The Effect of Ruminal Bypass Lysine and Methionine on Milk Yield and Composition of Lactating Cows

S. XU,* J. H. HARRISON,*[,1] W. CHALUPA,† C. SNIFFEN,‡
W. JULIEN,§ H. SATO,‖ T. FUJIEDA,‖ K. WATANABE,‖
T. UEDA,‖ and H. SUZUKI‖
*Department of Animal Science,
Washington State University, Puyallup 98371
†Center for Animal Health and Productivity,
University of Pennsylvania, Kennett Square 19348
‡William H. Miner Agricultural
Research Institute, Chazy, NY 12921
§Julien & Assoc., Omaha, NE 68122
‖Ajinomoto Co., Inc., Tokyo, Japan

ABSTRACT

Fifty-six multiparous Holstein cows were assigned at 3 wk prepartum to rations based on grass silage with 1) corn distillers grains to provide 86 and 90% of estimated required metabolizable Lys and Met, respectively; 2) a blend of blood meal, fish meal, and meat and bone meal as amino acid (AA) sources to provide 112 and 103% of required metabolizable Lys and Met, respectively; 3) ruminally protected Lys and Met added as a top-dressing to ration 1 to provide 27 g/d of Lys and 8 g/d of Met as available AA at the duodenum postpartum; and 4) ruminally protected AA for 8 wk postpartum as a top-dressing to ration 1 to provide 40 g/d of Lys and 13 g/d of Met as available AA at the duodenum. Cows fed rations 3 and 4 were offered 13.5 g/d of duodenally available Lys and 4 g/d of duodenally available Met for 3 wk prepartum. The total length of the study was 43 wk.

Cows fed ration 4 consumed 3 to 4 kg more dry matter than did cows fed the other three rations, and milk yield and the percentage of milk protein and fat were significantly increased during the first 8 wk of lactation. In early lactation, cows fed ration 3 had a greater milk fat percentage but similar dry matter intake, protein percentage, and yield of 4% fat-corrected milk compared with cows fed ration 2. The concentrations of blood serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, triglyceride, and nonesterified fatty acids were lower for cows fed ration 4 during the first 8 wk of lactation than they were for cows fed the other three rations. The mammary arteriovenous difference of whole blood AA indicated that Met along with His and Arg may be the most limiting AA for milk yield.

(**Key words**: prepartum feeding, ruminal bypass amino acids, milk protein)

Abbreviation key: **BUN** = blood urea N, **CNCPS** = Cornell Net Carbohydrate and Protein System, **HRPLys + Met** = high amount of RP Lys plus Met, **MUN** = milk urea N, **NCR** = negative control ration, **PCR** = positive control ration, **RP** = ruminally protected, **SGOT** = serum glutamic oxaloacetic transaminase, **SGPT** = serum glutamic pyruvic transaminase, **TG** = triglyceride.

INTRODUCTION

Research (7, 8, 16, 21, 34, 37, 38, 39) on feeding strategies for high yielding dairy cows over the last two decades has focused on an increase in the energy density of the ration and an increase in the postruminal supply of limiting AA for postparturient cows.

The enhancement of energy density is a conventional approach to overcome low feed intake during the first few weeks of lactation. Intake depression may be initiated by metabolic disorders in the liver (18). Metabolic alteration in liver function may commence well before calving (46), and supplementation of necessary AA can mitigate such disorders by exporting excess triglycerides (**TG**) as very low density lipoproteins (22). Van Saun (44) observed fewer postpartum disorders when cows were fed higher concentrations of CP and RUP before calving.

Most research that has evaluated ruminally protected (**RP**) AA has concentrated on Lys and Met, which are commonly considered to be the first-limiting and second-limiting AA for milk protein synthesis (34, 38), especially when the proportion of corn grain or corn silage in the ration is substantial because of the poor content of Lys in these two products

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[1]Correspondence and reprint requests.

(38). The AA that limits milk protein synthesis most is variable and is largely dependent on the quality and quantity of AA in the basal ration. Grass silage is the main forage source for lactating dairy cows in the Pacific Northwest, and the application of RP AA to rations based on grass silage and fed to high yielding dairy cows has not been documented for this region.

Models such as the Cornell Net Carbohydrate and Protein System (**CNCPS**) offer a mechanistic approach to formulate rations based on AA requirements. This approach is gaining acceptance because of 1) possible reductions of unnecessary CP in the ration and thus the avoidance of excess excretion of N to the environment and 2) increased availability of feed characteristics needed for the CNCPS model.

The objectives of this research were to determine 1) the effects of sources of AA from plant origin versus those from animal origin and 2) the effects of RP Lys and Met on milk yield and composition of high yielding dairy cows fed rations based on grass silage.

MATERIALS AND METHODS

Cows and Dietary Formulation

Fifty-six multiparous Holstein cows were used in this study. Fourteen cows were assigned to each of four treatment rations at 3 wk before predicted calving date based on BW, body condition score, and PTA.

Two dry cow rations were used prepartum, which resulted in four dietary treatment groups. Cows that were to be fed the negative control ration (**NCR**) and the positive control ration (**PCR**) during lactation were fed rations consisting of 80% grass silage and oat hay plus either 20% of the NCR grain mix or 20% of the PCR grain mix during the last 3 wk of the dry period (Table 1). Cows that were to be fed two different concentrations of RP AA during lactation were fed the NCR prepartum supplemented with RP Lys and Met to supply 13.5 g/d of duodenally available Lys and 4 g/d of duodenally available Met. All cows were fed 455 g/d of a premix containing Se and vitamins A, D, and E. Corn was used as the carrier. The premix contained 111 IU/g of vitamin A, 48 IU/g of vitamin D, 2.2 IU/g of vitamin E, and 0.015 ppm of Se. After parturition, cows were fed transition rations for 2 wk. During this period, 2.3 kg of grain mix were substituted with oat hay (Table 1).

Two basal rations were formulated using the CNCPS (25, 35, 41); corn distillers grains (negative control) or blood meal, fish meal, and meat and bone meal (positive control) were the two bypass AA sources. The ingredients of the two basal rations and the main parameters predicted by the CNCPS are shown in Table 1. The PCR provided 112 and 103% of the requirements for metabolizable Lys and Met, respectively, and the NCR provided 86 and 90% of the requirements for metabolizable Lys and Met, respectively.

The four treatment rations were 1) NCR, 2) PCR, 3) NCR plus RP Lys and Met [Ajinomoto Co. Inc., Tokyo, Japan] (the RP Lys and Met were supplemented to the NCR to provide 27 g/d of Lys and 8 g/d of Met that would be available for absorption at the duodenum), and 4) NCR plus a high amount of RP Lys and Met (**HRPLys + Met**). The higher amount of RP Lys and Met was added to provide 40 g/d of Lys and 13 g/d of Met that would be available for absorption at the duodenum. Our hypothesis was that supplementation of Lys and Met in excess of the amounts that are required (based on the CNCPS) would meet the demand for the most limiting AA for maximal milk yield of the cow even though DMI is inevitably low during the first few weeks of lactation. The HRPLys + Met in ration 4 was supplemented up to 8 wk, and then cows were switched to ration 2 (PCR). Available metabolizable protein and energy for lactation and metabolizable Lys and Met from each treatment ration were estimated by the CNCPS based on actual weekly means of DMI, milk yield, percentages of milk fat and protein, BW, and body condition score during the first 8 wk of lactation.

The experiment lasted through 43 wk of lactation for cows fed rations 1, 2, and 3 and through 24 wk for cows fed ration 4. The index to terminate supplementation of RP Lys and Met for cows fed ration 3 was DMI and yield of 4% FCM, which were monitored weekly. When the prediction executed by the CNCPS indicated that AA (Lys and Met) from the NCR alone could sustain milk yield, the supplementation of RP Lys and Met was stopped. Cows were changed from the PCR or NCR to the midlactation ration (Table 1) when DMI or energy was no longer a constraint for milk yield, which was determined when the body condition score was ≥3 [five-point scale where 1 = thin to 5 = fat; (47)], when DMI was 20 to 22 kg/d, and when the yield of 4% FCM was <30 kg/d. Cows were fed the late lactation ration when the body condition score was ≥4 after cows were fed the midlactation ration (Table 1).

Feeding and Management

Cows were individually fed the TMR once daily through a Calan® headgate system (American Calan, Northwood, NH) both prepartum and postpartum. Feed offered and orts were recorded daily using an autofeeder. Preweighed RP Lys and Met were blended with an equal amount of grain mix and top-dressed immediately after the ration was offered. The consumption of RP Lys and Met was closely monitored by the staff, and any visual residual of RP Lys and Met was recorded as a percentage of the total amount offered. Cows were milked twice daily, and milk yield was recorded by a computer system. Cows were weighed weekly, and body condition scores were obtained weekly by the herd manager using a five-point scale (47). The health of the cows during the experiment was recorded, including the type of treatments, costs of medicine, and labor.

Sampling and Analytical Procedures

Total mixed rations were sampled weekly and composited monthly for analyses of N by the macro-Kjeldahl procedure (1) (wet samples) and for DM by drying in a forced-air oven (55°C). Dried samples were ground through 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and the contents of ash (1), NDF, ADF, and lignin (17) were determined. Orts were sampled every other week to determine DM.

Milk samples were taken weekly on Monday evening and Tuesday morning and were composited for infrared analyses of milk protein and milk fat by the regional DHI laboratory (Burlington, WA). At wk 4, 8, 12, 16, 20, 24, 28, and 32, milk samples (p.m. and a.m) from 10 cows fed each treatment ration were obtained to determine total milk N by macro-Kjeldahl (1), casein N (33), and milk urea N (**MUN**) using a colorimetic procedure (13).

Blood samples were taken from the coccygeal and mammary veins before the a.m. feeding at 2 wk

TABLE 1. Ingredients of rations for dry cows and cows in early, mid, and late lactation.

	Ration[1]							
Ingredient	DNCR	DPCR	ETNCR	ETPCR	NCR	PCR	Mid-lactation	Late lactation
	(% of DM)							
Grass silage	40	40	22.74	22.43	22.74	22.43	39.99	49.99
Alfalfa hay	. . .	. . .	15.16	14.95	15.16	14.95	10.00	10.00
Oat hay	40	40	4.30	4.30	. . .	. . .	. . .	. . .
Corn distillers grains, dry solubles	4.0	0.5	14.11	1.74	15.16	1.87	12.78	10.22
Soybean meal, 49% CP	0.73	1.4	2.65	5.23	2.84	5.61	2.40	1.92
Cracked corn	8.9	9.8	17.63	20.56	18.95	22.06	16.00	12.8
Barley meal, heavy	4.9	6.0	17.63	21.78	18.95	23.36	16.00	12.8
Megalac®[2]	0.73	0.72	2.65	2.62	2.84	2.80	. . .	. . .
Blood meal	. . .	0.34	. . .	1.21	. . .	1.31	. . .	. . .
Meat and bone meal	. . .	0.48	. . .	1.74	. . .	1.87	. . .	. . .
Fish meal[3]	. . .	0.24	. . .	0.88	. . .	0.93	. . .	. . .
Minerals and vitamins[4]	0.8	0.7	3.13	2.62	3.35	2.80	2.84	2.28
Predictions by the model[5]								
N, % of DM					2.80	2.98		
RDP, % of CP					53.3	54.9		
RUP, % of CP					46.7	45.7		
NE_L, Mcal/kg of DM					1.76	1.78		
MP[6] from Bacteria, g/d					1384	1487		
MP from Undegraded feed, g/d					1350	1585		
Lys Absorbed at the duodenum, g/d					154.0 (86%)[7]	196.1 (112%)		
Met Absorbed at the duodenum, g/d					52.2 (90%)	58.4 (103%)		

[1]DNCR = Dry cow negative control ration (NCR), DPCR = dry cow positive control ration (PCR), ETNCR = early transition NCR, and ETPCR = early transition PCR.

[2]Church and Dwight Co., Inc. (Princeton, NJ).

[3]Mixed fish meal (James Farrell & Co., Seattle, WA).

[4]Minerals in ETNCR, NCR, and mid and late lactation rations were (percentage of grain mix) 2.47% sodium bicarbonate, 1.83% limestone, 0.65% trace-mineralized salt, and 0.46% dicalcium phosphate. Minerals in ETPCR and PCR were (percentage of grain mix) 2.42% sodium bicarbonate, 1.34% limestone, 0.49% trace-mineralized salt, and 0.09% dicalcium phosphate. The concentration of vitamins was 26.8 IU/kg of vitamin E, 2247 IU/kg of vitamin A, 396 IU/kg of vitamin D, 0.27 ppm of Se premix (Ca carbonate, Fe oxide, mineral oil, and sodium selenite to provide 1600 ppm of Se in premix), and 4.29 ppm of Zn.

[5]Using the following assumptions: cow BW, 614 kg; body condition score, 3.5 [five-point scale where 1 = thin to 5 = fat; (47)]; milk fat, 3.2%; milk protein, 3.2%; milk yield, 47 kg/d; and DMI, 24 kg.

[6]Metabolizable protein.

[7]Values in parentheses are percentages of requirements.

prepartum and at 2, 8, and 32 wk postpartum. Time between sampling of the two blood vessels was <5 min for each cow. Blood samples from the coccygeal vein were analyzed for blood urea N (**BUN**), glucose, serum glutamic oxaloacetic transaminase (**SGOT**), serum glutamic pyruvic transaminase (**SGPT**), TG, and NEFA (Phoenix Central Laboratory, Everett, WA). Concentrations of individual AA in whole blood from the coccygeal and mammary veins were determined at wk 8 using postcolumn derivitization with *o*-phthalaldehye ion-exchange HPLC (Interaction Chromatograph, Inc., San Jose, CA) and fluorescence detection. Arteriovenous differences across the mammary gland were estimated (20).

Statistical Analysis

Statistical analysis was performed using the general linear models procedure of SAS (35). A completely randomized block design was used, and the model included treatment, block, cow within treatment and block, week, and the interaction of treatment and week. Cow within treatment and block was used to test the effect of treatment. Data were analyzed separately for the periods from 3 wk prepartum to parturition 1 to 8 wk postpartum, 9 to 16 wk postpartum, 17 to 24 wk postpartum, and 25 to 43 wk postpartum for all variables except blood analyses. Health data were analyzed by chi-square analysis according to Proc Freq of SAS (36). Significance was declared at $P < 0.05$ unless otherwise indicated.

RESULTS

Ration Composition

The chemical composition of the TMR averaged monthly over a period of 18 mo is shown in Table 2. The contents of DM, N, NDF, and ADF were mainly affected by the proportion of grass silage, alfalfa hay, or oat hay in the rations. There was about a 14 percentage unit variation in DM content (40 to 54%) because the grass silage constituted 50 to 22% of the rations. Nitrogen content averaged 2.4% of DM for the NCR and PCR fed to the dry cows, and the N content averaged 3.0 and 3.2% of DM for the NCR and the PCR, respectively. The concentrations of NDF and ADF fluctuated from dry cow rations to late lactation rations, which reflected the different proportions of forage in the rations at different stages of lactation. Ash contents were similar.

TABLE 2. Chemical composition of TMR.

	Ration[1]															
	DNCR		DPCR		ETNCT		ETPCR		NCR		PCR		Mid-lactation		Late lactation	
	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
DM, %	42.16	6.93	41.35	5.31	50.64	6.28	51.24	4.26	54.04	6.07	52.78	6.20	44.05	4.27	38.90	4.57
	(% of DM)															
N	2.42	0.28	2.36	0.27	2.75	0.25	2.97	0.19	3.00	0.26	3.18	0.29	2.94	0.38	3.03	0.45
NDF	50.21	2.08	47.91	4.4	37.88	3.16	37.60	2.90	31.90	3.52	31.31	2.99	38.24	3.45	38.64	3.02
ADF	30.85	2.02	29.49	1.39	22.43	3.59	21.16	2.21	17.75	3.15	17.52	1.66	23.46	5.06	22.56	0.64
Lignin	3.47	1.23	2.53	0.61	2.98	1.2	2.86	1.12	3.40	1.49	2.93	0.80	4.35	1.5	4.40	0.98
Ash	10.35	0.90	10.23	0.89	10.27	0.88	10.47	0.63	10.29	0.69	10.13	0.70	10.37	1.00	10.72	0.73
Macrominerals																
Ca	0.67		0.69		0.98		1.52		1.20		1.49		1.30		1.08	
K	2.31		2.22		1.92		1.78		2.11		1.73		2.29		2.39	
Mg	0.21		0.20		0.22		0.24		0.25		0.23		0.25		0.25	
Na	0.34		0.31		0.59		0.75		0.72		0.76		0.69		0.49	
P	0.41		0.40		0.49		0.55		0.53		0.56		0.49		0.48	
	(μg/g of DM)															
Microminerals																
B	6.5		6.8		7.6		8.9		8.4		8.6		12.4		13.1	
Cu	4		4		7		7		8		7		11		9	
Fe	335		263		270		272		318		271		202		276	
Mn	63		60		58		58		66		57		74		81	
Zn	46		38		84		81		111		86		96		75	

[1]DNCR = Dry cow negative control ration (NCR), DPCR = dry cow positive control ration (PCR), ETNCR = early transition NCR, and ETPCR = early transition PCR.

DMI, Milk Yield, Milk Components, and Feed Efficiency

Mean DMI and the N intake of cows fed the PCR and the NCR plus HRPLys + Met during the prepartum period were greater than those for cows fed the NCR alone and the NCR plus RP Lys and Met ($P < 0.05$) (Table 3). The DMI and N intake expressed as percentages of BW were also significantly higher for cows fed those two treatment rations.

Daily DMI increased quickly after calving (wk 1 to 8); cows fed the NCR plus HRPLys + Met consumed 3 to 4 kg more feed than did cows fed the other rations, which resulted in a higher N intake for these cows. Milk yield was greatest for cows fed the PCR and the NCR plus HRPLys + Met (39 kg/d), the milk yield of cows fed the NCR plus RP Lys and Met was intermediate (38 kg/d), and cows fed the NCR alone had the lowest milk yield (34 kg/d). Milk fat percentage was increased ($P < 0.05$) when cows were fed rations supplemented with RP Lys and Met, which enhanced yields of 4% FCM and milk fat. Milk protein percentage was significantly greater when cows were fed HRPLys + Met. However, this response was not observed when cows were fed the NCR plus RP Lys and Met. The yield of energy-corrected milk was a function of milk yield and milk fat and protein percentages. Cows fed the NCR plus HRPLys + Met, PCR, or the NCR plus RP Lys and Met yielded a greater amount of energy-corrected milk (42 kg/d) than did cows fed the NCR alone. The efficiency of DMI and N intake for 4% FCM or milk N was higher for cows fed the PCR or the NCR plus RP Lys and Met than for cows fed the NCR alone or the NCR plus HRPLys + Met during early lactation.

The DMI of cows reached a maximum at 3.5 to 3.7% of BW during wk 9 to 16, and the DMI and N intake (kilograms per day or percentage of BW) were similar across treatment rations (Table 4). Cows fed rations supplemented with RP Lys and Met tended to sustain milk fat percentage, which resulted in almost

TABLE 3. Dry matter intake and milk yield responses of cows fed treatment rations 3 wk prepartum to 8 wk postpartum (early lactation).

	Ration[1]					
	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met	SE	$P<$
3 wk Prepartum						
BW,[2] kg	732	714	750	723	20	NS[3]
DMI, kg/d	9.3^{b}	11.1^{a}	9.4^{b}	10.8^{a}	0.52	0.06
DMI, % of BW	1.3^{b}	1.6^{a}	1.3^{b}	1.5^{a}	0.07	0.04
NI,[4] g/d	236^{b}	276^{a}	227^{b}	284^{a}	15	0.03
NI, g/100 kg of BW	32^{b}	39^{a}	30^{b}	40^{a}	2.0	0.02
wk 1 to 8						
BW, kg	599	602	616	631	15	NS
DMI, kg/d	16.6^{b}	17.1^{b}	17.4^{b}	21.0^{a}	0.93	0.01
DMI, % of BW	2.8^{b}	2.9^{b}	2.8^{b}	3.3^{a}	0.14	0.02
NI, g/d	483^{b}	530^{b}	525^{b}	610^{a}	28	0.04
NI, g/100 kg of BW	80^{d}	89^{bc}	85^{cd}	98^{a}	4	0.16
Milk, kg/d	33.8^{c}	39.4^{ab}	37.5^{b}	39.0^{ab}	1.48	0.08
4% FCM, kg/d	31.6^{c}	36.0^{b}	36.5^{b}	38.0^{ab}	1.49	0.05
Fat, %	3.66^{bc}	3.56^{c}	3.98^{a}	3.96^{a}	0.14	0.15
Fat yield, kg/d	1.21^{d}	1.35^{c}	1.43^{bc}	1.50^{ab}	0.07	NS
Protein, %	3.06^{b}	3.07^{b}	3.06^{b}	3.29^{a}	0.03	0.01
Protein yield, kg/d	1.03^{d}	1.19^{bc}	1.14^{c}	1.27^{ab}	0.05	0.13
ECM,[5] kg/d	34.1^{b}	39.0^{a}	39.0^{a}	41.3^{a}	1.58	0.03
4% FCM/DMI, kg/kg	2.01^{b}	2.22^{a}	2.23^{a}	1.96^{b}	0.09	0.12
Milk N/NI, %	35.2	37.2	35.5	34.4	1.2	NS

[a,b,c,d]Means within rows with no common superscripts differ according to P values given.

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Mean BW.

[3]$P \geq 0.20$.

[4]Nitrogen intake.

[5]Energy-corrected milk (43).

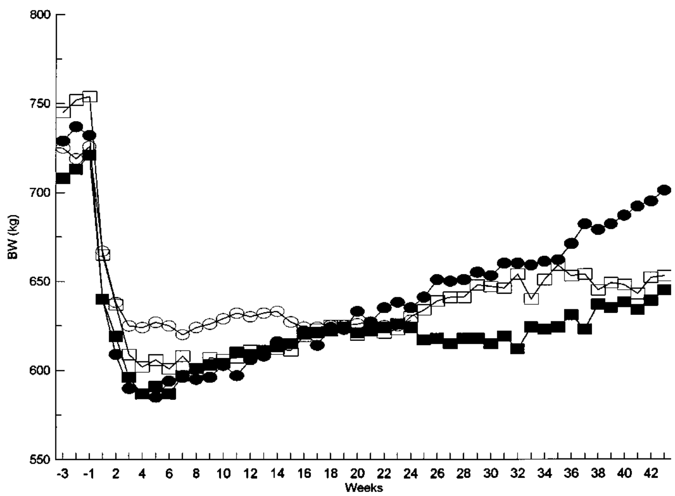

Figure 1. Change in BW during 43 wk of lactation. Pooled standard error = 21.6. Treatment rations: negative control ration (NCR), ●; positive control ration, ■; NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), □; and NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d), o. Cows fed the NCR plus HRPLys + Met were off of the experiment from wk 25 to 43 because of reasons unrelated to the experiment.

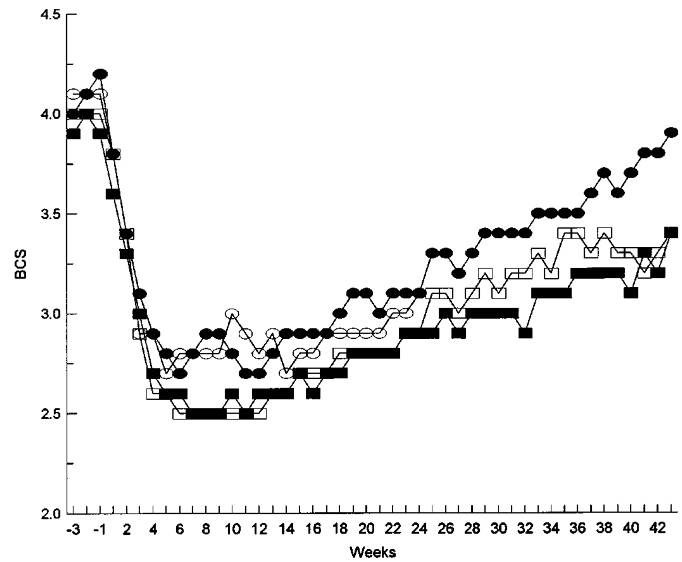

Figure 2. Change in body condition score [(BCS); five-point scale where 1 = thin to 5 = fat; (47)] during 43 wk of lactation. Pooled standard error = 0.21. Treatment rations: negative control ration (NCR), ●; positive control ration, ■; NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), □; and NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d), o. Cows fed the NCR plus HRPLys + Met were off of the experiment from wk 25 to 43 because of reasons unrelated to the experiment.

the same yield of 4% FCM as that of cows fed the PCR, although the latter had a numerically greater milk yield, but milk fat percentage was lower. Milk protein percentage was highest for cows fed the NCR plus HRPLys + Met, although cows in this group were fed the PCR from wk 9 to 16. Milk protein percentage did not increase when RP Lys and Met were supplemented during this period. The efficiency of DMI and N intake for 4% FCM and milk N decreased during this period as compared with that during early lactation.

During wk 17 to 24, cows generally gained BW with the exception of cows fed the NCR plus HRPLys + Met (Figures 1 and 2). Dry matter intake and N intake (Table 5) were similar to those of cows in peak lactation. The yields of milk and 4% FCM declined for all groups, but the yields for cows fed the PCR tended to decrease to a lesser extent. Milk protein yield and efficiency of transfer of feed N to milk N was greatest for cows fed the PCR during this period.

Cows continued to gain BW from wk 25 to 43, and DMI and N intake started to decrease (Table 6). Although not statistically different, cows fed the PCR and those fed the NCR plus RP Lys and Met yielded approximately 3 kg more milk, 4% FCM, and energy-corrected milk than did cows fed the NCR alone. The numerically higher milk yield coupled with equal intakes of DM resulted in a greater efficiency in conversion of DMI to 4% FCM for cows fed the PCR and the NCR plus RP Lys and Met.

Available Metabolizable Protein, Energy, Met, and Lys

Available metabolizable protein and energy for lactation and available metabolizable Met and Lys were estimated by the CNCPS (Tables 7 and 8). Intakes of metabolizable protein and energy from wk 1 to 8 were numerically highest for cows fed the NCR plus HRPLys + Met. The group fed the NCR plus RP Lys and Met had higher metabolizable protein intake than did the groups fed the PCR or the NCR alone, although the differences in metabolizable energy intake among these three groups were minimal. Cows fed the NCR plus HRPLys + Met had the greatest metabolizable Lys and Met intakes and consumed the required amount for milk yield during wk 1 and 2 after calving, respectively (Table 8). Cows fed the NCR plus RP Lys and Met had numerically higher metabolizable Met intake than did cows fed the PCR, although the difference in metabolizable Lys intake between the two groups was small. The group fed the NCR alone had the lowest metabolizable Lys and Met among the four treatment groups.

Concentrations of total milk N and casein N in early lactation were increased ($P < 0.05$) when cows were fed HRPLys + Met, and this increase was primarily attributed to casein N (Table 9). The group

TABLE 4. Dry matter intake and milk yield responses of cows fed treatment rations 9 to 16 wk postpartum (peak lactation).

	Ration[1]					
	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met	SE	*P*<
wk 9 to 16						
BW,[2] kg	608	611	610	629	14	NS[3]
DMI, kg/d	21.7	22.1	22.8	22.0	1.0	NS
DMI, % of BW	3.6	3.6	3.7	3.5	0.14	NS
NI,[4] g/d	632	670	673	647	30	NS
NI, g/100 kg of BW	104	110	110	102	4	NS
Milk, kg/d	37.0	43.3[ab]	41.1[b]	40.7[b]	1.34	0.06
4% FCM, kg/d	33.0[b]	37.5[a]	36.5[a]	36.0[a]	1.20	0.08
Fat, %	3.26	3.12	3.26	3.32	0.14	NS
Fat yield, kg/d	1.21	1.35	1.34	1.31	0.06	NS
Protein, %	3.09[bc]	3.01[cd]	2.98[d]	3.12[ab]	0.04	0.06
Protein yield, kg/d	1.14[c]	1.30[ab]	1.22[bc]	1.27[b]	0.04	0.05
ECM,[5] kg/d	36.0[b]	41.0[a]	39.6[a]	39.4[a]	1.26	0.05
4% FCM/DMI, kg/kg	1.52	1.75	1.64	1.75	0.08	NS
Milk N/NI, %	28.8	31.7	29.1	31.8	1.2	NS

[a,b,c,d]Means within rows with no common superscripts differ according to *P* values given.

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Mean BW.

[3]$P \geq 0.20$.

[4]Nitrogen intake.

[5]Energy-corrected milk (43).

TABLE 5. Dry matter intake and milk yield responses of cows fed treatment rations 17 to 24 wk postpartum (midlactation).

	Ration[1]					
	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met	SE	*P*<
wk 17 to 24						
BW,[2] kg	629	623	624	626	16	NS[3]
DMI, kg/d	22.6	21.3	22.2	20.0	1.2	NS
DMI, % of BW	3.6	3.4	3.5	3.1	0.18	NS
NI,[4] g/d	633	638	645	603	34	NS
NI, g/100 kg of BW	101	103	103	95	5	NS
Milk, kg/d	33.8[c]	39.8[ab]	36.1[bc]	34.2[c]	1.76	0.07
4% FCM, kg/d	30.1[b]	36.0[a]	32.6[b]	31.5[b]	1.39	0.08
Fat, %	3.27	3.39	3.39	3.60	0.14	NS
Fat yield, kg/d	1.10[c]	1.34[ab]	1.21[bc]	1.19[c]	0.06	0.06
Protein, %	3.23[a]	3.11[b]	3.12[b]	3.13[b]	0.04	0.05
Protein yield, kg/d	1.09[b]	1.24[a]	1.13[b]	1.07[b]	0.05	0.15
ECM,[5] kg/d	33.2[b]	39.3[a]	35.7[b]	34.3[b]	1.53	0.10
4% FCM/DMI, kg/kg	1.35[c]	1.81[ab]	1.51[bc]	1.68[bc]	0.10	0.05
Milk N/NI, g/g	27.4[b]	32.4[a]	28.0[b]	28.5[b]	1.50	0.07

[a,b,c,d]Means within rows with no common superscripts differ according to *P* values given.

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Mean BW.

[3]$P \geq 0.20$.

[4]Nitrogen intake.

[5]Energy-corrected milk (43).

TABLE 6. Dry matter intake and milk yield responses of cows fed treatment rations 25 to 43 wk postpartum (late lactation).

	Ration[1]					
	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met	SE	*P*<
wk 25 to 43						
BW,[2] kg	668	625	647	. . .	20	NS
DMI, kg/d	19.4	18.7	19.3	. . .	1.4	NS
DMI, % of BW	2.9	3.0	2.9	. . .	0.2	NS
NI,[3] g/d	575	594	561	. . .	40	NS
NI, g/100 kg of BW	87	95	86	. . .	5	NS
Milk, kg/d	24.5	27.5	27.6	. . .	1.93	NS
4% FCM, kg/d	23.2	26.1	26.3	. . .	1.94	NS
Fat, %	3.63	3.69	3.70	. . .	0.17	NS
Fat yield, kg/d	0.89	1.01	1.01	. . .	0.08	NS
Protein, %	3.37	3.28	3.30	. . .	0.06	NS
Protein yield, kg/d	0.83	0.89	0.91	. . .	0.07	NS
ECM,[4] kg/d	25.5	28.5	28.7	. . .	2.11	NS
4% FCM/DMI, kg/kg	1.19[b]	1.50[a]	1.43[a]	. . .	0.09	0.07
Milk N/NI, %	22.9	25.5	26.2	. . .	1.7	NS

[a,b]Means within rows with no common superscripts differ according to *P* values given.

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Mean BW.

[3]Nitrogen intake.

[4]Energy-corrected milk (43).

TABLE 7. Intakes of metabolizable protein and energy for lactation as estimated by the Cornell Net Carbohydrate and Protein System based on means of the first 8 wk of lactation.

Intake for lactation	Ration[1]							
	NCR		PCR		NCR Plus RP Lys + Met		NCR Plus HRPLys + Met	
Metabolizable protein, g/d								
wk 1	806	(57)[2]	1023	(69)	1145	(79)	1382	(84)
wk 2	944	(70)	1162	(78)	1266	(84)	1614	(96)
wk 3	1091	(84)	1407	(89)	1379	(97)	1962	(109)
wk 4	1265	(92)	1590	(92)	1552	(96)	2134	(110)
wk 5	1336	(90)	1616	(90)	1750	(103)	2246	(115)
wk 6	1480	(94)	1625	(92)	1870	(106)	2420	(126)
wk 7	1543	(98)	1806	(99)	1924	(107)	2460	(128)
wk 8	1617	(103)	1814	(100)	1990	(111)	2440	(126)
Metabolizable energy, Mcal/d								
wk 1	15.8	(46)	19.4	(55)	17.6	(44)	20.9	(52)
wk 2	20.6	(59)	23.2	(57)	21.8	(52)	28.1	(65)
wk 3	25.3	(72)	30.0	(75)	25.6	(65)	38.5	(88)
wk 4	30.6	(85)	34.9	(84)	30.9	(73)	43.4	(94)
wk 5	32.7	(91)	35.6	(83)	36.7	(86)	46.6	(98)
wk 6	36.9	(98)	35.9	(85)	40.2	(94)	51.3	(111)
wk 7	38.7	(100)	40.5	(92)	41.7	(93)	52.5	(116)
wk 8	40.8	(105)	40.7	(92)	43.6	(97)	51.9	(115)

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Values in parentheses are percentages of requirements.

TABLE 8. Intakes of metabolizable Met and Lys as estimated by the Cornell Net Carbohydrate and Protein System based on means of the first 8 wk of lactation.

	Ration[1]			
Lactation	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met
Metabolizable Lys, g/d				
wk 1	76.5 (68)[2]	100.3 (85)	104.9 (91)	123.7 (97)
wk 2	86.0 (76)	111.6 (92)	113.2 (93)	140.2 (102)
wk 3	96.1 (85)	130.4 (99)	120.7 (100)	164.1 (107)
wk 4	108.1 (89)	144.4 (100)	132.7 (98)	175.8 (106)
wk 5	113.0 (87)	146.5 (98)	146.5 (101)	183.2 (109)
wk 6	123.1 (89)	147.1 (100)	154.5 (102)	194.8 (114)
wk 7	127.4 (91)	161.2 (104)	158.4 (102)	197.3 (114)
wk 8	132.5 (93)	161.9 (105)	162.6 (104)	196.0 (113)
Metabolizable Met, g/d				
wk 1	26.2 (72)	30.7 (80)	35.2 (93)	41.3 (100)
wk 2	29.4 (80)	34.1 (86)	38.0 (96)	46.9 (105)
wk 3	32.8 (89)	39.5 (91)	40.6 (103)	54.9 (110)
wk 4	36.8 (92)	43.5 (92)	44.6 (101)	58.8 (109)
wk 5	38.5 (91)	44.1 (91)	49.2 (104)	61.3 (111)
wk 6	41.9 (93)	44.3 (92)	51.9 (105)	65.1 (116)
wk 7	43.3 (94)	48.4 (96)	53.2 (105)	66.0 (117)
wk 8	45.0 (97)	48.6 (96)	54.6 (107)	65.5 (116)

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Values in parentheses are percentages of requirements.

fed the NCR plus RP Lys and Met did not differ in total milk N and casein N from the groups fed the NCR alone or the PCR; however, the concentration of MUN was about 2 percentage units higher for cows fed the NCR plus RP Lys and Met than for cows fed the NCR alone during early lactation. Cows fed the PCR had a lower concentration of casein N and a higher concentration of MUN than did cows fed the NCR alone, although total milk N percentage was similar between cows fed the two treatment rations. No differences in milk N constituents were detected across treatment rations during peak lactation. During mid and late lactation, MUN was significantly higher for cows fed the PCR than for cows fed the NCR alone or the NCR plus RP Lys and Met.

Blood Enzymes and Metabolites

Concentrations of SGOT, SGPT, and glucose were similar across treatment rations at wk 2 prepartum (Table 10). The BUN was higher ($P < 0.05$) for cows fed the PCR than that for cows fed the NCR plus HRPLys + Met or that for cows fed the NCR plus RP Lys and Met. The concentration of TG was not significantly different across treatment rations, except that cows fed the NCR plus HRPLys + Met had remarkably lower concentrations at wk 2 prepartum. The concentration of NEFA varied from 380 to 630 meq/L, and no significant difference was detected. Concentrations of BUN, TG, and NEFA in cows fed the NCR plus HRPLys + Met tended to be lower numerically than those of cows fed the other treatment rations at wk 2 postpartum. The only significant difference detected at wk 8 postpartum was for BUN; cows fed the PCR had a higher concentration of BUN than did cows fed the other treatment rations. The same ranking of treatment rations was observed at wk 32 postpartum; however, the magnitude was larger.

AA Concentration in Whole Blood

At 8 wk postpartum, the concentrations of whole blood AA (Table 11) were different among treatment rations for Leu, Lys, Met, Glu, and Tyr. Coccygeal concentrations of Lys were highest when cows were fed rations supplemented with RP Lys at either concentration of supplementation. The coccygeal concentration of Met was numerically greatest for cows fed the ration supplemented with HRPLys + Met when compared with the Met concentration for cows fed the NCR, and was statistically greater than that for cows fed the PCR and the NCR plus RP Lys and Met. As was observed for coccygeal measurements, the concentrations of Lys in the mammary vein were greatest when cows were fed rations supplemented with RP AA; concentrations of Met in the mammary

TABLE 9. Mean concentration of milk N constituents at different stages of lactation.

	Ration[1]				
	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met	SE
Early lactation[2]					
Milk total N, g/L	5.25[b]	5.18[b]	5.26[b]	5.94[a]	0.13
Milk casein N, g/L	4.07[b]	3.83[b]	4.02[b]	4.53[a]	0.12
Casein N/total N, %	77.67	73.95	76.59	76.71	1.60
MUN,[3] mg/dl	13.0[b]	15.95[a]	15.35[a]	13.30[b]	0.58
Peak lactation[4]					
Milk total N, g/L	5.55	5.59	5.44	5.50	0.16
Milk casein N, g/L	4.18	4.15	4.17	4.31	0.14
Casein N/total N, %	75.22	74.44	76.70	78.36	1.62
MUN, mg/dl	13.85	16.10	14.05	15.00	0.68
Midlactation[5]					
Milk total N, g/L	5.92	5.52	5.66	5.57	0.18
Milk casein N, g/L	4.46	4.08	4.29	4.38	0.15
Casein N/total N, %	75.52	73.98	75.96	78.58	1.42
MUN, mg/dl	12.95[b]	17.05[a]	13.70[b]	18.30[a]	0.65
Late lactation[6]					
Milk total N, g/L	5.73	5.45	5.79	. . .	0.17
Milk casein N, g/L	4.29	4.06	4.49	. . .	0.18
Casein N/total N, %	74.95	74.33	77.62	. . .	1.63
MUN, mg/dl	14.45[b]	18.30[a]	14.10[b]	. . .	0.68

[a,b]Means within rows with no common superscripts differ ($P < 0.05$).

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Values were averaged from 10 cows fed each treatment ration at wk 4 and 8.

[3]Milk urea N.

[4]Values were averaged from 10 cows fed each treatment ration at wk 12 and 16.

[5]Values were averaged from 10 cows fed each treatment ration at wk 20 and 24.

[6]Values were averaged from 10 cows fed each treatment ration at wk 28 and 32. Cows fed the NCR plus HRPLys + Met were off of the experiment from wk 25 to 43 because of reasons unrelated to the experiment.

vein were greatest for cows fed the NCR plus HRPLys + Met and the NCR alone. Arteriovenous differences (Table 12) across the mammary gland were not statistically different for any AA; however, negative differences were noted for Arg, His, and Met (essential AA) and for Ala, Asp, Glu, Gly, and Ser (nonessential AA).

Record of Health and Dietary Shifts

The health status of cows during the first 24 wk of lactation was recorded (Table 13). In the group fed the NCR alone, 5 cows were sick during wk 2 prepartum to 3 wk postpartum, and 2 cows were sick during wk 4 to 24. The distribution of clinical ailments was 50% for metritis and 20% for mastitis; other problems contributed 30%. Three cows were sick in each of two comparison periods in the group fed the PCR. One cow suffered milk fever immediately after calving, and 4 cows had mastitis during wk 4 to 24 in the group fed the NCR plus HRPLys + Met. Six cows were sick during wk 2 prepartum to wk 3 postpartum, and 2 cows were sick during wk 4 to 24 in the group fed the NCR plus RP Lys and Met.

The shift in treatment rations as lactation advanced and the termination of the supply of RP Lys and Met were dependent on the performance of the cow, which was monitored weekly based on the criteria mentioned previously. Seventy-nine percent of cows fed the NCR alone were shifted to the midlactation ration at wk 27 and 31; 88% of cows in this group were fed the late lactation ration (Table 14). Sixty-nine percent of cows fed the PCR were shifted to the midlactation ration at wk 32, and 30% of cows in this group were fed the late lactation ration at wk 39. Cows fed the NCR plus RP Lys and Met had ceased receiving RP Lys and Met and were shifted to the midlactation ration at wk 26, and 26% of cows were fed the late lactation ration at wk 39.

DISCUSSION

DMI, Milk Yield, Milk Components, and Feed Efficiency

Most of the studies in the literature associated with RP AA in the ration start either a few weeks after parturition or during midlactation (5, 8, 9, 29, 30). Furthermore, the importance of dietary energy density in the fresh cow ration has been heavily emphasized while other nutrients in the ration for early prepartum cows have been neglected (6, 10). The feeding strategy of this study employed the supplementation of RP Lys and Met to the NCR in contrast to the PCR at 3 wk prior to calving. Results indicated that cows fed the NCR plus 13.5 g/d of duodenally available Lys and 4 g/d of duodenally available Met had similar DMI as cows fed the PCR, which included high quality bypass protein from animal origins.

Maximum feed intake lags behind milk yield during the first few weeks of lactation. The conventional feeding strategy is to increase energy density per unit of feed (7). The remarkably high DMI of cows fed the NCR plus HRPLys + Met from wk 1 to 8 in this study indicated that the energy density in the feed was not the only factor that affected DMI during the first few weeks of lactation and suggested that additional AA

TABLE 10. Blood parameters (2 wk before calving and 2, 8, and 32 wk after calving) of cows fed different treatment rations.

	Ration[1]				
	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met	SE
wk 2 Before calving					
SGOT,[2] IU/L	63.00	61.07	60.07	62.86	5.20
SGPT,[3] IU/L	15.71	15.93	15.07	16.00	0.82
BUN,[4] mg/dl	10.98[ab]	12.71[a]	10.14[b]	10.79[b]	0.70
Blood glucose, mg/dl	57.25	60.64	59.36	59.93	2.25
TG,[5] mg/dl	101.29[a]	88.54[a]	113.29[a]	31.93[b]	16.09
NEFA, meq/L	629.29	447.29	543.14	379.36	89.42
wk 2 After calving					
SGOT, IU/L	145.43	133.79	125.75	96.07	20.37
SGPT, IU/L	18.36	15.14	17.00	15.00	1.71
BUN, mg/dl	10.07	11.93	10.50	9.43	0.84
Blood glucose, mg/dl	49.17	50.54	47.73	51.86	3.28
TG, mg/dl	76.00	71.43	75.75	42.00	16.40
NEFA, meq/L	1307.86	1193.00	1335.67	1048.20	187.85
wk 8 After calving					
SGOT, IU/L	64.67	69.20	69.40	65.60	3.40
SGPT, IU/L	20.07	19.33	19.20	21.80	1.09
BUN, mg/dl	10.00[b]	13.53[a]	11.87[b]	11.20[b]	0.57
Blood glucose, mg/dl	63.73	60.73	61.60	64.13	1.25
TG, mg/dl	56.80	61.53	56.47	27.13	13.61
NEFA, meq/L	386.27	471.00	478.53	406.50	73.22
wk 32 After calving[6]					
SGOT, IU/L	84.50	72.29	75.77	. . .	4.97
SGPT, IU/L	27.00	25.86	28.77	. . .	2.04
BUN, mg/dl	11.71[b]	15.79[a]	11.88[b]	. . .	0.86
Blood glucose, mg/dl	64.18	64.71	66.58	. . .	1.65
TG, mg/dl	42.79	74.14	40.46	. . .	18.29
NEFA, meq/L	323.36	351.93	338.36	. . .	23.69

[a,b,c]Means within rows with no common superscripts differ ($P < 0.05$).

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Serum glutamic oxaloacetic transaminase.

[3]Serum glutamic pyruvic transaminase.

[4]Blood urea N.

[5]Triglyceride.

[6]Cows fed the NCR plus HPRLys + Met were off of the experiment from wk 25 to 43 because of reasons unrelated to the experiment.

are necessary for high yielding lactating dairy cows. Cows fed the PCR and the NCR plus RP Lys and Met presumably were well balanced for AA and seemed to mobilize more body fat than did the cows fed the NCR alone. Studies (3, 12) have confirmed that the cow mobilizes more fat than protein in the first few weeks of lactation.

The general expectations of the inclusion of RP Lys, Met, or a combination of these in the ration are that milk yield and milk protein content and yield will increase (9, 29, 37, 38). However, responses have been inconsistent (8, 26). Obviously, many other factors interact in this complex process. Among them, source of protein may play a very significant role (24). The positive responses of milk yield and milk protein content were consistent through different stages of lactation in this study when cows were fed RP Lys and Met. Supplementation of RP Lys and Met also had a positive impact on milk fat content during the first 8 wk of lactation as reported previously (5).

Available Metabolizable Protein, Energy, Met, and Lys

Increased intake of available metabolizable protein and energy for lactation and of metabolizable Lys and Met as estimated by the CNCPS was the most plausible explanation for the yield response in the first 8 wk of lactation. The increased intakes of metabolizable protein and energy by cows fed the NCR plus HRPLys + Met compared with that by cows in the other three groups was caused mainly by higher DMI; therefore, cows mobilized fewer body reserves (Figures 1 and 2). Cows fed the NCR plus RP Lys and Met and cows fed the PCR mobilized more body reserves for milk yield because of a lower DMI.

Milk N Constituents

The inclusion of RP limiting AA in the ration has increased milk casein N content, which resulted in an increase in total milk N content in conjunction with no change in milk NPN content (40). Similar findings were evident in this study when milk casein N was elevated >0.5 g/L and when total N was elevated >0.7 g/L without alteration of MUN when the rations of cows were supplemented with HRPLys + Met in early lactation. Supplementation of RP Lys and Met based on requirements did not alter milk casein N content in this study. A possible explanation is that cows were still in a negative energy balance because

TABLE 11. Concentrations of AA in whole blood from the coccygeal (C) and mammary (M) veins at 8 wk postpartum.

	Ration[1]									
	NCR		PCR		NCR Plus RP Lys + Met		NCR Plus HRPLys + Met			
AA	C	M	C	M	C	M	C	M	SE[2]	SE[3]
	(nmol/ml)									
Essential										
Arg	9.0	5.8	3.8	1.4	3.9	3.6	4.9	2.1	1.69	1.8
His	24.2	32.4	23.7	28.3	29.4	33.0	19.4	18.1	4.0	8.9
Ile	56.8	36.9	55.5	34.1	52.7	26.6	46.3	30.6	5.1	4.7
Leu	80.2[bc]	53.3[cd]	96.1[ab]	65.4[ab]	98.1[ab]	55.8[bc]	67.4[c]	45.0[d]	5.7	4.0
Lys	59.4[d]	45.9[d]	66.2[cd]	50.0[cd]	113.2[a]	73.3[ab]	86.2[bc]	64.2[bc]	9.7	6.5
Met	13.8[b]	16.9[a]	7.1[c]	4.9[b]	7.5[c]	7.5[b]	17.6[ab]	19.0[a]	2.24	3.2
Phe	38.9	28.4	31.8	23.5	36.0	7.5	30.4	21.7	3.2	2.7
Thr	96.2	90.7	72.5	53.9	87.4	66.3	66.7	57.0	9.4	13.4
Val	144.2	137.1	155.5	134.9	158.4	114.8	131.4	113.1	12.0	11.5
Nonessential										
Ala	173.6	183.7	319.7	363.6	444.7	123.6	144.4	231.0	148.7	112.6
Asp	131.3	146.2	137.2	135.0	142.1	163.9	137.2	127.9	27.1	27.0
Glu	462.7[b]	448.6	467.0[b]	465.7	665.3[a]	455.7	424.5[b]	394.7	46.3	63.6
Gly	338.3	403.0	450.1	520.1	656.8	426.0	304.2	313.7	118.4	96.5
Ser	111.0	107.6	108.2	109.0	138.2	109.2	85.7	70.9	12.8	18.9
Tyr	32.2[ab]	23.8[ab]	20.2[c]	14.3[c]	18.7[c]	10.9[c]	24.9[bc]	18.5[bc]	3.4	3.1

[a,b,c,d]Means within rows and sample type (C or M) with no common superscripts differ ($P < 0.05$).

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Standard error for coccygeal samples.

[3]Standard error for mammary samples.

of the lower DMI in early lactation (Table 3) and the priority of partition of RP Lys and Met was to the glucogenic pathway (Met) or to the ketogenic pathway (Lys) rather than to milk protein synthesis.

MUN

Variation of MUN across treatment rations and throughout lactation in this study ranged from 13 to 18 mg/dl and was in general agreement with previous research (2, 27, 32). The greatest amount of MUN was observed for cows fed the PCR at any stage of lactation except midlactation. Treatment differences in MUN can be related to differences in DMI, CP intake, and rations, which would be effective in supplying first-limiting AA (2, 32).

BUN

The concentration of BUN reflects the status of dietary CP such as the percentage of CP, digestibility of CP, and the energy level of a ration (32). A strong correlation exists among dietary CP, BUN, and MUN (2, 32). In most reports, the concentration of BUN is higher than that of MUN, but opposite results have been reported (31), which are in agreement with the results of this study. Gustafsson and Palmquist (19) delineated the relationship that BUN reached a peak concentration at 1 to 2 h after ruminal ammonia reached its maximal concentration, and MUN peaked 1 h later than did BUN. Therefore, the discrepancy between experiments may be simply explained by the variation of sampling time. Blood samples were taken before the a.m. feeding in our study, which contributed to a lower BUN than MUN concentration. The trend for a greater BUN concentration was consistent with MUN when cows were fed the PCR compared with that of cows fed the other rations (Tables 9 and 10).

Blood Enzymes and Metabolites

A number of blood constituents are useful as monitors for clinical or subclinical signs of metabolic disorders in high yielding cows around parturition. The elevation of SGPT and SGOT may indicate an accumulation of FFA transported from blood to hepatocytes (42). A high concentration of SGOT can also imply damages in organs other than the liver because SGOT exists in muscle, kidney, intestine, and brain. The two enzymes in this study did not differ across treatment rations. However, the concentration of both enzymes fluctuated greatly at different stages of lactation ($P < 0.05$; Table 10). For example, the SGOT increased 1 wk after calving, but, by 8 wk after calving, the enzyme concentration returned to precalving concentrations. West (46) suggested that

TABLE 12. Mammary arteriovenous differences of whole blood AA for cows fed different treatment rations at wk 8 postpartum.

	Ration[1]				
AA	NCR	PCR	NCR Plus RP Lys + Met	NCR Plus HRPLys + Met	SE
	(nmol/ml)				
Essential					
Arg	2.57	0.40	–1.86	0.64	4.64
His	–8.81	–2.54	0.02	–2.40	8.37
Ile	19.11	18.42	25.52	13.49	8.26
Leu	27.05	22.66	33.96	19.84	8.36
Lys	14.73	13.34	23.58	27.99	11.18
Met	–2.78	–2.54	2.20	–1.47	5.94
Phe	8.72	5.91	14.15	10.11	5.52
Thr	6.46	7.26	20.27	3.39	19.30
Val	8.41	15.55	40.09	13.52	14.77
Nonessential					
Ala	–11.82	–3.71	26.65	20.84	30.76
Asp	–14.96	8.17	59.18	5.18	33.74
Glu	20.76	–2.85	117.32	35.10	66.91
Gly	–62.80	–34.16	52.13	–8.83	50.44
Ser	2.92	–6.21	31.72	14.31	24.15
Tyr	6.81	4.54	9.13	7.28	5.67

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

TABLE 13. Health record of cows from 3 wk prepartum to 24 wk postpartum.

	Ration[1]			
	NCR	PCR	NCR + RP Lys + Met	NCR + HRPLys + Met
Sick cows, no.				
wk –2 to 3	5	3	6	1
wk 4 to 24	2	3	2	4
Total	7	6	8	5
% of Group	47	40	53	33
Clinical signs,[2] % of all cows				
Displaced abomasum	6.6	6.6	13.3	0
Foot rot	0	13.3	6.6	0
Ketosis	6.6	0	20	0
Mastitis	13.3	0	6.6	26.7
Metritis	26.7	13.3	26.7	0
Milk fever	0	0	0	6.6
Off feed	0	6.6	0	0
Unknown	6.6	0	0	0

[1]NCR = Negative control ration, PCR = positive control ration, NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d), and NCR plus HRPLys + Met = NCR plus high amount of RP Lys (40 g/d) plus Met (13 g/d).

[2]Clinical signs were based on diagnosis by an assigned veterinarian.

a rise in SGOT immediately after calving might reflect muscle damage.

Blood NEFA and liver TG are correlated in the peripartum period (r = 0.46) (42), and the increased lipolysis is hormonally regulated and not entirely caused by energy deficit or DMI depression (7). Vaquez-Anon et al. (45) concluded that plasma NEFA triggered an accumulation of TG in the liver. Roberts and Reid (28) suggested that the reduction in TG secretion might be related to suboptimal apolipoprotein production in the liver and might be caused by an inadequate supply of necessary AA. This finding has been confirmed by a report (15) that Lys plus Met (20 and 10 g/d, respectively) stimulated transportation of lipoproteins that were rich in TG from the liver to adipose tissue. However, that conclusion was based on data from only two cows. Supplementation of RP Lys and Met significantly decreased TG prepartum in one group, but did not change concentrations in another group, which is difficult to explain because they were fed the same ration and equal amounts of RP Lys and Met. The variation in DMI between the two groups might have

TABLE 14. Inventory of cows that shifted to an alternative ration and termination of the supply of ruminally protected AA.

Ration[1]	Shift to alternative ration			DMI	Milk	4% FCM	BCS[2]
	(wk)		(% of cows)	(kg/d)			
	$\overline{X}$	SD					
NCR							
Mid[3]	27	7	79	25.1	25.9	22.8	4.0
Rest of group	. . .		21	24.4	35.6	35.4	2.5
Late[4]	31	9	88	18.9	23.2	20.9	4.0
Rest of group	. . .		12	22.5	30.5	29.3	3.0
PCR							
Mid	32	8	69	18.9	25.7	24.6	3.5
Rest of group	. . .		31	16.1	32.8	30.9	2.5
Late	39	6	30	17.8	25.0	24.2	4.0
Rest of group	. . .		70	17.1	22.0	20.9	3.0
NCR Plus RP Lys + Met							
Off RP Lys + Met[5]	26	8	. . .	21.0	32.4	29.1	3.0
Mid	26	7	36	23.8	32.7	30.2	3.25
Rest of group	. . .		64	23.9	36.0	32.9	3.25
Late	39	6	20	22.2	22.9	20.5	4.0
Rest of group	. . .		80	16.9	24.5	24.1	3.0

[1]NCR = Negative control ration, PCR = positive control ration, and NCR plus RP Lys + Met = NCR plus ruminally protected (RP) Lys (28 g/d) and Met (8 g/d).

[2]Body condition score. Scored on a five-point scale where 1 = thin to 5 = fat (47).

[3]Midlactation ration.

[4]Late lactation ration.

[5]Termination of the supply of RP Lys + Met.

contributed to the difference. The group supplemented with HRPLys + Met had a significantly higher DMI and numerically lower TG and NEFA at wk 2 and 8 postpartum compared with the DMI and TG and NEFA concentrations of cows in the other groups. Blood glucose in this study was fairly consistent across treatment rations and weeks and fell in a normal physiological range between 40 and 60 mg/dl (11).

AA Concentration in Whole Blood

The uptake of AA by the mammary gland from blood plasma may quantitatively underestimate AA supply from whole blood because erythrocytes have a role in the transport of AA (20). The concentration of Lys (Table 11) responded to supplementation of RP Lys and Met, and the arteriovenous difference for Lys was greatest when RP Lys and Met were supplemented. However, there was only a marginal elevation in Met caused by supplementation, which might have been due to the fast removal rate of Met by the mammary gland (23). Arteriovenous differences (Table 12) for Arg and His were also negative, which might indicate that they were taken up by the mammary gland with maximal efficiency (23). Therefore, Met, His, and Arg presumably were the most limiting AA in this study. This conclusion may be sound because 1) grass silage rather than corn silage was the main forage in the basal ration of this study [Met rather than Lys was the primary limiting AA (38)], 2) companion metabolism trials to this study with early and midlactation cows (4, 48) consistently showed similar yield responses and blood AA profiles to AA supplementation, and 3) Met is transferred from blood to milk without alteration (14). Arteriovenous differences in nonessential AA in this study were variable, which were in accordance with the finding (23) that uptakes of nonessential AA vary considerably and frequently are deficit in uptake. The use of blood AA to calculate arteriovenous differences from a single time point and without estimates of blood flow should be performed with caution.

Health Status

No measure of health status (Table 13) was significantly different among treatment rations; however, incidence of ketosis and metritis approached statistical significance ($P < 0.09$ and $P < 0.12$, respectively). Although no statistical differences were noted for any given health category, the overall incidence of health-related disorders was numerically lowest for cows fed the NCR plus HRPLys + Met.

CONCLUSIONS

Supplementation of RP Lys and Met to the ration based on grass silage and corn distillers grains yielded similar and positive responses compared with the ration based on blood meal, fish meal, and meat and bone meal. Cows fed these treatment rations had similar DMI, milk protein percentages and yields, and yields of 4% FCM for the first 8 wk of lactation. These responses were even greater when cows were offered about 50% more RP Lys and Met than that projected to be required. Increased milk protein percentage was due to the increased milk casein N fraction and not increased MUN. High concentrations of AA (40 g of Lys and 13 g of RP Lys and Met) in the rations during early lactation (wk 1 to 8) may reduce the risk of metabolic disorders. According to AA concentrations in whole blood and milk yield responses, Met seemed to be limiting, and Lys was colimiting, for milk yield when cows were fed rations based on grass silage. Application of models like the CNCPS to formulate prepartum and postpartum rations increases yields of milk and milk protein and may benefit the health of cows.

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