Interactions of energy and predicted metabolizable protein in determining nitrogen efficiency in the lactating dairy cow

A. G. Rius, M. L. McGilliard, C. A. Umberger, and M. D. Hanigan¹

Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg 24061

ABSTRACT

Lactating cows are relatively inefficient in converting dietary N to milk N compared with the efficiency of N use for growth in simple-stomached animals. The majority of productive N losses occur in the postabsorptive system. The aim of the study was to test whether predicted metabolizable protein (MP) and dietary energy exerted independent effects on milk protein synthesis and postabsorptive N efficiency. If true, postabsorptive N efficiency would be expected to be greater when animals are fed high-energy diets. Forty mid-lactation cows (32 multiparous Holstein and 8 primiparous Holstein × Jersey crossbreds) were used in a complete randomized design with a 2 × 2 factorial arrangement of diets. Cows were assigned to 1 of 4 dietary treatments: high-energy, high-protein (HE/HP); high-energy, low-protein (HE/LP); low-energy, high-protein (LE/HP); and low-energy, low-protein (LE/LP). Energy concentrations were 1.55 (HE/HP and HE/LP) or 1.44 (LE/HP and LE/LP) Mcal of net energy for lactation (NE_L)/kg of dry matter (DM). Changes in predicted MP were achieved by feeding diets with 6.6 (HE/HP and LE/HP) or 4.6% (HE/LP and LE/LP) ruminally undegradable protein (DM basis). Ruminally degradable protein was held constant at 10.1% of DM. All cows were fed the HE/HP diet from d 1 to 21 followed by the respective treatments from d 22 to 43 (n = 10). Milk protein yield was reduced as dietary energy was reduced. Milk yield followed a similar pattern as milk protein yield. There was a trend for decreased milk yield as crude protein was reduced. There were no interactions between dietary energy and protein for either milk or protein yield. Plasma amino acid concentrations were not affected by treatment. Milk urea N was affected by energy and protein with a significant interaction (HE/HP = 17.2, HE/LP = 12.2, LE/HP = 21.0, LE/LP = 12.2 mg/dL). Nitrogen efficiency calculated from predicted MP supply was affected by energy and protein supplies with no apparent interaction and ranged from a low of 31% (LE/HP) to a

high of 43% (HE/LP). The National Research Council model would predict N efficiency more accurately if a representation of the effects of energy on N efficiency were included in the postabsorptive system.

Key words: metabolizable protein, energy, nitrogen efficiency

INTRODUCTION

The lactating dairy cow has a relatively low efficiency of converting dietary N to milk N when fed to NRC (2001) model requirements (N output in milk/N intake; Bequette et al., 1998; Castillo et al., 2000), and efficiency decreases as dietary CP content increases (Broderick, 2003; Ipharraguerre and Clark, 2005). An improvement in N efficiency would lead to increased N capture and reduced N losses in the lactating cow (Jonker et al., 2002). Most of the N losses occur in the postabsorptive tissues (e.g., gut, liver), and the magnitude of the losses is variable (Hanigan, 2005). The NRC (2001) assumes fixed conversion partial efficiencies in calculating N requirements for postabsorptive processes in the lactating cow. The efficiency of use of MP for lactation is assumed constant at 0.67, meaning that each gram of milk protein output requires 1.5 g of MP. Similarly, the efficiency of MP use is assumed constant for maintenance functions. The model thus predicts that an increase in milk protein output in response to increased dietary energy can occur only if additional MP is provided to support the increase in milk protein output for a given animal.

A meta-analysis demonstrated that energy supply affects the capacity for milk protein synthesis, but it did not suggest that the efficiency of conversion of dietary protein to milk protein was affected by energy supply when cows were fed at or below N requirements (Hanigan et al., 1998). It is possible that the effects of energy on milk protein synthesis are caused entirely by changes in microbial growth and outflow from the rumen. However, Castillo et al. (2001) reported that the efficiency of N utilization was improved and N excretion reduced when cows were fed diets low in degradable starch, which is not consistent with the hypothesis of a microbial protein response. Ruminal infusions of

Received October 5, 2008.

Accepted January 5, 2010.

¹Corresponding author: mhanigan@vt.edu

propionate were also observed to increase milk protein percentage and yield (Raggio et al., 2006), and post-ruminal infusions of starch stimulated milk protein output (Reynolds et al., 2001). These observations all support a role for energy supply in regulating postabsorptive N utilization and efficiency independent of the effects of metabolizable protein supply.

Rulquin et al. (2004) concluded that lactating cows fed isonitrogenous, isoenergetic diets and duodenally infused with glucose increased arterial flux and mammary uptake of essential amino acids (EAA), which led to increased milk protein output. Although Lapierre et al. (2006) concluded that the splanchnic tissues (liver and portal-drained viscera) were the primary cause of poor efficiency of postabsorptive AA use for milk protein, the relatively low affinity for AA exhibited by these tissues does not support that hypothesis. First-pass removal has been shown to be low, and thus these tissues are not preventing AA from reaching the mammary gland (MG). The problem lies with recycling of AA not used by mammary in a given pass. The splanchnic tissues receive 50% of the cardiac output (Davis et al., 1988). Therefore, a large proportion of AA not used by the MG is returned to the splanchnic tissues (Hanigan, 2005). This constant recycling of unused AA to splanchnic tissues results in a 60% loss of the postabsorptive AA supply on a daily basis (Hanigan, 2005). Thus, increased mammary uptake and use for protein synthesis would reduce recycling of AA to the splanchnic tissues and improve efficiency of AA use by the MG; that is, excessive splanchnic catabolism is an effect rather than a cause.

Certainly, the MG can be very efficient at extracting AA when they are needed. A dietary deficiency of a single EAA triggered a 43-fold increase in transport activity in the MG for that single EAA plus a 33% increase in blood flow reaching the MG (Bequette et al., 2000). Thus, it is clear that the MG is able to alter EAA transport activity, which will increase in response to decreased supply of EAA or increased use of EAA for protein output (i.e., increased milk protein synthesis). This results in variable postabsorptive transfer efficiencies from the gut to milk protein. Therefore, if energy supply can stimulate milk protein output, the MG should respond by increasing AA removal from blood resulting in reduced recycling of AA to other tissues and improved efficiency.

We hypothesized that milk protein yield could be maintained and N efficiency improved when reduced dietary MP (elicited by changes in RUP) was combined with high dietary energy as opposed to the single-limiting-nutrient paradigm assumed by the NRC (2001) model. This can only be true if energy and protein exert independent, additive effects on milk production.

The first objective of this study was to assess the efficiency of N utilization under predicted conditions of MP deficiency when dietary energy density was varied. A second objective was to test the NRC (2001) model predictions of energy and protein requirements for the lactating dairy cow under these circumstances.

MATERIALS AND METHODS

Animals and Diets

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Virginia Tech. Thirty-two multiparous Holstein and 8 primiparous Jersey $\times$ Holstein cross-bred cows (186 ± 89 DIM) were selected from the Virginia Tech dairy herd. Cows were housed in a freestall barn at the Virginia Tech dairy complex and individually fed using a Calan door system (American Calan Inc., Northwood, NH). Animals were milked twice daily at 0230 and 1500 h. Milk weights and BW were recorded automatically at each milking. Body condition scores were recorded for each cow at d 21 and 43 according to the methods of Wildman et al. (1982). Animals were balanced (stratified) in 4 groups based on DIM, parity, breed, BW, and milk production, and randomly assigned to 1 of the 4 treatments. The study was a complete randomized design with a 2×2 factorial arrangement of treatments. Dietary treatments were 2 levels of energy (high and low) and 2 levels of MP (high and low) indicated as following: high-energy, high-protein (HE/HP); high-energy, low-protein (HE/LP); low-energy, high-protein (LE/HP); and low-energy, low-protein (LE/LP; Table 1). Diets were formulated to meet NRC (2001) recommendations for RDP, minerals, and vitamins of a mid-lactation dairy cow weighing 635 kg (BCS = 3.0) and producing 36.3 kg of milk/d containing 3.5% fat and 3.0% protein and consuming 22.9 kg/d of DM. Final diets contained either 50% forage and 50% concentrate (LE/HP and LE/LP) or 39% forage and 61% concentrate (HE/HP and HE/LP) on a DM basis (Table 1). Ruminally undegradable protein was manipulated while holding RDP constant by varying the amounts of soybean meal and ruminally protected soybean meal (HiVap). The latter was manufactured by Land O'Lakes/Purina Feed (Gainesville, GA) using an extrusion-based process (Patent No. 5683739) resulting in conformational and chemical changes in the protein that confer ruminal stability. The resulting product was previously found to contain 51.6% CP, which was 15% soluble and 85% insoluble, and the insoluble portion had a ruminal degradation rate of 0.02%/h (Cyriac et al., 2008). Tallow was included in 2 treatments to maintain constant energy concentrations as dietary

Table 1. Predicted nutrient requirements as determined from NRC (2001) on % of DM basis

Item	Experimental diet ¹			
	HE/HP	HE/LP	LE/HP	LE/LP
Corn silage	34.97	34.89	45.91	45.92
Mixed grass + legume silage	4.81	4.80	4.80	4.80
Cottonseed hulls	—	—	20.53	17.89
Soybean hulls	25.80	31.62	10.70	19.65
Corn grain, ground, dry	22.17	22.13	—	—
Tallow	—	1.19	—	1.13
Urea	1.16	1.24	0.74	0.79
Soybean meal, solvent (48% CP)	3.16	2.64	9.37	8.35
Protected soybean meal ²	6.66	—	6.57	—
Calcium carbonate	0.30	0.30	0.30	0.30
Calcium phosphate (di-) ³	0.35	0.57	0.48	0.63
Sodium bicarbonate	0.20	0.20	0.20	0.20
Salt	0.24	0.24	0.24	0.22
Trace mineral and vitamin mix ⁴	0.10	0.10	0.10	0.10

¹HE and LE = high and low energy, respectively; HP and LP = high and low protein, respectively.

²HiVap, Land O' Lakes/Purina Feed (Gainesville, GA).

³Contained 22% Ca and 19.3% P.

⁴Land O' Lakes/Purina Feed; formulated to provide (per kg of dietary DM): 25×10^5 IU of vitamin A, 400,000 IU of vitamin D, and 10×10^5 IU of vitamin E, 0.1 mg of Co, 12 mg of Cu, 0.7 mg of I, 60 mg of Fe, 48 mg of Mn, 48 mg of Zn, 0.3 mg of Se.

protein was manipulated and to compensate for the fat present in the protected soybean meal. The percentages of forages and concentrate were adjusted weekly on an as-fed basis to reflect changes in the DM content of the forages and concentrates. Diets were mixed at 0800 h and fed once daily as a TMR. Feed offered and refused was recorded daily. Feed offered each day was adjusted to achieve between 5 and 10% refusals. The HE/HP diet was fed from d 1 through 21 (the covariate period) followed by the respective experimental diets from d 22 to 43 (the treatment period).

Sample Collection and Analyses

Samples of forages, concentrates, and Orts were collected twice weekly to assess DM content of the ingredients and refusals. Feed samples were dried overnight at 105°C for DM content (Mechanical Convection Oven, Freas 645, Thermo Electron Corp., Waltham, MA). During collection weeks, samples of ingredients were obtained daily and stored frozen at -20°C for later chemical analyses. Daily subsamples were dried at 50°C for 48 h and ground through a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA). Subsamples were combined by treatment and period on an equal weight basis, and submitted to Cumberland Valley Analytical Service Inc. (Hagerstown, MD) for analysis of ether extract (method 2003.05; AOAC, 2006) and N (method 990.03; AOAC, 2000), starch (Holm et al., 1986), sugars (Dubois et al., 1956), lignin, ADF, and NDF (Van Soest et al., 1991). Ash was determined us-

ing method 942.05 (AOAC, 2000), and Ca, P, Mg, and K were determined by inductively coupled plasma spectrometry. Sulfur was determined using a Leco analyzer (Leco Corp., St. Joseph, MI), and Na was determined using method 985.01 (AOAC, 2000).

Milk samples were collected from 6 consecutive milkings during d 19 through 21 for covariate period analysis and during d 41 through 43 for treatment period analysis (wk 3 and 6). Individual milk samples were analyzed for fat, true protein, lactose, TS, and somatic cells (AOAC, 1997) by infrared analyses (DHIA, Blacksburg, VA; Foss North America, Eden Prairie, MN). The MUN analyses were conducted using the Berthelot procedure on a ChemSpec 150 Analyzer (DHIA, Blacksburg, VA; Bentley Instruments, Chaska, MN). Daily milk composition was calculated from the weighted a.m. and p.m. observations.

Two blood samples were collected from the coccygeal vessel on d 21 and 43 into vacutainer tubes containing either sodium heparin or sodium fluoride plus EDTA and placed immediately on ice (10 or 5 mL; Becton Dickinson and Co., Franklin Lakes, NJ). Plasma was harvested by centrifugation at $1,300 \times g$ for 20 min at 4°C within 2 h of blood collection. Plasma was stored at -20°C until analyses were conducted for AA, glucose, acetate, lactate, and β -hydroxybutyrate. Free AA were determined by isotope dilution using a gas chromatograph-mass spectrometer (Focus-PolarisQ GC-MS, Thermo Electron Corp., Waltham, MA) according to the methods of El-Kadi et al. (2006) and Calder et al. (1999).

Proton nuclear magnetic resonance spectroscopy (NMR) was conducted on the NaF-treated plasma samples to assess glucose, acetate, lactate, and β -hydroxybutyrate concentrations (Beckonert et al., 2007). Briefly, 0.5 mL of plasma was deproteinized by addition of an equal volume of acetonitrile. Samples were vortexed and centrifuged at $14,000 \times g$ for 20 min at 4°C. The supernatant was removed and freeze-dried (Savant SpeedVac SC 110, GMI Inc., Ramsey MN). Dried samples were resuspended in 0.5 mL of phosphate-buffered (100 mM) D₂O (Sigma Aldrich, St. Louis, MO) with 0.5% sodium azide and 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) as an internal standard (0.45 mM final concentration). Proton NMR (Varian 400, Palo Alto, CA) was performed and the spectra were baseline adjusted, phase corrected, and the peak areas integrated using the Know-It-All Informatics System (Bio-Rad, Hercules, CA). Validation of this procedure using samples from a previous study indicated that the peak areas were linearly correlated with observed concentrations. However, apparent recoveries were not 100% when comparing the NMR-determined concentrations with enzymatically determined concentrations. Although the technique clearly detects relative differences among samples with reasonable precision, we are not confident that the method can accurately determine concentrations. Thus, metabolite values were presented as relative peak areas with respect to the DSS peak area.

Statistical Analysis

Data were tested for normality using the univariate procedure of SAS (SAS Institute, 2004). Nutrient and DM intake, BW, milk yield, and milk composition data were analyzed as repeated measures in time (days) using the Mixed procedure of SAS (SAS Institute, 2004) to test for differences in the treatment means. Changes over time are expected in these dependent variables; thus, a model including repeated measures analysis was considered appropriate. Excepting milk composition, data from the last 7 d of each period were analyzed to allow for complete diet adaptation. Milk composition data from d 19, 20, 21, 41, 42, and 43 were subjected to analyses. Body condition score and blood metabolites were analyzed using the Mixed procedure without the effect of time. The statistical model used is described in Table 2. Breed did not affect variables of interest, thus the effect of breed and breed interactions with all other effects were removed from the model. Interactions between main effects were removed from the model if they were not significant. The autoregressive covariance structure was used for each variable analyzed. This structure was chosen based on Akaike's informa-

Table 2. Definition of effects for the statistical model

Effect ¹	Type	df	ddfm ²
Energy (E)	Fixed	1	31
Crude protein (P)	Fixed	1	31
Breed (B)	Fixed	1	31
E $\times$ B	Fixed	1	31
P $\times$ B	Fixed	1	31
E $\times$ P	Fixed	1	31
E $\times$ P $\times$ B	Fixed	1	31
b	Fixed	1	31
Cow (E $\times$ P $\times$ B)	Random	31	
Day (D)	Fixed	6	192
E $\times$ D	Fixed	6	192
P $\times$ D	Fixed	6	192
E $\times$ P $\times$ D	Fixed	6	192
B $\times$ D	Fixed	6	192
E $\times$ B $\times$ D	Fixed	6	192
P $\times$ B $\times$ D	Fixed	6	192
E $\times$ P $\times$ B $\times$ D	Fixed	6	192
Residual	Random	192	
Total		279	

¹The energy effect (high or low E), the crude protein effect (high or low P), the breed effect (Holstein or crossbreed), the effect of the regression analysis of the covariate b for the variable of interest, and the effect of day (1 through 7).

²ddfm = balanced denominator degrees of freedom for *F*-test.

tion criterion (AIC) and corrected AIC (AICC) fit statistics and the expected relationship among days. The smallest value for AIC and AICC was used to identify the appropriate covariance structure. Observations from d 15 to 21 were used for covariate analysis of BW, DMI, and milk yield. Milk composition data on d 19, 20, and 21 were used for covariate analysis of milk component percentages and yield; however, they were removed from the model because they were not significant. The covariate was removed from the model if it was not significant. The effect and interactions of breed were removed from the model because they were not significant. Observations from the covariate period were averaged and included as a covariate adjustment in the model. Unless otherwise stated, significance was declared at $P < 0.05$. All results are reported as least squares means.

NRC Model Analysis

Predictions of NE_L and MP by the NRC (2001) model were assessed for accuracy using the observed values for production and N efficiency. Model predictions were compared with the LSM values from the statistical analysis of DMI, milk yield, milk composition, and BW for each dietary treatment. Diet composition was set to values listed in Table 3, and observed DMI was used to set the feeding rate of each ingredient. The nutrient content of each grain mix was then calculated using tabular values and compared with the observed chemi-

Table 3. Analyzed chemical composition of the feed ingredients used in the experimental diets (% of DM basis)

Item	Ingredient ¹					
	Corn silage	Haylage	Conc. mix A	Conc. mix B	Conc. mix C	Conc. mix D
DM, % of feed	28.5	50.9	90.3	89.5	89.8	90.2
NDF, % of DM	43.7	48.8	36.0	41.5	50.1	54.6
ADF, % of DM	26.3	38.0	22.5	27.2	36.1	40.9
CP, % of DM	8.9	17.6	24.2	20.0	30.6	21.8
Fat, % of DM	3.6	2.5	2.8	3.7	2.1	4.0
NFC, % of DM	42.1	22.1	34.1	32.2	11.9	14.6
Lignin, % of DM	3.0	7.4	1.8	1.8	6.0	5.5
Starch, % of DM	30.0	1.2	28.5	24.3	4.0	2.8
Sugar, % of DM	1.2	4.3	3.7	3.9	5.1	3.6
Ash, % of DM	3.1	10.8	5.6	5.8	8.6	8.6
Calcium, % of DM	0.17	0.86	0.61	0.83	0.98	0.97
Phosphorous, % of DM	0.20	0.29	0.33	0.47	0.49	0.50
Magnesium, % of DM	0.15	0.23	0.19	0.20	0.27	0.25
Potassium, % of DM	1.04	3.01	1.07	0.94	1.50	1.23
Sulfur, % of DM	0.13	0.26	0.19	0.13	0.25	0.19
Sodium, % of DM	0.002	0.027	0.479	0.497	0.849	0.663

¹Concentrate mix A was used to formulate the high-energy high-protein diet, concentrate B was used to formulate the high-energy low-protein diet, concentrate C was used to formulate the low-energy high-protein diet, and concentrate D was used to formulate the low-energy low-protein diet.

cal composition of the grain mix in Table 3. Adjustment was needed because the tabular values of CP, ADF, and NDF differed from the observed values obtained by chemical analysis. Because ruminally protected soybean meal (SBM) and SBM were the major contributors of CP in the grain mixes, the tabular CP content of these 2 ingredients was increased to bring the average CP of the grain mixes in line with the predicted values (from 52.2 to 65% of DM for ruminally protected SBM and from 53.8 to 54.9% of DM for SBM). The large adjustment required for the ruminally protected SBM to bring observed and predicted grain mix CP contents in line with observed values likely reflects mixing or formulation errors at the plant, given that it would be highly improbable that a ruminally protected SBM ingredient could have such high CP content. It is also possible that the chemical analyses of the grain mixes were in error, but reanalysis confirmed the original observations. The ADF and NDF content of cottonseed hulls and soybean hulls were modified in a similar manner to reflect the observed chemical analysis of the grain mixes. The tabular ADF content for cottonseed hulls was reduced from 65.0 to 55.0% DM, and the tabular value for soybean hulls was increased from 44.6 to 47.0% DM. The tabular value for NDF in soybean hulls was increased from 60.3 to 70.0% DM.

RESULTS

Chemical Composition of Diets and Feed Ingredients

The nutrient requirements as predicted by NRC (2001) are shown in Table 1. The observed nutrient

concentrations of dietary components are listed in Table 3. The CP, ADF, NDF, and NFC contents of the experimental diets differed from the formulated values because of nutrient variation in the ingredients compared with tabular values used for formulation and possible variation in formulation as noted above. The predicted energy content of the treatments was slightly lower than expected probably because of the greater ADF and NDF contents in the concentrate mixes (Table 4), but the differences among treatments was maintained. As intended, the CP difference between the HP and LP treatments was greater than 3.0 percentage units (Table 4).

Animal Performance

Least squares means for DMI, BW, BCS, milk yield, and milk composition are presented in Table 5. Dry matter intake was not affected by treatment. As anticipated, CP intake was greater for cows consuming the HE/HP and LE/HP diets than for those consuming the HE/LP and LE/LP diets (4.7 and 4.6 vs. 3.7 and 3.6 kg/d, respectively). Reduced dietary energy decreased milk production ($P < 0.003$), and there was a trend for decreased production in association with inadequate dietary CP ($P < 0.08$). There was no apparent interaction of energy and CP effect on milk production. Lactose concentration and lactose yield decreased in association with feeding the LE diets ($P < 0.01$ and 0.01 , respectively). Cows that received HE diets (HE/HP and HE/LP) produced more milk protein ($P < 0.001$; 1.13 and 1.03 kg/d, respectively).

Table 4. Composition of experimental diets and predicted nutrient requirements as determined from the NRC (2001) model¹

Item	Experimental diet ²			
	HE/HP	HE/LP	LE/HP	LE/LP
DM, ³ %	49.6	47.8	46.0	44.8
CP, % of DM	18.7	15.2	19.1	15.5
NDF, % of DM	38.7	42.1	48.9	51.8
ADF, % of DM	24.5	26.8	31.2	33.4
RDP, % of DM	11.5	10.5	11.2	10.3
RUP, % of DM	7.2	4.7	7.9	5.2
NFC, % of DM	35.6	34.9	25.3	25.0
Crude fat, % of DM	3.3	4.4	2.9	4.0
NE _L , Mcal/kg	1.54	1.53	1.45	1.45
RDP required, g/d	2,463	2,453	2,355	2,141
RDP supplied, g/d	2,907	2,639	2,866	2,364
RDP balance, g/d	444	186	511	223
RUP required, g/d	1,333	1,265	1,203	1,075
RUP supplied, g/d	1,826	1,176	2,026	1,203
RUP balance, g/d	493	-89	823	128
MP required, g/d	2,574	2,424	2,395	2,089
MP balanced, g/d	412	-68	679	97
MP supplied, g/d	2,986	2,356	3,074	2,186
MP allowable milk, kg/d	43.5	32.0	45.6	28.9
NE _L allowable milk, kg/d	41.0	39.6	37.1	30.3

¹Values predicted using actual DMI, milk yield, and components for each treatment. NRC (2001) ingredient composition was modified to reflect the actual chemical values for CP, ADF, and NDF or, in the case of the grain mixes, the composition that would be required to achieve the observed mix values.

²HE and LE = high and low energy, respectively; HP and LP = high and low protein, respectively.

³Actual DM of TMR for each treatment.

than those fed LE diets (LE/HP and LE/LP; 0.91 and 0.87 kg/d, respectively). Milk protein percentage was not affected by dietary energy, but there was a trend for a CP effect to increased protein percentage ($P < 0.10$). Energy and CP both affected milk fat percentage ($P < 0.004$ and 0.01 , respectively), and there was no interaction between treatments. However, milk fat yield was not different among treatments. Nonfat milk solids percentage and yield were reduced ($P < 0.001$ and 0.01 , respectively) when dietary energy was inadequate (LE/HP and LE/LP). Milk urea N was reduced in cows fed low CP diets (HE/LP and LE/LP; 12.2 and 12.0 mg/dL, respectively) compared with cows fed high CP diets (HE/HP and LE/HP; 17.2 and 21.0 mg/dL) but at low CP, provision of addition energy did not result in a reduction in MUN concentrations. The interaction between energy and CP was significant for MUN ($P < 0.005$). Somatic cell counts were reduced in cows fed HE diets ($P < 0.02$). Reduced CP intake was associated with decreased ending BW ($P < 0.04$). Body condition scores were not affected by energy or protein and there was no interaction.

NRC Model Analysis

Observed DMI was greater than that used in formulating the diets, resulting in a nutrient supply greater than expected (Table 4). After adjusting for observed

intakes, ingredient composition, and milk performance, the NRC (2001) model overpredicted allowable milk yields for the HE/HP, LE/HP, and LE/LP diets and slightly underpredicted yields for cows fed the HE/LP diets. The relative predicted responses to energy on the high CP diets were close to observed, with the model predicting a 3.9 kg/d response and an observed response of 5.4 kg/d. This difference is well within the error of predicting for the model. However, the relative responses to CP were greatly overpredicted with predicted responses of 9.0 [(HE/HP) - (HE/LP), Table 4] and 8.2 kg/d [(LE/HP) - (LE/LP)] for the high- and low-energy diets, respectively, and observed responses of 2.8 and 4.7 kg/d (Table 5), respectively.

Amino Acids and Blood Metabolites

Although DMI, CP, and milk protein yield were affected by treatments, concentrations of plasma free nonessential AA and EAA did not change across treatments (Table 6). There was an effect of energy on plasma β -hydroxybutyrate concentrations ($P < 0.001$) and an interaction between energy and CP on blood glucose concentrations ($P < 0.01$; Table 7).

Nitrogen Utilization

Nitrogen supply was reduced for LP diets (Table 8). Cows that received the HE/HP and LE/HP diets had

Table 5. Least squares means of intake, milk production, BW, and BCS for cows fed varying amounts of energy and protein

Item	Experimental diet ¹				SEM	Effect ($P < $)		
	HE/HP	HE/LP	LE/HP	LE/LP		Energy (E)	Protein (P)	E $\times$ P
Intake, kg/d								
DM	24.8	24.4	24.9	23.2	0.8	0.50	0.21	0.39
CP	4.7	3.7	4.6	3.6	0.1	0.001	0.001	0.84
NDF	9.5	10.2	12.2	12.0	0.1	0.001	0.25	0.02
ADF	6.0	6.5	7.8	7.7	0.1	0.001	0.12	0.04
NE _L	38.8	37.8	35.3	33.9	1.5	0.01	0.39	0.90
Milk production								
Milk yield, kg/d	36.1	33.3	30.7	26.0	2.0	0.003	0.08	0.64
Lactose, kg/d	1.71	1.69	1.49	1.28	0.12	0.01	0.32	0.43
True protein, kg/d	1.13	1.03	0.91	0.87	0.05	0.001	0.16	0.57
Fat, kg/d	1.14	1.26	1.13	1.18	0.09	0.61	0.32	0.72
MSNF, ² kg/d	3.13	3.04	2.72	2.41	0.20	0.01	0.29	0.58
Lactose, %	4.97	4.94	4.76	4.79	0.03	0.001	0.95	0.34
True protein, %	3.24	3.12	3.19	3.16	0.04	0.90	0.10	0.29
Fat, %	3.46	3.75	3.82	4.30	0.15	0.004	0.01	0.54
MSNF, %	9.12	8.98	8.85	8.88	0.04	0.001	0.24	0.07
MUN, mg/dL	17.2	12.2	21.0	12.0	0.81	0.01	0.001	0.005
SCC, $\times 1,000$ cells/mL	32.7	23.5	132.8	161.3	56.0	0.02	0.85	0.71
Initial BW, kg	650	629	591	625	21.0	0.09	0.81	0.12
Final BW, kg	642	636	651	643	4.0	0.13	0.04	0.80
Initial BCS	3.01	3.02	3.00	3.01	0.01	0.89	0.75	0.64
Final BCS	3.09	3.11	2.90	2.97	0.08	0.24	0.35	0.54

¹HE and LE = high and low energy, respectively; HP and LP = high and low protein, respectively.

²Milk solids nonfat.

greater predicted urinary N excretion (292 and 338 g/d, respectively) relative to those that received the HE/LP and LE/LP treatments (216 and 202 g/d, respectively), and there was a significant interaction ($P < 0.01$). High CP diets increased predicted urinary N excretion but especially so at low dietary energy. There was an effect of energy on milk N output ($P < 0.002$) with cows fed HE diets increasing N output in milk relative to those

animals fed LE diets. Consistent with our hypothesis, postabsorptive N efficiency improved as energy increased, with the greatest efficiency observed for cows fed HE diets ($P < 0.001$; HE/HP and HE/LP; 37.1 and 43.0%, respectively) relative to cows fed LE diets (LE/HP = 31.0 and LE/LP = 38.5%). Likewise, cows fed LP diets had greater N efficiency compare with cows fed HP diets ($P < 0.001$).

Table 6. Concentrations of free AA (μM) in plasma of cows fed varying amounts of energy and protein

Item	Experimental diet ¹				SEM	Effect ($P < $)		
	HE/HP	HE/LP	LE/HP	LE/LP		Energy (E)	Protein (P)	E $\times$ P
Total essential AA	907	924	913	997	53	0.47	0.36	0.53
Arg	109	118	100	116	14	0.71	0.38	0.81
Ile	100	100	104	100	6	0.79	0.72	0.72
Leu	150	145	150	221	29	0.19	0.26	0.19
Lys	82	85	93	91	7	0.26	0.92	0.75
Met	10	17	13	14	2	0.92	0.23	0.27
Phe	96	56	67	62	18	0.56	0.24	0.37
Thr	85	93	86	92	7	0.99	0.35	0.91
Trp	40	44	47	46	3	0.13	0.68	0.44
Val	207	236	211	221	16	0.73	0.27	0.57
Total nonessential AA	623	622	605	635	42	0.95	0.74	0.72
Ala	184	199	196	187	10	0.99	0.80	0.25
Asp	14	15	15	14	1	0.80	0.50	0.34
Glu	52	50	46	45	3	0.18	0.69	0.92
Gly	262	239	246	252	34	0.96	0.79	0.67
Pro	82	90	85	93	4	0.64	0.09	0.98
Ser	89	97	99	100	7	0.40	0.60	0.66
Tyr	50	52	46	50	3	0.49	0.46	0.87

¹HE and LE = high and low energy, respectively; HP and LP = high and low protein, respectively.

Table 7. Relative amount of plasma metabolites with regard to 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS)¹ concentration (100%) as analyzed by nuclear magnetic resonance method in cows fed the experimental diets

Metabolite	Experimental diet ²				SEM	Effect (<i>P</i> <)		
	HE/HP	HE/LP	LE/HP	LE/LP		Energy (E)	Protein (P)	E × P
Acetate	59.9	70.4	75.1	91.2	14.0	0.18	0.32	0.83
β-Hydroxybutyrate	18.9	16.3	31.0	30.1	3.2	0.001	0.56	0.78
Lactate	75.3	89.4	70.5	91.7	21.1	0.95	0.40	0.86
Glucose	26.2	23.1	23.2	27.4	1.4	0.62	0.70	0.01

¹The DSS peak area (0.45 mM) was set to 100% and the other metabolite peak areas were expressed as a percentage of the DSS peak area.

²HE and LE = high and low energy, respectively; HP and LP = high and low protein, respectively.

DISCUSSION

Lactation Response

Cows fed the low CP diet were apparently protein deficient based on the trend for a milk yield response to increased CP supply ($P < 0.08$) and decreased ending BW compared with beginning BW ($P < 0.04$). Based on the single-limiting-nutrient paradigm used in the NRC, these protein-deficient animals should not have been able to respond to increased energy supply, yet they clearly did. The lack of an interaction between energy and CP for both milk and milk protein yields supports this conclusion. Therefore, the effects of energy and CP were additive and independent. Greater efficiency of N use in the postabsorptive system can be expected when animal are fed a high-energy diet as compared with a low-energy diet. The apparent independent effects of energy and CP are consistent with observations at the tissue level wherein increased rates of MG protein synthesis resulted in increased removal of AA from blood and reduced recycling of AA to and catabolism by the splanchnic bed and other peripheral tissues (Bequette et al., 2000, 2001; Hanigan et al., 2000; Hanigan, 2005). Our observations are also consistent with the observations of Raggio and coworkers (2006) who concluded that propionate infusions promoted milk protein concentration and yield by increasing AA uptake by the MG. The observed improvement in N efficiency in the current study from 38.5% for the LE/LP diet to 43.0%

for the HE/LP diet (Table 8) are also consistent with this conclusion. Finally, the N efficiency results are consistent with those of Broderick (2003) if one assumes the responses in the prior work resulted from changes in RUP rather than RDP as concluded by the authors. The lack of an interaction between energy and protein supply was also consistent with the observations of Broderick (2003) and supportive of a postabsorptive mechanism given that dietary RDP concentrations exceed NRC requirements in both studies and were controlled in the current work.

Milk urea nitrogen was significantly affected by both energy and CP supply, and there was a significant interaction. Provision of additional protein in the diet when dietary energy was low resulted in the greatest MUN concentrations. The HE/HP diet had intermediate MUN concentrations consistent with increased use of CP for milk protein. Low dietary CP resulted in comparably low MUN regardless of dietary energy. It was anticipated that the HE/LP diet would result in the lowest MUN concentration given the comparable N intakes and the significant effects of energy on milk protein output and N efficiency (Table 8).

Concentrations of MUN in our study were greater than those reported by other authors (Broderick, 2003; Ipharraguerre and Clark, 2005). Kohn and coworkers (2004) compared different methods to determine MUN and concluded that the Bentley, CL10, Foss 6000, and Skalar instruments explained more than 98% of the

Table 8. Nitrogen utilization in cows fed varying amounts of energy and protein

Item	Experimental diet ¹				SEM	Effect (<i>P</i> <)		
	HE/HP	HE/LP	LE/HP	LE/LP		Energy (E)	Protein (P)	E × P
N supplied in MP, g/d	470	372	468	364	14	0.71	0.001	0.84
Milk protein N, g/d	176	160	143	136	8	0.002	0.19	0.63
Predicted urinary N, ² g/d	292	216	338	202	11	0.20	0.001	0.01
N efficiency, ³ %	37.1	43.0	31.0	38.5	1.4	0.001	0.001	0.53

¹HE and LE = high and low energy, respectively; HP and LP = high and low protein, respectively.

²Estimated urine N output = $0.026 \times \text{MUN (mg/dL)} \times \text{BW (kg)}$; Kauffman and St-Pierre (2001).

³N efficiency (%) = $100 \times \text{milk N (g/d)} / \text{N supplied in MP (g/d)}$.

variance that was due to farm-to-farm variance. Machine accuracy and precision in DHIA laboratories is checked monthly against known standards (the CL10 method), and results from a split-sample test indicated the machine was properly calibrated and reporting accurately.

Plasma β -hydroxybutyrate concentrations were affected by dietary energy content. The large changes in ruminally fermentable carbohydrate content may have contributed to this finding; however, endogenous production from partial oxidation of fatty acids in the liver was likely stimulated by the dramatic decrease in energy metabolite supply to the animal, which would be expected to elicit a change in fatty acid mobilization from adipose. The liver would also be expected to release acetate under these conditions (Hanigan et al., 2004), which may have been adequate to offset any reductions in acetate supply associated with lower diet digestibility. Low milk and lactose yield would be likely to reflect reductions in mammary glucose uptake. Thus, reduced mammary removal would have caused increased glycemia.

Nitrogen Utilization

Predicted postabsorptive N efficiency was affected by dietary energy and CP independently with the greatest efficiency observed for cows fed the HE/LP diet (43.0%) and lesser efficiency for HE/HP and LE/HP diets (37.1 and 31.0%, respectively). These results are in agreement with those presented by Broderick (2003) that reported an increase in N efficiency as CP in the diet was decreased and energy density increased. Additionally, Broderick (2003) reported no significant interaction between CP and energy. Reducing N intake by approximately 100 g resulted in a <16-g reduction in milk N output, whereas changing energy density in the diet changed milk N output by approximately 30 g/d. The present study is consistent with observations of Castillo et al., (2000) who reported that reductions in N intake from 750 to 400 g/d had a much bigger effect on N partitioning to urinary output ($R^2 = 0.62$) than to milk protein output ($R^2 = 0.14$). These findings suggest that postabsorptive N efficiency in lactating cows is variable and independently affected by N and energy supply. Feeding diets with less RUP than recommended by the NRC (2001) in combination with high concentrations of dietary energy may be a useful strategy to improve the N capture in milk protein.

NRC Analysis

The partitioning of absorbed AA is represented by a fixed set of transfer coefficients in the NRC (2001)

model. Furthermore, this model assumes that a single nutrient limits animal performance. Thus, if CP is the first-limiting nutrient, as appears to be the case herein for the HE/LP and LE/LP diets (Table 5), then the model predicts that the animals cannot respond to provision of any other nutrient. However, milk production increased by 6.6 kg/d and milk protein output by 160 g/d when additional energy was provided with fixed dietary CP concentrations (LE/LP vs. HE/LP) resulting in a 4.5-percentage-unit increase in the efficiency of MP use for milk production. These observations are consistent with those of Bequette et al. (2000), in which the efficiency of transfer of AA into milk protein in the MG was found to be variable. Not only did the model fail to predict the response to energy in a protein-limiting situation, but it also overpredicted responses to CP in general. The model predicted a 9 kg/d milk response to provision of additional CP to the HE/LP diet and an 8.2 kg/d response for the LE/LP diet, but the cows exhibited a 2.8 kg/d response for the HE/LP diet and a 4.7 kg/d response for the LE/LP diet; that is, the observed response was, at best, 50% of the predicted response. These observations are consistent with the independent effects of energy and N supply and the variable N capture efficiencies observed by Hanigan et al. (1998). Because this variable efficiency is not captured in the NRC (2001) model, an improved representation of postabsorptive partitioning of N should result in a model that more accurately predicts N requirements. Additionally, the new representation would result in improved feeding programs and reduced N release to the environment.

CONCLUSIONS

The effects of postabsorptive energy and CP supplies on milk and protein yields were independent, and greater energy or CP was able to stimulate production. The efficiency of N utilization was maximal when feeding the combination of high energy and low CP in the diet. Therefore, the single-limiting-nutrient concept used in the NRC should be replaced with a multi-nutrient representation that accommodates at least energy and CP supply to better predict milk protein output and N efficiency.

ACKNOWLEDGMENTS

This material is based upon work supported by the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, under Project No. NC-1009. The authors acknowledge funding provided by the Virginia Agricultural Council (Richmond) and Land O' Lakes/Purina Feed LLC (Gray Summit, MO)

in support of this study, general departmental funding provided by the Virginia State Dairymen's Association (Bridgewater), and the Virginia Tech Dairy Complex crew for help in conducting the study. The contributions of J. Cyriac, A. Elgin, G. Moyer, H. Weeks, N. Ianni, and A. Cornman (all from Virginia Tech Dairy Complex) are gratefully acknowledged. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the US Department of Agriculture, the Virginia State Dairymen's Association, or Land O' Lakes/Purina Feed, Inc.

REFERENCES

- Association of Official Analytical Chemists. 1997. Official Methods of Analysis, 16th edition. AOAC Intl., Gaithersburg, MD.
- Association of Official Analytical Chemists. 2000. Official Methods of Analysis, 17th edition. AOAC Intl., Gaithersburg, MD.
- Association of Official Analytical Chemists. 2006. Official Methods of Analysis, 18th edition. AOAC Intl., Gaithersburg, MD.
- Beckonert, O., H. C. Keun, T. M. D. Ebbels, J. Bundy, E. Holmes, J. C. Lindon, and J. K. Nicholson. 2007. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat. Protoc.* 2:2692–2703.
- Bequette, B. J., F. R. C. Backwell, and L. A. Crompton. 1998. Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. *J. Dairy Sci.* 81:2540–2559.
- Bequette, B. J., M. D. Hanigan, A. G. Calder, C. K. Reynolds, G. E. Lobley, and J. C. MacRae. 2000. Amino acid exchange by the mammary gland of lactating goats when histidine limits milk production. *J. Dairy Sci.* 83:765–775.
- Bequette, B. J., C. E. Kyle, L. A. Crompton, V. Buchan, and M. D. Hanigan. 2001. Insulin regulates milk production and mammary gland and hind-leg amino acid fluxes and blood flow in lactating goats. *J. Dairy Sci.* 84:241–255.
- Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86:1370–1381.
- Calder, A. G., K. E. Garden, S. E. Anderson, and G. E. Lobley. 1999. Quantitation of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-(13)C amino acids as internal standards. *Rapid Commun. Mass Spectrom.* 13:2080–2083.
- Castillo, A. R., E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby, and J. France. 2001. The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.* 79:240–246.
- Castillo, A. R., E. Kebreab, D. E. Beever, and J. France. 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *J. Anim. Feed Sci.* 9:1–27.
- Cyriac, J., A. G. Rius, M. L. McGilliard, R. E. Pearson, B. J. Bequette, and M. D. Hanigan. 2008. Lactation performance of mid-lactation dairy cows fed ruminally degradable protein at concentrations lower than NRC recommendations. *J. Dairy Sci.* 91:4704–4713.
- Davis, S. R., R. J. Collier, J. P. McNamara, H. H. Head, and W. Sussman. 1988. Effects of thyroxine and growth hormone treatment of dairy cows on milk yield, cardiac output and mammary blood flow. *J. Anim. Sci.* 66:70–79.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350–356.
- El-Kadi, S. W., R. L. Baldwin, N. E. Sunny, S. L. Owens, and B. J. Bequette. 2006. Intestinal protein supply alters amino acid, but not glucose, metabolism by the sheep gastrointestinal tract. *J. Nutr.* 136:1261–1269.
- Hanigan, M. D. 2005. Quantitative aspects of ruminant splanchnic metabolism as related to predicting animal performance. *Anim. Sci.* 80:23–32.
- Hanigan, M. D., J. P. Cant, D. C. Weakley, and J. L. Beckett. 1998. An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. *J. Dairy Sci.* 81:3385–3401.
- Hanigan, M. D., L. A. Crompton, C. K. Reynolds, D. Wray-Cahen, M. A. Lomax, and J. France. 2004. An integrative model of amino acid metabolism in the liver of the lactating dairy cow. *J. Theor. Biol.* 228:271–289.
- Hanigan, M. D., J. France, L. A. Crompton, and B. J. Bequette. 2000. Evaluation of a representation of the limiting amino acid theory for milk protein synthesis. Page 127th in *Modelling Nutrient Utilization in Farm Animals*. J. P. McNamara, J. France, and D. E. Beever, ed. CABI Publishing, Wallingford, UK.
- Holm, J., I. Björck, A. Drews, and N. G. Asp. 1986. A rapid method for the analysis of starch. *Starch/Die Starke* 7:224–226.
- Ipharraguerre, I. R., and J. H. Clark. 2005. Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. *J. Dairy Sci.* 88:2556–2570.
- Jonker, J. S., R. A. Kohn, and J. High. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. *J. Dairy Sci.* 85:1218–1226.
- Kauffman, A. J., and N. R. St-Pierre. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *J. Dairy Sci.* 84:2284–2294.
- Kohn, R. A., K. R. French, and E. Russek-Cohen. 2004. A comparison of instruments and laboratories used to measure milk urea nitrogen in bulk-tank milk samples. *J. Dairy Sci.* 87:1848–1853.
- Lapierre, H., D. Pacheco, R. Berthiaume, D. R. Ouellet, C. G. Schwab, P. Dubreuil, G. Holtrop, and G. E. Lobley. 2006. What is the true supply of amino acids for a dairy cow? *J. Dairy Sci.* 89(E Suppl.) E1–E14.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Raggio, G., S. Lemosquet, G. E. Lobley, H. Rulquin, and H. Lapierre. 2006. Effect of casein and propionate supply on mammary protein metabolism in lactating dairy cows. *J. Dairy Sci.* 89:4340–4351.
- Reynolds, C. K., S. B. Cammell, D. J. Humphries, D. E. Beever, J. D. Sutton, and J. R. Newbold. 2001. Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. *J. Dairy Sci.* 84:2250–2259.
- Rulquin, H., S. Rigout, S. Lemosquet, and A. Bach. 2004. Infusion of glucose directs circulating amino acids to the mammary gland in well-fed dairy cows. *J. Dairy Sci.* 87:340–349.
- SAS Institute. 2004. *User's Guide*. Version 9.1.2. SAS Institute Inc., Cary, NC.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495–501.