Effects of Rumen-Undegradable Protein Sources and Supplemental 2-Hydroxy-4-(Methylthio)-Butanoic Acid and Lysine·HCl on Lactation Performance in Dairy Cows

L. M. Johnson-VanWieringen,* J. H. Harrison,* D. Davidson,* M. L. Swift,† M. A. G. von Keyserlingk,‡ M. Vazquez-Anon,§ D. Wright,|| and W. Chalupa#

*Department of Animal Sciences, Washington State University, Puyallup 98371

†Abbotsford Veterinary Clinic, Abbotsford V2S 5Z5, Canada

‡The University of British Columbia, Vancouver V6T 1Z6, Canada

§Novus International, St. Louis, MO 63141

||H. J. Baker Company, Stamford, CT 06901

#University of Pennsylvania, Kennett Square 19348

ABSTRACT

One hundred primiparous and multiparous Holstein cows were used in an experiment to evaluate the effect of supplementing diets with either a plant- or an animal-based source of rumen-undegradable protein (RUP), with or without AA supplementation, during the transition period and early lactation on milk production response. The experimental design was a randomized block design with approximately one-third of the cows being primiparous. Cows were assigned to 1 of 4 prepartum diets introduced 3 wk before the expected calving date and switched to the corresponding postpartum diet at calving. Diets 1 (AMI) and 2 (AMI+) included a vegetable RUP source (heat- and lignosulfonate-treated canola meal), with diet 2 containing supplemental Lys·HCl and Met hydroxy analog sources [D,L-2 hydroxy-4-(methylthio)-butanoic acid; Alimet feed supplement]. Diets 3 (PRO) and 4 (PRO+) consisted of a blend of animal RUP sources (blood meal, fish meal, feather meal, and porcine meat and bone meal), with diet 4 containing supplemental Lys·HCl and Met hydroxy analog sources [D,L-2 hydroxy-4-(methylthio)-butanoic acid; Alimet]. During the first 4 wk of lactation, dry matter intake was less when synthetic Lys·HCl and Alimet were supplemented, but this effect was no longer evident in wk 5 to 9 of the experiment. Interestingly, despite the initial decrease in dry matter intake in the cows fed AA-supplemented diets, there was no effect of treatment on milk production or the ratio of fat-corrected milk to dry matter intake throughout the 17 wk of the study. Undegradable protein source (vegetable vs. animal) did not affect dry matter intake, milk pro-

duction, or 3.5% fat-corrected milk production for the first 17 wk of lactation. The results of this study indicate that heat- and lignosulfonate-treated canola meal can be used as a source of undegradable protein in place of high-quality rumen-undegradable animal protein sources without negative effects on milk production when diets are equivalent in rumen degradable protein, RUP, and metabolizable Met and Lys. Despite other reports citing clear benefits to feeding supplemental synthetic Lys or Met in diets fed to high-producing lactating dairy cows, we were unable to provide additional evidence to support these findings. Additionally, there was a trend for whole-blood Lys concentrations to be greater for diets supplemented with Lys·HCl.

Key words: amino acid, rumen-undegradable protein, methionine, lysine

INTRODUCTION

Advances in dairy cattle nutrition in the last 20 yr include balancing dairy cattle diets for the correct amounts of the 2 most limiting AA, Lys and Met, to enable sufficient amounts to leave the rumen undegraded and be absorbed in the small intestine of high-producing lactating dairy cattle rations (Schwab et al., 1992). Two common methods used to achieve balanced diets for the correct amounts of rumen-undegradable Lys and Met are to incorporate animal and vegetable protein sources that are highly undegradable in the rumen but available for absorption in the small intestine, or to supplement synthetic Lys and Met sources that will leave the rumen undegraded and be absorbed across the small intestine. Numerous experiments have evaluated the benefits of adding different types and forms of animal and vegetable protein sources and synthetic Lys and Met sources to the diet to improve milk and milk component production. Unfortunately, the variable responses from previous research include no

Received November 7, 2006.

Accepted July 23, 2007.

¹Corresponding author: jhharrison@wsu.edu

differences in DMI (Rode et al., 1998; Liu et al., 2000; Piepenbrink et al., 2004), varying results in milk production response (Xu et al., 1998; Piepenbrink et al., 2004; Socha et al., 2005), improved milk fat content (Rode et al., 1998), increased milk protein content (Nichols et al., 1998; Socha et al., 2005; St-Pierre and Sylvester, 2005), and increased milk protein yield (Nichols et al., 1998; Rode et al., 1998; Socha et al., 2005).

No work to date has incorporated addition of both different types and forms of animal and vegetable protein sources and synthetic Lys and Met sources. In an experiment by Wright et al. (2005), cows fed diets formulated with heat- and lignosulfonate-treated canola meal had significantly greater milk yields than cows fed untreated canola meal. In addition, ruminal ammonia N, BUN, urinary N (as a percentage of N intake), and MUN were reduced in cows fed diets supplemented with heat- and lignosulfonate-treated canola meal (Wright et al., 2005), indicating that treating canola meal with heat and lignosulfonate is an effective method of increasing the proportion of CP digested in the lower tract. In an experiment by Xu et al. (1998), milk production was significantly greater for cows fed diets supplemented with a commercially available blend of animal protein sources than a control diet balanced with corn distillers grains in wk 1 through 24 of lactation. Energy-corrected milk was also significantly greater for the cows fed diets supplemented with a commercially available blend of animal protein sources than a control diet from wk 9 through 24 of lactation (Xu et al., 1998).

There has been a trend in recent years for most countries to order the removal of animal-based protein sources in ruminant feeds. This increases the need for additional plant protein sources, such as heat- and lignosulfonate-treated canola meal (e.g., Amipro, Agro Pacific Industries Ltd., Chilliwack, British Columbia, Canada; Wright et al., 2005), to be evaluated as potential replacements of animal protein sources that have traditionally been used as RUP supplements. The main objective of this experiment was to determine whether heat- and lignosulfonate-treated canola meal could be used as a source of RUP while maintaining milk production in high-producing lactating cows when compared with diets supplemented with a high-quality rumen-undegradable animal protein source. The secondary objective was to determine whether milk production would be further enhanced if the diets were also supplemented with synthetic Lys and **HMTBA** [Alimet; contains 88% D,L-2 hydroxy-4-(methylthio)-butanoic acid and is a trademark of Novus International Inc., St. Louis, MO].

MATERIALS AND METHODS

Cows and Diets

One hundred primi- and multiparous Holstein cows were used in a randomized block design grouped according to PTA and randomly assigned to 1 of 4 dietary treatments approximately 21 d prepartum until wk 17 postpartum. Approximately 30% of the cows were primiparous, with a similar number of primiparous cows being assigned to each dietary treatment. Cows received 1 of 4 prepartum diets, which consisted of a similar proportion of alfalfa hay, corn silage, and grass silage (forage content was approximately 70.2% of the ration DM; Table 1). The grain portion of the 4 rations consisted primarily of barley and corn grains. Varying amounts of Amipro (commercially available source of heat- and lignosulfonate-treated solvent-extracted canola meal, Agro Pacific Industries Ltd.), Prolak (commercially available source of blood meal, fish meal, feather meal, and meat and bone meal, H. J. Baker, Stamford, CT), canola meal, Lys·HCl, and Alimet feed supplement [contains 88% D,L-2 hydroxy-4-(methylthio)-butanoic acid and is a trademark of Novus International Inc.] were included, depending on the dietary treatment (Table 1).

The 4 dietary prepartum treatments included 1) **AMI** (Amipro supplemented at 5.9% of dietary DM), 2) **AMI+** (Amipro plus Lys·HCl and Alimet supplemented at 5.8, 0.11, and 0.05% of dietary DM, respectively), 3) **PRO** (Prolak supplemented at 2.6% of dietary DM), and 4) **PRO+** (Prolak plus Lys·HCl and Alimet supplemented at 2.6, 0.11, and 0.05% of dietary DM, respectively). Prepartum intake of Lys·HCl and Alimet was estimated by using the Cornell-Penn-Miner (**CPM**) ration-balancing model (Boston et al., 2000) at 14 and 7 g, respectively, for the AMI+ and PRO+ diets. Actual prepartum intakes of Lys·HCl (13.7 g) and Alimet (5.9 g) were slightly lower than estimated prepartum intakes.

At parturition, cows were switched from a prepartum diet to a postpartum diet. Two basal rations were formulated by using the CPM model. Amipro and Prolak were the 2 RUP sources. The chemical composition (% of DM) of Amipro was DM, 87.7; CP, 39.1; RUP, 70 (% of CP); neutral detergent-insoluble CP, 16.1; ADF, 20.4; NDF, 45.2; crude fat, 3.2; ash, 6.9; lignin, 7.6; Met, 0.56; and Lys, 1.71. The chemical composition (% of DM) of Prolak was DM, 93.3; CP, 76.9; RUP, 72 (% of CP); neutral detergent-insoluble CP, 23.1; ADF, 5.0; NDF, 17.7; crude fat, 10.3; ash, 10.8; lignin, 0 AA as a percentage of RUP; Met, 1.57; Lys, 6.05; and fatty acids as a percentage of total fatty acid DM: C₁₆, 27.6; C₁₈, 11.3; 18:1 *trans*, 1.3; 18:1 *cis*, 30.5; 18:2, 9.1; 18:3, 0.7. The ingredients for the 2 basal diets are shown in Table 2. The 4 postpartum treatments were 1) AMI, 2) PRO, 3) AMI+,

Table 1. Ingredients, actual chemical composition, and estimated chemical composition of prepartum diets introduced 21 d prior to calving and supplemented with AMI (Amipro), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid], PRO (Prolak), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid] (% of DM basis)

Item	Plant protein		Animal protein	
	AMI	AMI+	PRO	PRO+
Ingredient ¹				
Alfalfa hay	32.4	32.5	32.3	32.3
Corn silage	29.4	29.4	29.5	29.5
Grass silage	8.4	8.4	8.4	8.4
Barley grain	10.4	10.4	13.4	13.4
Amipro ²	5.9	5.8	—	—
Soybean meal, 46%	3.5	3.3	1.5	1.5
Corn grain	2.9	2.9	2.9	2.9
Prolak ³	—	—	2.6	2.6
Canola meal	—	—	2.4	2.2
Limestone	2.5	2.5	2.3	2.3
Lys·HCl	—	0.11	—	0.11
Alimet ⁴	—	0.05	—	0.05
Magnesium oxide	0.08	0.12	0.19	0.23
Mineral premix ⁵	4.28	4.28	4.28	4.28
Vitamin premix ⁶	0.24	0.24	0.23	0.23
Chemical composition (% of DM):actual (estimated ⁷)				
DM, %	53.3 (52.1)	51.6 (52.1)	51.8 (52.1)	50.8 (52.1)
CP, %	19.1 (17.4)	19.1 (17.4)	19.5 (17.4)	19.0 (17.4)
ADICP, ⁸ %	1.8	1.9	1.9	2.4
NDICP, ⁹ %	4.5	4.5	4.8	4.7
Soluble protein, % of CP	37.9 (26.8)	38.2 (27.5)	37.6 (27.1)	35.6 (27.6)
Ash, %	10.2 (12.2)	11.6 (12.3)	10.9 (12.1)	10.7 (12.2)
NDF, %	41.5 (35.6)	41.3 (35.5)	40.9 (34.4)	39.9 (34.3)
ADF, %	26.6 (23.0)	25.1 (22.9)	25.3 (22.4)	22.9 (22.4)
NFC, ¹⁰ %	26.6 (36.3)	25.6 (37.3)	26.1 (38.2)	27.9 (38.1)
RUP, % CP	(38.5)	(38.2)	(32.3)	(38.8)
RDP, % CP	(61.5)	(61.8)	(67.7)	(61.2)
Metabolizable Met, g/d	(18.3)	(20.3)	(19.7)	(20.0)
Metabolizable Lys, g/d	(60.3)	(65.9)	(67.8)	(68.2)

¹Samples of TMR were sampled weekly, dried, and pooled to provide one representative sample for each month of the experiment. The monthly samples were pooled to result in 2 TMR samples that represented the first and second halves, respectively, of the entire study period.

²Amipro, Agro Pacific Industries, Ltd. (Chilliwack, British Columbia, Canada).

³Prolak, H. J. Baker Company (Stamford, CT).

⁴Alimet, Novus International (St. Louis, MO).

⁵Mineral premix provided (% of DM) magnesium sulfate, 1.3; ammonium chloride, 0.8; molasses, 0.5; Diamond V yeast (Diamond V Mills Inc., Cedar Rapids, IA) 0.5; Megalac (Church and Dwight, Princeton, NJ), 0.3; niacin, 0.26; dicalcium phosphate, 0.2; calcium sulfate, 0.16; calcium chloride, 0.16; selenium, 0.10.

⁶Vitamin premix provided 41,666 IU/kg of vitamin A, 10,000 IU/kg of vitamin D, and 277 IU/kg of vitamin E.

⁷Calculations based on the CPM Dairy (version 3.0.8) model, using a 584-kg prepartum cow consuming 10.8 kg of DM/d.

⁸ADICP = acid detergent insoluble CP.

⁹NDICP = neutral detergent insoluble CP.

¹⁰NFC = 100 - (CP + NDF + ash + ether extract).

and 4) PRO+. The postpartum levels of Met and Lys were targeted to be at approximately 100% of the requirement for the AMI and PRO diets. However, there are different ways to evaluate AA requirements for lactating dairy cattle, such as a percentage of the requirement and the Rulquin ratio (Rulquin and Verite, 1993). The CPM model uses the Rulquin ratio as one of the methods to evaluate Lys and Met requirements of lactating dairy cattle. The AMI+ and PRO+ diets were

formulated based on the Rulquin ratio (Rulquin and Verite, 1993).

The Rulquin ratio (Rulquin and Verite, 1993) is a technique for expressing individual AA requirements in lactating dairy cows by evaluating AA as a percentage of MP. The optimum level of Met, as a percentage of MP, is 2.50%, and the optimum level of Lys, as a percentage of MP, is 7.20% (Boston et al., 2000). Because excess Met may cause negative effects, it is recommended that

Table 2. Ingredients, actual chemical composition, and estimated chemical composition of postpartum diets introduced on the day of calving and fed for 17 wk¹

Item	Plant protein		Animal protein	
	AMI	AMI+	PRO	PRO+
Ingredient ²				
Corn silage	19.2	19.2	19.2	19.2
Grass silage	11.6	11.6	11.6	11.6
Alfalfa hay	11.2	11.2	11.2	11.2
Barley grain	17.2	17.1	17.8	17.7
Corn grain	11.1	11.1	12.7	12.6
Amipro ³	9.9	9.8	—	—
Whole cottonseed	8.6	8.6	8.6	8.6
Canola meal	—	—	8.3	8.3
Soybean meal, 46%	6.7	6.6	2.3	2.3
Prolak ⁴	—	—	4.3	4.3
Limestone	1.4	1.4	1.1	1.0
Vitamin premix ⁵	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.2	0.2	—	—
Lys·HCl	—	0.2	—	0.2
Alimet ⁶	—	0.1	—	0.1
Other ⁷	2.4	2.4	2.4	2.4
Chemical composition (% of DM):actual (estimated ⁸)				
DM	55.1 (54.8)	53.7 (54.9)	55.6 (55.0)	54.7 (55.7)
CP	19.2 (18.4)	18.9 (18.5)	20.2 (18.8)	20.4 (19.0)
ADICP ⁹	2.6	1.9	1.9	2.3
NDICP ¹⁰	5.2	5.2	5.9	5.9
Soluble protein, % of CP	27.3 (24.5)	28.8 (25.5)	30.0 (26.1)	30.0 (27.0)
ADF	21.8 (20.6)	21.8 (20.6)	22.5 (20.3)	23.3 (20.3)
NDF	38.0 (33.6)	39.4 (33.5)	40.1 (31.0)	39.8 (30.9)
NFC ¹¹	30.4 (40.0)	29.1 (39.9)	27.0 (41.0)	26.6 (40.9)
Ash	8.1 (8.6)	8.1 (8.6)	8.0 (8.4)	8.1 (8.3)
RUP, % CP	(39.6)	(39.4)	(39.5)	(39.4)
RDP, % CP	(60.4)	(60.6)	(60.5)	(60.6)
Metabolizable Met (g/d)	(43.6)	(50.5)	(42.1)	(49.1)
Metabolizable Lys (g/d)	(135.6)	(141.5)	(138.3)	(144.3)

¹Diets were supplemented with AMI (Amipro), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid], PRO (Prolak), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid] (% of DM).

²Dry weekly samples of TMR were composited by calendar month. Monthly composites for each diet were further composited to result in 2 TMR samples that represented the first half and second half of the entire study period. The mean of the 2 chemical analyses for each diet are presented above.

³Amipro, Agro Pacific Industries Ltd. (Chilliwack, British Columbia, Canada).

⁴Prolak, H. J. Baker Company (Stamford, CT).

⁵Vitamin premix provided 10,000 IU/kg of vitamin A, 3,300 IU/kg of vitamin D, and 277 IU/kg of vitamin E.

⁶Alimet, Novus International (St. Louis, MO).

⁷Other (% of DM): molasses, 0.8; sodium sesquicarbonate, 0.7; Megalac (Church and Dwight, Princeton, NJ), 0.5; salt, 0.4.

⁸Calculations based on CPM Dairy (version 1.0) model using a 584-kg cow producing 36.3 kg of milk at 3.65% fat, 2.90% true protein, and consuming 19.5 kg of DM/d.

⁹ADICP = acid detergent insoluble CP.

¹⁰NDICP = neutral detergent insoluble CP.

¹¹NFC = 100 – (CP + NDF + ash + ether extract).

the Lys:Met ratio be 3.1:1 (Boston et al., 2000). The Rulquin ratio was 2.05 and 6.38% of MP for Met and Lys, respectively, for the AMI diet, and 1.95 and 6.42% of MP for Met and Lys, respectively, for the PRO diet. The Lys:Met ratios for the AMI diet and the PRO diet were 3.11:1 and 3.29:1, respectively.

Postpartum intakes of Lys·HCl and Alimet were estimated to be 40 and 20 g, respectively, for both the AMI+ and PRO+ diets. Lysine·HCL provided 8 g of ruminal escape Lys (Velle et al., 1998), and Alimet provided

7 g of ruminal escape Met (Koenig et al., 1999). The postpartum levels of Lys and Met were targeted to be at approximately 104 and 118% of requirements, respectively, for the AMI+ diet, and approximately 108 and 116% of requirements, respectively, for the PRO+ diet. The Rulquin ratio was 2.36 and 6.62% of MP for Met and Lys, respectively, for the AMI+ diet, and 2.26 and 6.65% of MP for Met and Lys, respectively, for the PRO+ diet. The Lys:Met ratios for the AMI+ and PRO+ diets were 2.81:1 and 2.94:1, respectively. The AMI+

diet was formulated to improve Met, as a percentage of MP, and Lys, as a percentage of MP, by 0.31 and 0.24, respectively, for the AMI+ diet compared with the AMI diet. The PRO+ diet was formulated to improve Met, as a percentage of MP, and Lys, as a percentage of MP, by 0.31 and 0.23, respectively, for the PRO+ diet compared with the PRO diet.

Feeding and Management

Cows were group housed, but were individually fed their respective TMR once daily through a Calan head-gate system (American Calan, Northwood, NH) both pre- and postpartum. Total mixed rations were offered to provide 10% orts, and the actual weights of TMR offered and of the orts collected were recorded daily. Cows were milked twice daily, and milk yield was recorded at each milking. Body weights and BCS were taken 3 wk prior to calving, and were recorded weekly thereafter until the end of the study. Body condition score measures were taken by the same individual throughout the course of the trial to minimize observer bias.

All cows were injected with recombinant bST (**rbST**; Posilac, Monsanto, St. Louis, MO) beginning at 63 DIM. The Institutional Animal Care and Use Committee at Washington State University approved this study.

Sampling and Analytical Procedures

Total mixed rations, grass silage, corn silage, and grain mixes were sampled weekly and analyzed for DM by drying in a forced-air oven (55°C). The dried samples were composited by calendar month and ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Monthly composites were further pooled to result in 2 TMR samples that represented the first half and second half of the entire study period per treatment (AMI, AMI+, PRO, PRO+) for all pre- and postpartum TMR. All TMR samples were analyzed for CP, neutral detergent CP, acid detergent CP [NDF and ADF fiber recovered on Whatman 7-cm 934-AH glass microfilters from the NDF and ADF procedure were pelletized (filter and residue) and followed method 990.03; AOAC, 2000], soluble protein (Krishnamoorthy et al., 1982), ADF (method 973.18, AOAC, 1990), NDF (Goering and Van Soest, 1970, with procedure modified per D. R. Mertens, USDA-ARS, US Dairy Forage Research Center, Madison, WI; 1992, personal communication; "Use of Whatman 934-AH glass microfiber filters with 1.5 µm particle retention in place of Gooch crucibles for recovery"), crude fat (method 920.39, AOAC, 1990), ash (method 942.05, AOAC, 1990), lignin (Goering and Van Soest, 1970), and Ca, P, Mg, K, and Na

(method 985.01, AOAC, 1990). Orts were sampled weekly and analyzed for DM.

Milk samples were taken weekly on Monday evening and Tuesday morning and were composited for infrared analyses of milk protein, milk fat, and milk lactose by the regional DHI laboratory (Burlington, WA). Additional milk samples were obtained at 2, 6, 10, and 14 wk postpartum for 9 cows per treatment and analyzed for MUN (Crocker, 1967), milk total N (AOAC, 1990), CN N (Rowland, 1938), and milk allantoin (Chen, 1989). Milk samples obtained from the 9 cows per treatment at 2 and 6 wk postpartum were also analyzed for milk fatty acids (Sukhija and Palmquist, 1988). Blood samples were taken from the coccygeal vein before the a.m. feeding at 2 and 6 wk postpartum for 9 cows per treatment (the same 9 cows from which additional milk samples were obtained), and concentrations of individual AA in whole blood were determined (Beckman, 1975, 1980).

Statistical Analysis

Statistical analyses were performed by using PROC MIXED of SAS (SAS, 1999). A randomized block design with repeated measures was used. Data were analyzed separately for the periods from 3 wk prepartum to parturition, 1 to 4 wk postpartum, 5 to 9 wk postpartum, and 10 to 17 wk postpartum. The first 4 wk of lactation were evaluated separately because this is a time of transition from prepartum to postpartum. Weeks 5 to 9 postpartum were evaluated as the time prior to rbST administration, and wk 10 to 17 postpartum were evaluated to observe the effects of AA supplementation post-rbST administration. Results are reported as least squares means. Significance was declared at $P < 0.05$ and trends were discussed when $P < 0.10$.

The model used to evaluate prepartum DMI and BW, and postpartum DMI, milk, and milk components for this experiment was

$$Y_{ijklm} = \mu + B_i + P_j + S_k + (P \times S)_{jk} + (B \times P \times S)_{ijk} + W_l + (P \times W)_{jl} + (S \times W)_{kl} + (P \times S \times W)_{jkl} + E_{ijklm},$$

where μ is the overall mean; B_i is the random effect of block ($i = 1$ to 25); P_j is the fixed effect of protein source ($j = 1$ to 2); S_k is the fixed effect of supplement source ($k = 1$ to 2); $(P \times S)_{jk}$ is the interaction effect of P_j and S_k ; $(B \times P \times S)_{ijk}$ is the random interaction effect of B_i , P_j , and S_k used to test the whole-plot effects [protein, supplement, and interaction (error a)]; W_l is the fixed effect of week ($m = -3$ to 0; $m = 1$ to 4; $m = 5$ to 9; or $m = 10$ to 17); $(P \times W)_{jl}$ is the interaction effect of P_j and W_l ; $(S \times W)_{kl}$ is the interaction effect of S_k and W_l ;

$(P \times S \times W)_{jkl}$ is the interaction effect of P_j , S_k , and W_l ; and E_{ijklm} is the residual error (error b).

The model used to analyze milk total N, CN N, MUN, milk allantoin, milk fatty acids, and blood AA for this randomized block experiment was

$$Y_{ijkl} = \mu + B_i + P_j + S_k + (P \times S)_{jk} + E_{ijkl},$$

where μ is the overall mean, B_i is the random effect of block ($i = 1$ to 9), P_j is the fixed effect of protein source ($j = 1$ to 2), S_k is the fixed effect of supplement source ($k = 1$ to 2), $(P \times S)_{jk}$ is the interaction effect of P_j and S_k , and E_{ijkl} is the residual error.

RESULTS

Diets and Feed Composition

Actual dietary formulation and chemical composition for the pre- and postpartum rations are shown in Tables 1 and 2, respectively. By design, the proportions of corn silage, grass silage, and alfalfa hay were similar between treatments, with forage making up approximately 70 and 42% of the dietary DM for the pre- and postpartum diets, respectively. The prepartum diets contained approximately 5% lignin (% of DM) and 2.7% crude fat (% of DM). As expected, prepartum diets were similar in mineral composition, containing on average (% of DM) Ca, 1.8; P, 0.4; Mg, 0.5; K, 1.9; and Na, 0.1.

A comparison of estimated and actual chemical composition of the test diets fed during the first 17 wk of lactation is shown in Table 2. In summary, CP, ADF, and NDF concentrations differed between estimated and actual values. Actual CP concentrations were approximately 0.5 and 1.5% greater for the Amipro- and Prolak-based diets, respectively (Table 2). Actual ADF contents were approximately 1 and 2.5% higher than estimated for the Amipro- and Prolak-based diets, respectively (Table 2). Discrepancies between actual and estimated NDF concentrations were 5 and 9% for the Amipro- and Prolak-based diets, respectively (Table 2). The average lignin was 5.0% of DM and crude fat was 4.7% of DM for each of the actual treatment diets. The postpartum diets were similar in mineral composition (% of DM), with each diet containing on average Ca, 1.0; P, 0.5; Mg, 0.4; K, 1.6; and Na, 0.4.

Postpartum requirements of Met and Lys were approximately 43 and 136 g/d, respectively. Estimated total Met and Lys available from the AMI diet were 44 and 136 g/d, respectively; from the PRO diet were 42 and 138 g/d, respectively; from the AMI+ diet were 50 and 142 g/d, respectively; and from the PRO+ diet were 49 and 144 g/d, respectively.

Intake, BW, and Milk Production

Prepartum DMI did not differ among treatments, with cows consuming on average 12.2 ± 3.8 kg/d. As expected, there were no differences in DMI during the first 4 wk, 5 to 9 wk, or 10 to 17 wk of lactation due to the source of protein (plant vs. animal; Table 3). However, DMI was significantly less ($P = 0.03$) when supplemented with additional Lys·HCl and HMTBA during the first 4 wk postpartum (Table 3). The reduced DMI during wk 1 through 4, when cows were supplemented with Lys·HCl and HMTBA, disappeared at wk 5 through 9 postpartum and wk 10 through 17 postpartum (Table 3).

Cows fed diets containing an animal protein source of RUP tended to have greater pre- and postpartum BW; however, there was no effect of dietary supplementation of Lys·HCl and HMTBA on pre- or postpartum BW in this study (Figure 1).

Milk production ($P = 0.38$ for protein supplements, $P = 0.89$ for AA supplements, $P = 0.96$ for protein by supplement interaction) and 3.5% FCM production ($P = 0.08$ for protein supplements, $P = 0.47$ for AA supplements, $P = 0.73$ for protein by supplement interaction) did not differ among treatments for the first 17 wk of lactation (Table 3). However, when the data was plotted, we observed that 3.5% FCM production (Figure 2) was greater for cows fed diets supplemented with Lys·HCl and HMTBA from 14 to 17 wk postpartum. Fat-corrected milk production (3.5%; kg/d) was 39.6, 41.3, 40.1, and 42.7, respectively, for the AMI, AMI+, PRO, PRO+ groups. Milk fat production was (kg/d) 1.38, 1.45, 1.42, and 1.53, respectively for the AMI, AMI+, PRO, and PRO+ groups.

The FCM to DMI ratio and milk protein content were not affected by treatment from wk 1 to 17 of lactation (Table 3). Milk fat content was greater ($P = 0.03$) and milk lactose content was lower ($P = 0.03$) for cows supplemented with Lys·HCl and HMTBA from wk 1 to 4 of lactation (Table 3). However, by wk 5 to 9 and wk 10 to 17 of lactation, cows supplemented with additional dietary Lys·HCl and HMTBA had milk fat and milk lactose content similar to that of the unsupplemented cows (Table 3). There was a trend for milk fat content ($P = 0.10$) to be greater during wk 10 to 17 of lactation for cows fed animal sources of RUP compared with vegetable sources or RUP (Table 3).

Milk Total Nitrogen, CN Nitrogen, MUN, and Milk Allantoin

Milk total N was greater ($P = 0.03$) for cows fed the nonsupplemented (HMTBA and Lys·HCl) treatments at wk 14 (Table 4). Interestingly, supplementation of

Table 3. Dry matter intake, milk production, and milk composition from cows fed diets supplemented with AMI (Amipro), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4 (methylthio)-butanoic acid], PRO (Prolak), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4 (methylthio)-butanoic acid] for wk 1 to 4, wk 5 to 9, and wk 10 to 17 (n = 25 per treatment)

	Plant protein		Animal protein			<i>P</i> <		
Item	AMI	AMI+	PRO	PRO+	SE	Protein	Supplement	Protein × supplement
Weeks 1–4								
DMI, kg/d	18.0	16.9	18.1	16.5	0.7	NS	0.03	NS
Milk, kg/d	34.7	32.7	35.9	34.4	1.7	NS	NS	NS
3.5% FCM, kg/d	37.6	36.4	39.0	38.5	1.8	NS	NS	NS
FCM:DMI	2.32	2.24	2.22	2.53	0.1	NS	NS	NS
Milk fat, %	4.07	4.30	4.04	4.33	0.1	NS	0.03	NS
Milk protein, %	3.34	3.42	3.39	3.19	0.1	NS	NS	0.02
Milk lactose, %	4.86	4.67	4.81	4.77	0.1	NS	0.03	NS
Weeks 5–9								
DMI, kg/d	22.2	22.2	22.9	22.3	0.7	NS	NS	NS
Milk, kg/d	40.1	40.2	41.3	41.2	1.6	NS	NS	NS
3.5% FCM, kg/d	40.2	40.4	41.1	41.6	1.6	NS	NS	NS
FCM:DMI	1.88	1.72	1.86	1.95	0.1	NS	NS	NS
Milk fat, %	3.52	3.55	3.50	3.59	0.1	NS	NS	NS
Milk protein, %	2.97	3.00	2.99	2.93	0.04	NS	NS	NS
Milk lactose, %	4.96	4.85	4.94	4.94	0.04	NS	NS	NS
Weeks 10–17								
DMI, kg/d	23.8	24.1	23.7	23.9	0.7	NS	NS	NS
Milk, kg/d	40.1	41.2	40.3	41.5	1.4	NS	NS	NS
3.5% FCM, kg/d	40.2	41.1	41.0	42.7	1.3	NS	NS	NS
FCM:DMI	1.80	1.75	1.76	1.83	0.1	NS	NS	NS
Milk fat, %	3.53	3.51	3.62	3.68	0.1	0.10	NS	NS
Milk protein, %	3.07	3.08	3.10	3.05	0.04	NS	NS	NS
Milk lactose, %	4.89	4.85	4.87	4.86	0.1	NS	NS	NS

Lys·HCl and HMTBA decreased ($P = 0.05$) CN N in milk samples collected at wk 2, regardless of protein source provided to the cows (Table 4). A significant protein by supplement interaction ($P = 0.04$) was noted at wk 14 for MUN (Table 4); supplementation of syn-

thetic AA in the AMI+ diet resulted in a decrease in MUN concentration, whereas supplementation of these AA caused an increase in MUN concentration in milk from cows fed the PRO+ diet (Table 4). There was a trend for milk allantoin concentration to be greater at

Figure 1. Average weekly BW for cows on AMI (Amipro, Agro Pacific Industries Ltd., Chilliwack, British Columbia, Canada; ●), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid; ◆], PRO (Prolak, H. J. Baker Company, Stamford, CT; ■), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid; ▲]-based diets. SEM = 13.1 kg/d; P -value for the protein × supplement × week interaction was 0.99.

Figure 2. Average weekly 3.5% FCM production for cows on AMI (Amipro, Agro Pacific Industries Ltd., Chilliwack, British Columbia, Canada; ●), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid; ◆], PRO (Prolak, H. J. Baker Company, Stamford, CT; ■), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid; ▲]-based diets. SEM = 1.3 kg/d; *P*-value for the protein × supplement × week interaction was 0.33.

wk 10 (*P* = 0.07) in cows supplemented with HMTBA and Lys·HCl (Table 4).

Milk Fatty Acids

There were no differences in the concentration of short chain (<C₁₆), C₁₆, C₁₈, or 18:2 fatty acids in milk

samples collected at 2 or 6 wk postpartum (*P* > 0.05; Table 5). In milk samples collected at 2 wk postpartum, supplementation of Lys·HCl and HMTBA to the AMI+ diet increased the concentration of 18:3 (Table 5). In contrast, the same supplementation to the PRO+ diet decreased the concentration of this particular fatty acid, resulting in a protein by supplement interaction (Table

Table 4. Milk total N, CN N, MUN, and milk allantoin from cows fed diets supplemented with AMI (Amipro), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid], PRO (Prolak), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid] for wk 2, 6, 10, and 14 postpartum (n = 9 per treatment)

Item	Plant protein		Animal protein		SE	<i>P</i> <		
	AMI	AMI+	PRO	PRO+		Protein	Supplement	Protein
								× supplement
Week 2								
Milk total N, %	0.56	0.53	0.55	0.54	0.02	NS	NS	NS
CN N, %	0.42	0.39	0.44	0.40	0.02	NS	0.05	NS
MUN, mg/dL	14.2	13.6	15.4	14.6	1.2	NS	NS	NS
Milk allantoin, mmol/d	13.2	15.0	17.2	14.8	1.3	NS	NS	0.10
Week 6								
Milk total N, %	0.50	0.45	0.47	0.48	0.02	NS	NS	0.10
CN N, %	0.37	0.34	0.34	0.35	0.02	NS	NS	NS
MUN, mg/dL	16.1	15.6	16.1	17.9	0.9	NS	NS	NS
Milk allantoin, mmol/d	15.4	17.1	16.0	16.0	0.9	NS	NS	NS
Week 10								
Milk total N, %	0.50	0.47	0.48	0.44	0.04	NS	NS	NS
CN N, %	0.36	0.35	0.36	0.36	0.02	NS	NS	NS
MUN, mg/dL	18.2 ^a	15.0 ^b	18.6 ^a	19.1 ^a	1.2	0.05	NS	0.08
Milk allantoin, mmol/d	13.6	15.1	14.7	17.4	1.3	NS	0.07	NS
Week 14								
Milk total N, %	0.50	0.45	0.51	0.49	0.02	NS	0.03	NS
CN N, %	0.38	0.36	0.38	0.35	0.02	NS	NS	NS
MUN, mg/dL	17.9 ^a	14.0 ^b	17.3 ^a	19.1 ^a	1.4	0.09	NS	0.04
Milk allantoin, mmol/d	12.7	14.3	13.7	15.9	1.4	NS	NS	NS

^{a,b}Means within rows with no common superscripts differ (*P* < 0.10).

Table 5. Milk fatty acid concentrations from cows fed diets supplemented with AMI (Amipro), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid], PRO (Prolak), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid] (% of total fatty acids) for wk 2 and 6 postpartum (n = 9 per treatment)

Item	Plant protein		Animal protein		SE	<i>P</i> <		
	AMI	AMI+	PRO	PRO+		Protein	Supplement	Protein × supplement
Week 2								
Short (<C ₁₆)	18.9	17.9	17.3	17.9	1.6	NS	NS	NS
C ₁₆	29.2	27.9	28.9	28.9	0.6	NS	NS	NS
C ₁₈	15.6	16.3	15.3	15.9	1.1	NS	NS	NS
18:1 (<i>trans</i>)	2.0	1.6	2.6	2.0	0.3	NS	NS	NS
18:1 (<i>cis</i>)	26.9	28.3	27.7	27.5	1.6	NS	NS	NS
18:2	2.9	3.1	2.9	3.0	0.1	NS	NS	NS
18:3	0.41 ^b	0.48 ^a	0.41 ^b	0.39 ^b	0.02	0.04	NS	0.05
Conjugated linoleic acid	0.61	0.54	0.77	0.65	0.05	0.02	0.07	NS
Week 6								
Short (<C ₁₆)	23.2	24.5	21.4	22.2	1.6	NS	NS	NS
C ₁₆	29.4	29.7	29.1	28.0	0.9	NS	NS	NS
C ₁₈	15.1	15.3	14.5	16.2	1.1	NS	NS	NS
18:1 (<i>trans</i>)	1.7	1.7	2.4	1.6	0.4	NS	NS	NS
18:1 (<i>cis</i>)	23.1	21.3	24.7	24.6	1.4	0.06	NS	NS
18:2	2.8	3.1	3.0	2.7	0.2	NS	NS	0.10
18:3	0.42	0.45	0.40	0.39	0.03	NS	NS	NS
Conjugated linoleic acid	0.59	0.58	0.81	0.67	0.10	0.08	NS	NS

^{a,b}Means within rows with no common superscripts differ (*P* < 0.10).

5). This interaction was no longer evident at wk 6 postpartum because the dietary treatment did not affect the concentration of 18:3 in milk (Table 5). The concentration of conjugated linoleic acid, expressed as a percentage of total fatty acids, was greater at 2 wk (*P* = 0.02) postpartum for cows fed diets containing Prolak (Table 5). We also noted a similar trend at 6 wk postpartum (*P* = 0.08; Table 5). At 6 wk postpartum, 20:4 milk fatty acid concentration (*P* = 0.006) was greater for cows fed diets containing an animal protein source of RUP (data not shown). Milk fatty acid 20:4 was also greater (*P* = 0.02) for cows fed diets supplemented with Lys·HCl and HMTBA 6 wk postpartum compared with cows fed unsupplemented diets (data not shown).

Whole-Blood AA

There were minimal differences in whole-blood AA concentrations 2 wk postpartum (Table 6). The whole-blood Gly concentration was greater (*P* = 0.05), and there was a trend for the Ala concentration to be lower (*P* = 0.06) for cows fed diets containing animal sources of RUP (Table 6). The essential AA Ile was greater (*P* = 0.05) for cows fed diets supplemented with Lys·HCl and HMTBA at 2 wk postpartum (Table 6).

At 6 wk postpartum, the concentrations of the essential AA Arg (*P* = 0.05) and the nonessential AA Gln (*P* = 0.04) were greater for cows fed diets supplemented with Lys·HCl and HMTBA (Table 6). We also noted a similar trend for Trp (*P* = 0.07).

DISCUSSION

At the time this study was conducted, the possibility existed that sources of bovine animal proteins used to balance lactating dairy cattle diets for RUP would be banned as a ruminant feedstuff in North America. Not surprisingly, in 1997 regulations governing the use of animal feeds were amended such that the majority of feedstuffs of bovine origin, with the exception of blood meal and tallow, would no longer be available as possible feed sources for ruminants. To our knowledge, this is the only comprehensive study that assesses the effects of feeding either plant- or animal-based RUP sources to lactating dairy cows for the first 17 wk of lactation.

Cows fed the Amipro-based diets were able to maintain DMI, milk production, and milk components when compared with cows fed the Prolak diets throughout the 17 wk of lactation when diets were equivalent in RDP, RUP, and metabolizable Met and Lys. Liu et al. (2000) investigated the effects of feeding corn distillers grains as the sole source of RUP compared with feeding a blend of plant- and animal-based RUP sources (corn distillers grains, soybean meal, and fish meal). Similar to our findings, these authors (Liu et al., 2000) reported no differences in milk production or 3.5% FCM production.

Some researchers have investigated whether the quantity and quality of CP in the diet can affect milk production. Xu et al. (1998) evaluated 2 diets of similar

CP, RDP, and RUP contents, one containing a vegetable-based source of RUP (corn distillers grains) and the other containing animal sources of RUP (blood meal, meat and bone meal, feather meal, and fish meal). These authors (Xu et al., 1998) reported no difference in DMI but an increase in milk production from cows fed diets containing animal sources of RUP. Noftsker and St-Pierre (2003) altered the digestibility of the RUP fraction by using different animal sources (porcine meal, blood meal, hydrolyzed feather meal, and poultry meal) and monitored DMI and milk production. These authors (Noftsker and St-Pierre, 2003) reported that DMI and milk production were decreased in cows fed diets containing the less digestible sources of RUP. Interestingly, in our study, there was no difference in DMI and milk production between the PRO and AMI treatments. In the study by Noftsker and St-Pierre (2003), CP content of the diet could be reduced by 1% without affecting DMI and milk production if highly digestible sources of RUP and a synthetic source of Met were included in the diet. Unfortunately, we could not validate this finding because the CP content of the AMI+ diet was only 0.3% less than the CP content of the AMI diet and there was no difference in CP content between the PRO and PRO+ diets.

As mentioned above, we observed no significant differences in milk fat concentration between the cows fed the Amipro-based diets and those consuming the Prolak-based diets. This finding is similar to the results reported by Xu et al. (1998). Wright et al. (2005), who compared the effects of heat- and lignosulfonate-treated canola meal with untreated canola meal, also found no differences in milk fat concentration. Similar to the studies by Xu et al. (1998) and Wright et al. (2005), we observed no effect of RUP source on milk protein concentration, although these authors did report differences in milk protein yield due to differences in milk yield between treatments. Supplementation of Lys·HCl and HMTBA did affect animal performance in this study; however, this effect appeared to be limited to the first 4 wk of lactation, when DMI was lower and milk fat concentration was higher, and during wk 14 to 17 postpartum, when increases in FCM production and milk fat production were observed in cows provided supplemental AA compared with cows that received unsupplemented diets. Xu et al. (1998) also reported that milk fat composition tended to be elevated for cows fed diets supplemented with ruminally protected Lys and Met during the first 8 wk of lactation. Rode et al. (1998) and Piepenbrink et al. (2004) reported higher milk yield and FCM when feeding 50 and 25 g of HMTBA, respectively. Similar to the current study, HMTBA was fed prior to precalving and early lactation

in both of these studies (Rode et al., 1998; Piepenbrink et al., 2004).

The rise in FCM and fat production during wk 14 to 17 in the AA-supplemented animals may indicate that the combination of rbST and supplementation with Lys·HCl and HMTBA resulted in a greater use of AA for milk production, despite no differences in DMI being observed between treatment groups. Although this is only speculative at the time, we feel that this observation warrants further investigation in the future.

St-Pierre and Sylvester (2005) reported that milk production was significantly greater when high-producing cows were supplemented with an isopropyl ester of HMTBA compared with HMTBA supplementation and control. Further, these authors report that the response to milk production was progressive across the week of experiment with cows introduced to the experimental rations 4 wk postpartum. However, caution is warranted when comparing our results with those of St. Pierre and Sylvester (2005) because the sources of synthetic Met were different. Moreover, the percentage of Lys in the diet, as a percentage of microbial protein (6.8%), was considerably higher than that used in our study (6.6%). As St-Pierre and Sylvester (2005) concluded, the dietary adequacy of Lys may be a limiting factor in milk production, and thus may have been a limiting factor affecting milk production in our study.

An explanation regarding the differences in 18:3 content of milk collected from cows fed an AA supplement is not readily apparent. The increase in conjugated linoleic acid content of milk at 2 wk postpartum could be attributed to the inclusion of fish meal in the Prolak supplement, as reviewed by Chilliard et al. (2001).

Although we found that milk CN N concentration decreased 2 wk postpartum for cows supplemented with Lys·HCl and HMTBA, this difference was no longer apparent at 6, 10, or 14 wk postpartum. This finding agrees with the work of Xu et al. (1998), who reported no difference in milk CN N 4 and 8 wk postpartum between cows fed diets balanced with primarily corn distillers grains as the RUP source, diets balanced with animal protein sources for the RUP, or when synthetic Lys and Met were supplemented at 27 and 8 g/d, respectively, to the corn distillers grains-based diet. Others reported no difference in milk CN N between cows fed unsupplemented diets and ruminally available AA-supplemented diets (Bateman et al., 1999; Liu et al., 2000).

The lower values for MUN in milk from cows fed the AMI+ diet as compared with the AMI, PRO, and PRO+ diets are of interest because MUN has been linked to urinary excretion of N and reproductive performance (Jonker et al., 1998). At wk 10 and 14, MUN values in milk from AMI+-fed cows decreased, whereas those fed PRO+ produced milk with higher MUN values. Ac-

Table 6. Whole-blood AA profile from cows fed diets supplemented with AMI (Amipro), AMI+ [Amipro plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid], PRO (Prolak), and PRO+ [Prolak plus Lys·HCl and 2-hydroxy-4(methylthio)-butanoic acid] (nmol/mL) for wk 2 and 6 postpartum (n = 9 per treatment)

	Plant protein		Animal protein			<i>P</i> <		
Item	AMI	AMI+	PRO	PRO+	SE	Protein	Supplement	Protein × supplement
Week 2								
Essential AA, nmol/mL								
Arg	31.4	30.6	26.4	28.2	3.2	NS	NS	NS
His	80.7	75.1	103.5	81.7	20.4	NS	NS	NS
Lys	86.4	90.2	83.1	90.4	7.0	NS	NS	NS
Trp	0.0	1.13	0.21	0.27	0.39	NS	NS	NS
Phe	34.3	32.3	31.9	33.3	3.0	NS	NS	NS
Leu	90.1	112.9	98.2	115.3	12.9	NS	NS	NS
Ile	59.0	78.2	61.6	76.2	8.2	NS	0.05	NS
Val	139.4	161.1	151.6	182.9	21.8	NS	NS	NS
Thr	114.3	111.4	108.9	105.0	10.9	NS	NS	NS
Total essential AA	635.9	693.1	665.5	714.6	56.3	NS	NS	NS
Nonessential AA, nmol/mL								
Tyr	29.3	37.0	29.4	29.8	3.3	NS	NS	NS
Ala	143.8	138.0	119.5	106.1	14.2	0.06	NS	NS
Gly	333.5	325.1	395.5	353.7	23.9	0.05	NS	NS
Pro	55.0	46.5	57.4	50.9	5.3	NS	NS	NS
Gln	150.0	149.4	140.8	161.4	13.7	NS	NS	NS
Glu	136.4	82.8	88.4	92.6	17.6	NS	NS	0.10
Asn	22.4	26.7	24.1	22.7	3.5	NS	NS	NS
Ser	89.9	84.5	87.3	76.0	10	NS	NS	NS
Total nonessential AA	980.1	907.0	960.2	915.7	53.7	NS	NS	NS
Week 6								
Essential AA, nmol/mL								
Arg	30.0	34.3	30.9	36.5	3.1	NS	0.05	NS
His	79.1	70.7	72.6	75.7	4.2	NS	NS	NS
Lys	88.2	84.5	81.9	88.9	5.8	NS	NS	NS
Trp	0.03	1.15	0.55	2.12	0.81	NS	0.07	NS
Phe	30.8	30.9	32.5	33.2	1.7	NS	NS	NS
Leu	93.8	98.6	99.8	113.9	8.0	NS	NS	NS
Ile	65.4	70.9	60.9	70.2	6.3	NS	NS	NS
Val	154.8	160.9	168.9	200.5	15.5	0.05	NS	NS
Thr	127.7	111.6	115.0	116.9	8.5	NS	NS	NS
Total essential AA	663.6	653.7	661.6	733.5	38.7	NS	NS	NS
Nonessential AA, nmol/mL								
Tyr	35.0	36.0	33.8	33.7	2.3	NS	NS	NS
Ala	139.6	141.7	138.1	140.8	9.3	NS	NS	NS
Gly	287.7	288.4	304.6	314.5	19.9	NS	NS	NS
Pro	53.9	53.1	49.3	52.8	4.9	NS	NS	NS
Gln	137.4	156.1	143.6	155.5	8.2	NS	0.04	NS
Glu	90.4	161.7	77.5	84.9	30.0	0.09	NS	NS
Asn	25.0 ^a	18.5 ^b	26.3 ^a	27.6 ^a	2.3	0.02	NS	0.04
Ser	79.7 ^a	69.7 ^b	79.9 ^a	81.0 ^a	4.0	0.08	NS	0.10
Total nonessential AA	879.7	944.3	872.6	916.4	45.0	NS	NS	NS

^{a,b}Means within rows with no common superscripts differ (*P* < 0.10).

cording to Broderick and Clayton (1997) and Jonker et al. (1998), MUN is closely related to dietary CP concentration, with high MUN values reflecting overfeeding of CP. The protein content of the AMI+ diet was 0.3% lower (18.9 vs. 19.2%) than that of the AMI diet. However, the AMI diet was 1% lower than that of the PRO diet, with no difference in MUN values in milk collected from cows fed these treatments. The lower values of MUN in milk from the AMI+-fed cows would infer improved N efficiency, but the fate of the N is not readily apparent because no differences in BW or milk production were noted. In retrospect, it would have been of

interest to monitor the reproductive performance of cows fed the different dietary treatments fed in this trial.

Considerable research has attempted to elucidate the effects on productivity of shifting the rumen microbial populations in ruminants (Vazquez-Anon et al., 2001; Noftsger et al., 2005). Milk allantoin tended to be elevated in cows fed diets supplemented with Lys·HCl and HMTBA at wk 10, which is an indication that there was an increase in microbial protein production. This trend may be due to a shift in rumen microbial populations caused by HMTBA (Vazquez-Anon et al., 2001). In

lactating dairy cows, the increase in microbial protein production is a beneficial response, because the increased microbial supply of protein will provide the cow with the necessary AA to facilitate milk production.

The trend in this study for whole-blood Lys concentrations to be greater for diets supplemented with Lys·HCl suggests that further research should be undertaken to determine and validate the benefits of feeding supplemental Lys·HCl. One limitation of the current study is that we were unable to measure whole-blood Met concentrations. However, elevation of the plasma Met concentration would not have been expected when feeding HMTBA, because it is absorbed and transported in blood in the form of HMTBA and converted to L-Met within the tissues (Lobley et al., 2006).

CONCLUSIONS

The findings of this study clearly showed that heat- and lignosulfonate-treated canola meal could be used as a source of RUP to maintain milk production in high-producing lactating cows when compared with diets supplemented with a high-quality rumen-undegradable animal protein source. As evidence supporting this result, providing an RUP derived from canola meal did not affect DMI, milk production, or 3.5% FCM production for the first 17 wk of lactation when compared with cows fed Prolak, and when diets were equivalent in RDP, RUP, and metabolizable Met and Lys.

The addition of synthetic Lys·HCl and HMTBA to diets containing either animal- or vegetable-based RUP sources did reduce DMI during the first 4 wk of lactation. However, neither milk production nor the ratio of FCM to DMI was adversely affected throughout the first 17 wk of lactation. Despite previous reports citing clear benefits of feeding supplemental synthetic AA in diets fed to high-producing dairy cows, we were unable to provide additional evidence to support these findings in the current study.

ACKNOWLEDGMENT

The authors acknowledge financial support from Novus International (St. Louis, MO). We acknowledge the donation of Prolak from H. J. Baker Company (Stamford, CT) and the donation of Alimet for study diets from Novus International.

REFERENCES

- AOAC. 1990. Official Methods of Analysis. 15th ed. AOAC Int., Arlington, VA.
- AOAC. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Arlington, VA.
- Bateman, H. G., II, J. N. Spain, M. S. Kerley, R. L. Belyea, and R. T. Marshall. 1999. Evaluation of ruminally protected methionine and lysine or blood meal and fish meal as protein sources for lactating Holsteins. *J. Dairy Sci.* 82:2115–2120.
- Beckman. 1975, March. Beckman 121-M Application Notes: 121-M-TB-004A. Beckman Instruments, Palo Alto, CA.
- Beckman. 1980, January. Beckman 121-M Application Notes: 121-MB-TB-018. Beckman Instruments, Palo Alto, CA.
- Boston, R., D. Fox, C. Sniffen, E. Janczewski, R. Munson, and W. Chalupa. 2000. The conversion of a scientific model describing dairy cow nutrition and production to an industry tool: The CPM-Dairy project. Pages 361–377 in *Modeling Nutrition of Farm Animals*. J. P. McNamara, J. France, and D. E. Beever, ed. CAB Int., Oxford, UK.
- Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80:2964–2971.
- Chen, X. B. 1989. Excretion of purine derivatives by sheep and cattle and its use for the estimation of absorbed microbial protein. PhD Dissertation, Univ. Aberdeen, Aberdeen, UK.
- Chilliard, Y., A. Ferlay, and M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest. Prod. Sci.* 70:31–48.
- Crocker, C. L. 1967. Rapid determination of urea nitrogen in serum on plasma without deproteinization. *Am. J. Med. Technol.* 33:361–365.
- Goering, H. K., and P. J. VanSoest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). *Agric. Handb.* 379. USDA-ARS, Washington, DC.
- Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *J. Dairy Sci.* 81:2681–2692.
- Koenig, K. M., L. M. Rode, C. D. Knight, and P. R. McCullough. 1999. Ruminant escape, gastrointestinal absorption, and response of serum methionine to supplementation of liquid methionine hydroxy analog in dairy cows. *J. Dairy Sci.* 82:355–361.
- Krishnamoorthy, U., T. V. Muscato, C. J. Sniffen, and P. J. Van Soest. 1982. Borate-phosphate procedure as detailed in nitrogen fractions in selected feedstuffs. *J. Dairy Sci.* 65:217–225.
- Liu, C., J. Schingoethe, and G. A. Stegeman. 2000. Corn distillers grains versus a blend of protein supplements with or without ruminally protected amino acids for lactation cows. *J. Dairy Sci.* 83:2075–2084.
- Lobley, G. E., T. J. Wester, A. G. Calder, D. S. Parker, J. J. Dibner, and M. Vazquez-Anon. 2006. Absorption of 2-hydroxy-4-methylthiobutyrate (HMTBA) and conversion to Met in lambs. *J. Dairy Sci.* 89:1072–1080.
- Nichols, J. R., D. J. Schingoethe, H. A. Maiga, M. J. Brouk, and M. S. Piepenbrink. 1998. Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating cows. *J. Dairy Sci.* 81:482–491.
- Noftsker, S., and N. R. St-Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. *J. Dairy Sci.* 86:958–969.
- Noftsker, S., N. R. St-Pierre, and J. T. Sylvester. 2005. Determination of rumen degradability and ruminal effects of three sources of methionine in lactating cows. *J. Dairy Sci.* 88:223–237.
- Piepenbrink, M. S., A. L. Marr, M. R. Waldron, W. R. Butler, T. R. Overton, M. Vazquez-Anon, and M. D. Holt. 2004. Feeding 2-hydroxy-4(methylthio)-butanoic acid to periparturient dairy cows improves milk production but not hepatic metabolism. *J. Dairy Sci.* 87:1071–1084.
- Rode, L. M., C. D. Knight, K. A. Andrews, and K. M. Koenig. 1998. Effects of pre- and post-partum Alimet® supplementation on milk production of dairy cows. *J. Dairy Sci.* 81(Suppl. 1):294 (Abstr.)
- Rowland, S. J. 1938. The protein distribution in normal and abnormal milk. *J. Dairy Res.* 9:47–57.
- Rulquin, H., and R. Verite. 1993. Recent Advances in Animal Nutrition. Univ. Nottingham Press, Nottingham, UK.
- SAS Institute. 1999. SAS/STAT User's Guide, Version 8.2. SAS Inst. Inc., Cary, NC.

- Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and V. M. Olson. 1992. Amino acid limitation and flow to the duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. *J. Dairy Sci.* 75:3486–3502.
- Socha, M. T., D. E. Putnam, B. D. Garthwaite, N. L. Whitehouse, N. A. Kierstead, C. G. Schwab, G. A. Ducharme, and J. C. Robert. 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. *J. Dairy Sci.* 88:1113–1126.
- St-Pierre, N. R., and J. T. Sylvester. 2005. Effects of 2-hydroxy-4(methylthio) butanoic acid (HMB) and its isopropyl ester on milk production and composition by Holstein cows. *J. Dairy Sci.* 88:2487–2497.
- Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* 36:1202–1206.
- Vazquez-Anon, M., T. Cassidy, P. McCullough, and G. A. Varga. 2001. Effects of Alimet on nutrient digestibility, bacterial protein synthesis, and ruminal disappearance during continuous culture. *J. Dairy Sci.* 84:159–166.
- Velle, W., T. I. Kanui, A. Aulie, and O. C. Sjaastad. 1998. Ruminant escape and apparent degradation of amino acids administered intraruminally in mixtures to cows. *J. Dairy Sci.* 81:3231–3238.
- Wright, C. F., M. A. G. von Keyserlingk, M. L. Swift, L. J. Fisher, J. A. Shelford, and N. E. Dinn. 2005. Heat and lignosulfonate treated canola meal as a source of ruminal undegradable protein for lactating dairy cows. *J. Dairy Sci.* 88:238–243.
- Xu, S., J. H. Harrison, W. Chalupa, C. Sniffen, W. Julien, H. Sato, T. Fujieda, K. Watanabe, T. Ueda, and H. Suzuki. 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. *J. Dairy Sci.* 81:1062–1077.