiDRUP+Met. There was no significant change in ruminal pH, NH_3-N and volatile fatty acid (VFA) concentration among all treatments. Apparent digestibility of dry matter (DM), CP, NDF and ADF and estimated bacterial CP synthesis were similar for all treatments. Nitrogen intakes, blood and milk urea-N concentrations were significantly higher for cows receiving HiRUP diets. Urine volume and total urinary N excretion were significantly lowered by LoRUP diets. Lowering dietary RUP level while supplementing the highly digestible RUP source with rumen escape sources of Met resulted in similar milk production, maximal milk fat and protein concentration and maximum N efficiency, indicating that post-ruminal digestibility of RUP and amino acid balance in the small intestine can be more important than total RUP supplementation. (**Key Words :** Rumen Undegradable Protein, Methionine, Nitrogen Efficiency)

INTRODUCTION

Due to increasing societal pressure to improve the environmental performance of livestock operations, dairy research is currently being directed towards improving the efficiency of nitrogen (N) use of lactating cows to reduce losses of N (NRC, 2001). One of the first steps in diet formulation for lactating dairy cows is to provide rumen-degradable protein (RDP) to meet the requirements of rumen microorganisms. The total metabolizable protein (MP) requirement of the cow is met by supplementing rumen-undegradable protein (RUP) when microbial protein synthesis alone is insufficient to meet the MP requirements (Kalscheur et al., 2006). Efficiency of N utilization could be improved by increasing post-ruminal digestibility and/or providing a pattern of absorbed amino acids (AA) that more closely matches the AA requirements for milk synthesis (Noftsger and St-Pierre, 2003). Erasmus et al. (1993) found significant differences among feedstuffs in the digestibility of RUP and the AA profile of RUP. Metabolizable protein supply and AA balance can be manipulated through selection of highly digestible RUP sources and protected methionine (Met) supplementation (Noftsger and St-Pierre, 2003). 2-Hydroxy-4-(methylthio)-butanoic acid (HMB), is a common source of bypass Met (Schwab, 1998). The most consistent response to feeding HMB has been an increase in

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Table 1. Composition of feeds

Item	Corn	Wheat bran	Rapeseed meal	Cottonseed meal	Soybean meal	Corn silage	Alfalfa hay	Chinese wildrye hay
CP (% of DM)	9.4	15.6	37.1	38.0	48.1	6.7	13.6	6.5
RDP[1] (% of CP)	55	76	41	43	58	66	61	59
RUP[1] (% of CP)	45	24	59	57	42	34	39	41
NDF (% of DM)	15.7	40.1	29.8	30.8	15.9	54.2	51.5	67.1
ADF (% of DM)	7.3	11.6	20.5	19.9	7.2	35.1	29.7	41.2
RUP digestibility % of RUP[2]	88	74	64	89	94	64	71	61
AA (% of CP)								
Aspartic acid	7.23	5.51	6.20	9.05	9.23	7.16	13.68	8.92
Threonine	3.30	2.50	3.77	3.00	3.04	3.58	4.49	4.00
Serine	4.89	3.85	4.04	4.53	4.37	3.58	5.15	4.46
Glutamic acid	17.45	20.13	17.47	20.34	15.11	10.15	10.44	9.85
Proline	3.72	3.21	3.07	1.89	2.12	2.99	2.87	2.31
Glycine	3.62	3.72	4.47	3.87	3.26	5.67	4.93	4.62
Alanine	6.38	3.46	4.10	4.05	3.41	14.33	5.66	5.54
Cysteine	3.40	3.21	2.86	2.05	1.83	5.52	3.46	6.15
Valine	4.47	3.59	4.58	3.89	3.53	6.27	5.59	4.62
Methionine	2.55	1.92	2.48	2.03	1.58	2.84	2.28	2.62
Isoleucine	3.19	2.63	3.45	2.74	3.37	4.48	4.34	3.38
Leucine	10.53	5.45	6.66	5.79	6.22	9.25	8.01	6.92
Tyrosine	2.98	2.44	2.26	2.71	2.66	1.79	2.94	2.46
Phenylalanine	4.47	3.53	3.72	5.21	3.99	4.48	4.85	4.31
Lysine	2.98	2.88	1.86	3.92	5.07	3.73	5.44	4.31
Histidine	3.51	2.63	4.12	3.63	2.74	3.73	4.12	4.62
Arginine	4.68	5.38	4.15	11.00	6.36	2.99	4.63	4.15
Tryptophan	0.74	1.47	1.46	1.45	1.31	0.60	1.40	0.62

[1] Determined by *in situ* method.
[2] Percentage of RUP that is digested using the Minnesota three-step enzymatic assay for protein digestion (Calsamiglia and Stern, 1995).

milk fat percentage (Huber et al., 1984; Koenig et al., 2002).

This experiment was designed to assess N utilization and milk production responses to changes in RUP level, post-ruminal RUP digestibility and AA balance in the intestine. We hypothesized that milk production and composition could be maintained through selection of high digestibility RUP feedstuffs and supplementation of HMB when we decreased RUP concentration, and the efficiency of N utilization would increase.

MATERIALS AND METHODS

Location

This study was conducted at the State Key Laboratory of Animal Nutrition of China Agricultural University. All experimental procedures were approved by the University of China Agriculture Animal Care and Use Committee.

Experimental design and diets

Four ruminally-cannulated, multiparous Holstein cows averaging 193±13 days in milk (DIM) were used in a 4×4 Latin square design to assess N utilization and milk production responses to changes in RUP level, post-ruminal RUP digestibility and protected Met supplementation. Average body weight (BW) for cows at the beginning of the experiment was 585±14 kg. The length of each experimental period was 28 days, the first 7 days of which was an adaptation period and the last 7 days was for data and sample collection. Treatment diets were fed as total mixed rations (TMR) for *ad libitum* intake to allow 5 to 10% feed refusal. The total mixed ration was offered in equal portions three times daily at 0600, 1200, and 1730 h. Cows were held in tie stalls, fed individually, and had free access to water. Cows were weighed on 3 consecutive days at the beginning and end of each period.

Composition of feeds is presented in Table 1. The RDP and RUP contents of different feedstuffs in the diets were determined by an *in situ* method using dacron bags before the experiment (Flis and Wattiaux, 2005). Three lactating cows fed a diet of 25% corn silage, 20% alfalfa hay, 10% Chinese wildrye hay and 50% concentrate on a DM basis were used to incubate the bags. Intestinal digestibility of RUP was determined using the Minnesota three-step enzymatic assay for protein digestion before trial initiation (Calsamiglia and Stern, 1995). Diets were formulated using the NRC model (2001). Experimental diets were formulated to have similar concentrations of RDP, net energy of lactation (NE_L), NDF, ADF, calcium, phosphorus and ether extract (Table 2). Diets contained 25.8% corn silage, 20.2% alfalfa hay, 10.1% Chinese wildrye hay and 44%

Table 2. Ingredient and nutrient composition of experimental diets for lactating dairy cows

Item	Treatments[1]				SEM
	A	B	C	D	
Ingredient, (% DM basis)					
Corn silage	25.80	25.80	25.70	25.70	
Alfalfa hay	20.20	20.20	20.20	20.20	
Chinese wildrye hay	10.10	10.10	10.10	10.10	
Corn, ground shelled	23.20	23.10	26.10	26.10	
Wheat Bran	5.10	5.10	7.10	7.10	
Rapeseed meal	7.10	0	0	0	
Cottonseed meal	0	7.10	5.10	5.10	
Soybean meal	7.10	7.10	4.00	4.00	
Sodium bicarbonate	0.36	0.36	0.36	0.36	
Di-calcium phosphate	0.30	0.30	0.30	0.30	
Premix[2]	0.50	0.50	0.50	0.50	
Urea	0	0	0.20	0.20	
Salt	0.36	0.36	0.36	0.36	
MHA[3]	0	0	0	0.085	
Nutrient contents of the diets					
DM (%)	53.2	54.2	52.5	53.5	1.3
NE_L[4] (Mcal/kg of DM)	1.54	1.53	1.52	1.52	
CP (% of DM)	14.00	14.10	13.10	13.10	0.1
RDP (% of DM)	8.00	8.10	7.90	7.90	0.12
RUP (% of DM)	6.00	6.00	5.20	5.20	0.06
NDF (% of DM)	39.65	39.73	39.88	39.88	1.1
ADF (% of DM)	23.21	23.17	23.00	23.00	0.6
Calcium (% of DM)	0.76	0.72	0.79	0.79	0.03
Phosphorus (% of DM)	0.40	0.40	0.42	0.42	0.02
Ether extract (% of DM)	3.42	3.45	3.39	3.37	0.2

[1] A = HiRUP-LoDRUP diet; B = HiRUP-HiDRUP diet; C = LoRUP-HiDRUP diet; D = LoRUP-HiDRUP+Met diet.
[2] Contained 100 mg/kg of I_2; 4,000 mg/kg of Fe; 2,000 mg/kg of Cu; 2,500 mg/kg of Mn; 8,000 mg/kg of Zn; 60 mg/kg of Se; 20 mg/kg of Co; 950,000 IU/kg of vitamin A; 200,000 IU/kg of vitamin D; 5,500 IU/kg of vitamin E.
[3] MHA contains 86% metabolizable Met, 40% bypass rate. [4] Estimated using NE_L values for feedstuffs from NRC (2001).
[a-c] Means in rows with different superscripts differ ($p<0.05$).

concentrate on a DM basis.

Treatments were A) 14.0% crude protein (CP), 8.0% rumen degradable protein (RDP) and 6.0% RUP of low intestinal digestibility (HiRUP-LoDRUP); B) 14.1% CP, 8.1% RDP and 6.0% RUP of high intestinal digestibility (HiRUP-HiDRUP); C) 13.1% CP, 7.9% RDP and 5.2% RUP of high intestinal digestibility (LoRUP-HiDRUP), and D) 13.1% CP, 7.9% RDP and 5.2% RUP of high intestinal digestibility plus supplemental Met from HMB (LoRUP-HiDRUP+Met). All treatments were similar in concentration of RDP. Supplemental undegradable protein was provided by plant protein sources that were selected to be either highly digestible in the intestine or of lower digestibility in the intestine. Rapeseed meal was selected as low intestinal digestibility (64%) RUP feedstuff. Soybean meal and cottonseed meal were selected as high intestinal digestibility (94% and 89%) RUP feedstuffs using the Minnesota three-step enzymatic analysis of RUP (Calsamiglia and Stern, 1995). The difference of RUP digestibility among the diets was mostly due to the different content of rapeseed meal, cottonseed meal and soybean meal. Methionine was supplied in the form of dry calcium salt of the analog (86% HMB; MHA, Novus Inc US) and was formulated to contain 40% Met bypass rate (Koenig et al., 2002).

Diets HiRUP-LoDRUP, LoRUP-HiDRUP and LoRUP-HiDRUP+Met provided similar balance of MP, and diet HiRUP-HiDRUP exceeded the requirement according to the NRC (2001) model using predicted intakes, BW, milk yield and composition (Table 3). Diets HiRUP-LoDRUP, HiRUP-HiDRUP and LoRUP-HiDRUP contained similar Lys:Met ratio. The addition of supplemental Met in diet LoRUP-HiDRUP+Met brought this ratio to 3.2:1, which is closer to the optimum ratio derived by the NRC (2001). Estimates by the NRC (2001) model are shown in Table 3.

Sample collection and analysis

All feedstuffs were analyzed for AA content using a Beckman 7300 amino acid analyzer (Beckman Instruments Inc) before the experiment, and samples were prepared

Table 3. Estimates of dietary nutrients at standard production (23 kg) and intake (16.5 kg DMI) in diets that vary in CP and digestibility of RUP

Predicted nutrients	Treatments[1]			
	A	B	C	D
NE_l balance[2] (Mcal/d)	0.70	0.6	0.30	0.30
MP[3] balance (g/d)	-101	12	-90	-90
Met (% of MP)	1.75	1.82	1.71	2.02
Lys (% of MP)	6.25	6.65	6.47	6.47
Lys:Met in the MP	3.6	3.7	3.8	3.2

[1] A = HiRUP-LoDRUP diet; B = HiRUP-HiDRUP diet; C = LoRUP-HiDRUP diet; D = LoRUP-HiDRUP+Met diet.
[2] Balance = Amount above requirement. [3] MP = Metabolizable protein (protein that is digestible in the small intestine).

according to AOAC (1990). Feed was sampled weekly, frozen and composited on a 4-wk basis. The composited sample was mixed thoroughly and sub-sampled for chemical analysis. Daily feed offered and refusals for individual cows were recorded. Feed samples were dried at 60°C in a forced air oven for 48 h and then ground through a 1-mm screen of a standard Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA). The NE_L content of the diet was calculated by using NE_L values for individual ingredients (NRC, 2001). Analytical DM content of TMR, orts, and fecal samples was determined by oven drying at 105°C for 48 h (AOAC, 1990). The total N content of TMR and orts were determined using the Kjeldahl procedure according to AOAC (1990). Crude protein content was calculated as N×6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the modified filter bag method of Van Soest et al. (1991) and AOAC (1990). The NDF and ADF procedures were adapted for use in an ANKOM^{200} Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). Ether extracts in feed ingredients and diets were conducted with a Soxlec system HT6 apparatus (Tecator, Fisher Scientific) according to AOAC (1990).

Cows were milked three times daily at 0630, 1230 and 1800 h, with individual milk weights recorded at each milking. Composite milk samples were obtained from the last 5 consecutive days at the end of each period. The samples were split into two portions (without preservative) for analysis. One portion was stored at 4°C and analyzed for fat, protein, lactose and solids-non-fat (SNF) by the Beijing Dairy Cattle Center laboratory using near-infrared spectroscopy (Foss MikoScan 4000; Foss Technology, Eden Prairie, MN). Milk urea nitrogen (MUN) in defatted milk samples was determined using a colorimetric diagnostic kit (Sigma Diagnostics, St. Louis, MO, procedure 535) via a procedure described by Wattiaux and Karg (2004). Fat corrected milk (FCM) was calculated using NRC (2001) equations: 3.5% FCM = (0.432×kg of milk yield+16.23×kg of fat). The remaining portion was stored at -20°C for later analysis of total nitrogen in milk by macro-Kjeldahl (AOAC, 1990).

Total feces and urine were collected separately from cows for five consecutive days during the last week of each period. On d 21 of each experimental period, cows were fitted with harnesses and tubes allowing the collection of feces and urine separately. Feces were collected from a rubber mat placed behind the animal and stored in a plastic container. Mass of feces was recorded on an individual cow basis. For 5 consecutive days, feces were weighed and mixed daily, and a representative sample (2%) was taken, stored at -20°C, and subsequently thawed, dried at 55°C for 48 h, then ground through a 1-mm screen (Wiley mill) and analyzed for DM, NDF, ADF, ether extract and N as described earlier for feed. Total urine was collected into stainless steel containers via a plastic tube attached to the cow with a nylon netting covered with neoprene affixed to the vulva. Urine was acidified daily with H_2SO_4 (50% vol/vol) to maintain pH<3.0. A representative sample (2%) was taken and kept frozen at -20°C until analysis. Urine samples were composited on a yield basis and stored at -20°C until analysis for total N by micro-Kjeldahl (AOAC, 1990).

The concentration of N in acidified urine samples was determined by micro-Kjeldahl analysis (AOAC, 1990). Uric acid concentration of urine was determined colorimetrically using a diagnostic uric acid reagent (No.685, Sigma) as described by Reynal et al. (2003). Allantoin concentration of urine was determined colorimetrically using the method described by Reynal et al. (2003). Bacterial CP synthesis in the rumen was computed from urinary allantoin as described by Vagnoni et al. (1997) using the bacterial N: total purine ratio as reported by Reynal et al. (2003).

Samples of ruminal digesta were collected on d 25 from various parts of the rumen of each cow using a vacuum pump prior to the morning feeding (0 h) and then at 2 h intervals for 24 h. The pH was immediately determined using a portable pH meter (pH/temp meter 199 Model No 3D; Fisher Scientific, Pittsburgh, PA), and samples were acidified to pH<2 with 50% H_2SO_4 and frozen at -20°C for later determination of volatile fatty acid (VFA) and NH_3-N concentrations. An aliquot of ruminal fluid was used to determine NH_3-N concentration following the procedure

Table 4. BW, DMI, milk yield and milk composition from cows fed the experimental diets

	Treatments[1]				SEM	Contrasts (p value)		
	A	B	C	D		A vs. B	B vs. D	C vs. D
BW (kg)	586	609	598	601	11	0.25	0.27	0.84
DMI (kg/d)	17.4	17.2	16.6	16.1	0.27	0.45	0.27	0.34
Milk yield								
Actual (kg/d)	23.3[a]	23.5[a]	21.7[b]	23.1[a]	0.56	0.34	0.21	0.04
3.5% FCM[2] (kg/d)	22.89	23.62	21.55	23.45	0.55	0.09	0.26	0.07
Feed efficiency[3]	1.34[a]	1.37[a]	1.31[a]	1.43[b]	0.04	0.27	0.04	0.01
Milk composition								
Fat (%)	3.37[a]	3.53[b]	3.46[b]	3.57[b]	0.056	0.04	0.29	0.11
Fat yield (kg/d)	0.79	0.83	0.75	0.83	0.03	0.14	0.88	0.07
Protein (%)	3.14[a]	3.19[b]	3.21[b]	3.26[b]	0.037	0.04	0.43	0.09
Protein yield (kg/d)	0.73	0.75	0.70	0.78	0.05	0.32	0.18	0.07
Lactose (%)	4.65	4.69	4.72	4.78	0.032	0.59	0.92	0.82
Lactose (kg/d)	1.08	1.10	1.02	1.10	0.02	0.67	0.87	0.18
SNF (%)	8.60	8.38	8.68	8.73	0.16	0.47	0.14	0.56
SNF yield (kg/d)	2.00	1.97	1.88	2.02	0.08	0.28	0.29	0.12

[1] A = HiRUP-LoDRUP diet; B = HiRUP-HiDRUP diet; C = LoRUP-HiDRUP diet; D = LoRUP-HiDRUP+Met diet.
[2] 3.5% FCM = 0.432×kg milk+16.23×kg fat. [3] Feed efficiency (%) = milk yield (kg/d)/DMI (kg/d).
[a-c] Means in rows with different superscripts differ ($p<0.05$).

described by Broderick and Kang (1980). The remaining ruminal fluid was acidified with 25% metaphosphoric acid (wt/vol) and analysed for VFA according to Erwin et al. (1961).

A blood sample from each cow was also collected on d 27 of each period from the jugular vein into heparinized evacuated tubes (Becton Dickinson and Cie, Rutherford, NJ). Blood samples were de-proteinized by mixing 1.25 ml of 25% (wt/vol) TCA with 5 ml of whole blood, then centrifuged (15,000×g at 4°C for 15 min) and supernatants were stored at -20°C until analysis for urea (Broderick and Clayton, 1997).

Statistical analyses

All data were analyzed as a Latin square design using the Proc Mixed procedures of SAS (SAS Institute, 1999). Model sums of squares were separated into overall mean, cow, square, period, treatment (effect of diet), square× treatment interaction and overall error. All variables were considered fixed, except cow and overall error, which were considered random. The interaction term square×treatment was removed from the model when $p>0.25$.

Model sums of squares for ruminal data collected at different times (pH and concentrations of NH_3-N and VFA) were separated into overall mean, cow, period, treatment (effect of diet), square×treatment interaction, whole plot error, hours postfeeding (repeated measures), hours postfeeding×treatment interaction, and subplot error. The repeated measures analyses were performed using the SP (POW) structure of SAS.

Statistical significance was declared at $p<0.05$ and tendencies at $0.05<p<0.10$.

RESULTS AND DISCUSSION

Body weight, intake, production and milk composition

Results for BW, DMI and milk performance measurements are shown in Table 4. Average BW was not significantly affected by dietary treatment. Intake of DM was not significantly affected by dietary treatment. This observation is consistent with a review by Santos et al. (1998) who found that feeding a high digestible RUP (fish meal) with a Lys:Met ratio of approximately 3:1 had no effects on DMI over a low digestible RUP (corn gluten meal) in eight out of nine studies reviewed.

Milk yield was similar for diets HiRUP-LoDRUP, HiRUP-HiDRUP and LoRUP-HiDRUP+Met, and significantly higher than diet LoRUP-HiDRUP, which may be due to insufficient MP and Met in diet LoRUP-HiDRUP. Feed efficiency (kilograms milk per kilogram DMI) was significantly improved for diet LoRUP-HiDRUP+Met when supplemented with Met, and there was no significant difference among the other three diets. Higher feed efficiency on diet LoRUP-HiDRUP+Met indicates a better utilization of the nutrients ingested through improved Lys and Met balance by supplementing Met and using a higher quality RUP source. In this trial, milk fat percentage was higher for cows receiving HiDRUP treatments, with the greatest increase on diet LoRUP-HiDRUP+Met, although it was not significantly different from other HiDRUP diets. McCarthy et al. (1968) reported that Met might be important for synthesis of serum lipoproteins and as a methyl donor for synthesis of phospholipids, suggesting a possible post-absorptive effect of Met on lipid metabolism. Lundquist et al. (1983) found that supplementation of high-

Table 5. Effect of diet treatment on ruminal metabolites and apparent total tract digestibility of nutrients

	Treatments[1]				SEM	Contrasts (p value)		
	A	B	C	D		A vs. B	B vs. D	C vs. D
pH	6.57	6.65	6.63	6.61	0.06	0.48	0.63	0.69
NH_3N (mg/dl)	7.35	7.43	7.48	7.57	0.25	0.29	0.08	0.12
VFA (%)								
Acetate (A)	53.7	54.5	53.4	55.2	3.21	0.83	0.64	0.32
Propionate (P)	13.8	14.4	13.7	14.6	0.61	0.68	0.81	0.26
Isobutyrate	0.66	0.70	0.69	0.71	0.04	0.54	0.75	0.45
Butyrate	7.71	7.75	7.83	7.73	0.22	0.72	0.65	0.44
Isovalerate	0.85	0.90	0.89	0.88	0.06	0.37	0.57	0.65
Valerate	0.96	0.94	0.90	0.93	0.05	0.36	0.96	0.42
A:P	3.89	3.78	3.90	3.78	0.08	0.35	0.56	0.29
Total VFA (mM)	82.4	84.4	81.5	84.1	3.75	0.53	0.66	0.45
Apparent total tract digestibility (% of intake)								
DM	67.3	67.8	66.1	66.7	0.4	0.93	0.66	0.77
CP	69.7	70.9	71.4	71.8	1.2	0.47	0.67	0.79
NDF	55.4	56.1	53.8	54.7	1.2	0.29	0.14	0.23
ADF	53.4	53.8	52.5	53.1	0.7	0.64	0.65	0.54

[1] A = HiRUP-LoDRUP diet; B = HiRUP-HiDRUP diet; C = LoRUP-HiDRUP diet; D = LoRUP-HiDRUP+Met diet.

quality RUP and HMB increased fat concentration at several concentrations of dietary protein and two forage to concentrate ratios. In the current study, milk fat production was not significantly different among the diets, but it tended ($p = 0.07$) to be higher for diet LoRUP-HiDRUP+Met with the addition of Met compared with diet LoRUP-HiDRUP. The FCM (3.5%) yield followed a similar trend to milk fat production. Noftsger and St-Pierre (2003) found fat production in primiparous cows was higher for the high digestible RUP treatments; however, for multiparous cows, fat production was not significantly different between high digestible RUP treatment and low digestible RUP treatment.

Milk protein percentage was significantly higher for cows receiving HiDRUP treatments compared with the HiRUP-LoDRUP diet. The cows fed diet LoRUP-HiDRUP +Met had similar milk protein percentage and production compared with cows fed diet HiRUP-HiDRUP, but tended to increase milk protein percentage ($p = 0.09$) and production ($p = 0.07$) compared with cows fed diet LoRUP-HiDRUP. An increase in milk protein production is common on diets containing undegradable sources of Met (Armentano et al., 1997; Rulquin and Delaby, 1997). Increasing AA availability, either by supplementing Met or using a high-quality RUP source, may have influenced the concentration and production of milk protein (Noftsger and St-Pierre, 2003). Milk lactose and SNF percentage were not significantly affected by dietary treatment in the current study.

Ruminal fermentation and total tract digestibility

In this trial, dietary treatment had no significant effects on ruminal pH, ammonia and VFA concentration (Table 5). Ruminal pH for the 4 experimental diets remained above 6.0 at all time points throughout the daily feeding cycle, indicating that all cows had a satisfactory ruminal environment for nutrient fermentation. Ruminal ammonia concentration was not significantly different among the four treatments because of similar RDP in the diets, but it tended to increase comparing diet LoRUP-HiDRUP+Met with HiRUP-LoDRUP and HiRUP-HiDRUP, which may have been due to the fast release of urea and partial degradation of HMB in the rumen. There were no significant effects of treatment on VFA concentration in the rumen and apparent total tract digestibility of nutrients. These observations are consistent with Noftsger and St-Pierre (2003) who found that DM digestibility, N and NDF digestibility were not significantly affected by using a higher-quality RUP source or supplementing Met. Apparent digestibility of CP did not differ between diets HiRUP-LoDRUP and HiRUP-HiDRUP (Table 5). The only difference between the two diets was the use of rapeseed meal or cottonseed meal. The RUP content provided by rapeseed meal or cottonseed meal provided approximately 11% of total CP in diets HiRUP-LoDRUP and HiRUP-HiDRUP. Therefore, a 25% increase in the intestinal digestibility of RUP source would only increase overall CP digestibility by 2.7%. With an SEM for apparent CP digestibility of 1.2, the power of the test was too low to detect differences. Belal et al. (2008) found that supplementing the diet of growing Awassi lambs with rumen-protected methionine at 0, 7 or 14 g/d per head did not affect nutrient intake and nutrient digestibilities.

Nitrogen metabolism

Nitrogen intakes were significantly higher for cows fed the HiRUP diet than cows fed the LoRUP diet (Table 6). Cows fed diet LoRUP-HiDRUP+Met had significantly

Table 6. Effect of diet treatment on N metabolism

	Treatments[1]				SEM	contrasts (p value)		
	A	B	C	D		A vs. B	B vs. D	C vs. D
N intake (g/d)	390[a]	388[a]	348[b]	337[b]	17.7	0.17	0.03	0.12
Total milk N (g/d)	117[a]	120[a]	111[b]	120[a]	4.5	0.14	0.57	0.01
N efficiency[2] (%)	30.0[a]	30.9[a]	32.0[b]	35.7[c]	2.2	0.17	0.01	0.04
Blood urea-N (mg/dl)	13.6[a]	14.1[a]	13.0[b]	13.1[b]	1.0	0.14	0.03	0.33
Milk urea-N (mg/dl)	9.79[a]	9.99[a]	9.30[b]	9.05[b]	0.33	0.13	0.01	0.08
Urinary excretion								
Urine volume (L/d)	24.4[a]	23.6[a]	22.0[b]	22.3[b]	1.4	0.22	0.04	0.16
Total Urinary N (g/d)	141[a]	145[a]	117[b]	120[b]	8.9	0.16	0.01	0.12
N (% of N intake)	36.2	37.4	33.6	35.6	1.6	0.17	0.08	0.12
Fecal excretion								
DM (kg/d)	5.52	5.47	5.38	5.33	0.14	0.77	0.13	0.29
N (g/d)	113[a]	111[a]	98[b]	95[b]	7.1	0.35	0.02	0.28
N (% of N intake)	29.1	28.6	28.2	28.2	1.1	0.32	0.26	0.69
Environmental N efficiency[3]	2.17[a]	2.13[a]	1.94[b]	1.79[b]	0.07	0.08	0.01	0.04
Allantoin (mmol/d)	220	224	213	217	4.3	0.32	0.12	0.34
Uric acid (mmol/d)	56	54	50	53	2.2	0.18	0.48	0.12
Purine derivative (mmol/d)	276	278	263	270	7	0.26	0.16	0.15
Bacterial CP synthesis[4] (g/d)	1,678	1,690	1,599	1,642	32	0.22	0.16	0.12

[1] A = HiRUP-LoDRUP diet; B = HiRUP-HiDRUP diet; C = LoRUP-HiDRUP diet; D = LoRUP-HiDRUP+Met diet.
[2] N efficiency (%) = 100×total milk N (g/d)/N intake (g/d). [3] Calculated as excreted N (Urinary N+fecal N)/milk N
[4] Calculation based on the bacterial N:purines ratios reported by Reynal et al. (2003). [a-c] Means in rows with different superscripts differ ($p<0.05$).

increased total milk N production compared to those fed diet LoRUP-HiDRUP due to the supply of Met, and was similar to those fed diets HiRUP-LoDRUP HiRUP-HiDRUP. Gross N efficiency (milk N/N intake) was increased on all diets containing low RUP level and high digestible RUP compared to the diets containing high RUP, with the greatest increase occurring on the Met-supplemented diet. Because of similar ingredient composition and N intake for diets LoRUP-HiDRUP and LoRUP-HiDRUP+Met, Met supplementation appeared to improve the efficiency of conversion of MP into milk N. High RUP diets had greater concentrations of MUN and BUN, probably due to the higher N intake, which is consistent with the study of Broderick and Clayton (1997).

In the present study, urine volume and total urinary N excretion were significantly lowered by low RUP diets. The greater urine volumes on high CP diets were consistent with earlier results (Olmos Colmenero and Broderick, 2006), indicating that increased volumes were required for excreting excess N in dairy cows. The ratio of urinary N to N intake tended to decrease from 37.4 to 33.6% comparing the LoRUP diet with the HiRUP diet. The fecal N excretion decreased significantly on the LoRUP diet compared with the HiRUP diet, but the ratio of fecal N to N intake was similar among the diets. St-Pierre and Thraen (1999) proposed using excreted N (feces N+urinary N) over milk N as a measure of environmental N efficiency. Decreasing the environmental N ratio has the potential of lowering the impact of dairy production on the environment. In the current study, environmental N ratio was lower for diets LoRUP-HiDRUP and LoRUP-HiDRUP+Met, with the greatest decrease occurring on the diet supplemented with Met. This indicates that feeding diets supplemented with RUP of low level and high digestibility could decrease the urinary and fecal N released into the environment. The result was consistent with Noftsger and St-Pierre (2003). In this trial, bacterial CP synthesis, estimated from purine derivatives, was not significantly different among the diets, probable due to the similar RDP and energy supply.

IMPLICATIONS

Under the current situation, lowering dietary RUP level while improving intestinal digestibility of RUP and supplementing with rumen escape sources of Met maintained similar milk production and allowed some increases in milk fat and protein concentration, indicating that post-ruminal digestibility of RUP and AA balance can be more important than total RUP supplementation. Estimates of environmental N efficiency indicate that lowering RUP while balancing AA supply properly in the small intestine can significantly decrease the amount of N released into the environment.

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