J. Dairy Sci. 86:3634–3647

Adaptations in Body Muscle and Fat in Transition Dairy Cattle Fed Differing Amounts of Protein and Methionine Hydroxy Analog[1]

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ABSTRACT

The objectives were to determine effects of prepartum protein intake and dietary amino acid balance on production, adaptations in body fat and protein, amino acid concentrations, and, indirectly, body protein breakdown in early lactation. Multiparous Holstein cows (n = 42) were fed diets containing 11 or 14% crude protein with or without 20 g/d of methionine hydroxy analog for 21 d prepartum and then fed a common diet of 17% crude protein for 120 d postpartum, with or without 50 g/d of methionine hydroxy analog. Dry matter intake postpartum averaged 25.4 kg and milk production 41.6 kg. Cows fed the 14% CP diet ate 0.7 kg more dry matter and gave 1.7 kg more milk than those fed the 11% diet postpartum, but this difference was not significant. Cows fed methionine hydroxy analog prepartum lost less body protein from −14 to 60 d in milk. From d 60 to 120, body fat increased 8.5 and 11.5 kg for low and high protein groups and body protein increased 0.5 and 1.0 kg. Serum concentrations of branched chain amino acids fell 17% in the first few weeks postpartum, lysine fell 15%, histidine fell 16%, methionine increased 20%, and cysteine increased 30%. The ratio of serum 3-methylhistidine to creatinine was determined to indicate muscle protein degradation. An increase in this ratio at 7 d postpartum indicated increased body protein breakdown, there was no effect of prepartum ration. Increased protein intake prepartum may allow more feed intake and milk production postpartum, and supplementing a methionine analog on a ration already balanced in methionine by contemporary models may spare body protein.
(**Key words:** methionine analog, body fat, body protein, transition)

Abbreviation key: BF = body fat, **BP** = body protein, **HMB** = D,L-2-hydroxy-4-(methylthio)-butanoic acid, **MHA** = methionine hydroxy analog, **MP** = metabolizable protein, **3MH** = 3-methyl histidine.

INTRODUCTION

The dairy cow requires sufficient dietary protein to meet the demands for milk and calf production, to maintain health through muscle tissue replacement and growth, and to provide adequate immune response. Another major component to protein feeding efficiency is to minimize nitrogenous waste of protein metabolism. Protein not used by the animal to produce milk, tissue, or other body proteins (**BP**) represents a decrease in overall efficiency of dietary protein use and a potential environmental liability for the farmer. The dairy cow in late gestation and especially early lactation is in amino acid and glucose deficit. These result in mobilization of body fat (**BF**) to supply energy to various organs and for milk fat, and mobilization of BP to supply amino acids for glucose production. Our understanding of the mechanisms and kinetics of these processes is still limited, especially within a range of dietary intake of carbohydrates, fats, and amino acid-yielding components and under a range of milk yields.

The rates of intake of protein prepartum have been studied as a ways to optimize postpartum DMI and milk yield. Results are mixed, with many finding that some increased CP content of diets increases postpar-

Received November 25, 2002.
Accepted May 29, 2003.
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[1]This paper published with partial support from Consolidated Nutrition, Decatur, IN; Cargill, Inc., Minneapolis, MN, and the Agricultural Research Center, Washington State University. This paper published in conjunction with the NC-185 CREES Regional Research Project "Protein Synthesis in Lactating Dairy Cattle."

tum DMI and milk yield (Park et al., 2002 and references therein), whereas others have shown no effect (VandeHaar et al., 1999) or perhaps a decrease in postpartum DMI with an increase in prepartum protein (Greenfield et al., 2000; Hartwell et al., 2000). The amount of RUP and, more specifically, the amino acid balance may actually be the critical factor (Park et al., 2002). Lysine and methionine rumen-protected products can increase milk and milk protein production in high-producing dairy cows (Schwab et al., 1992).

Another method used to supply additional methionine is the use of various analogs of methionine. A commonly used methionine analog is D,L-2-hydroxy-4-(methylthio)-butanoic acid (**HMB**), also commonly known as **MHA**, methionine hydroxy analog. Methionine hydroxy analog is not a true amino acid; however, it can be metabolized to methionine upon absorption. However, this is probably dose dependent (Belasco, 1980; Koenig et al., 1998). It has been used successfully in chickens and pigs to increase muscle growth in both species and egg production in chickens. It has also been studied for its effects on dairy cattle (Griel et al., 1968; Lobley et al., 1980; Schwab, 1998) with a wide variation in response. The rumen undegradability of MHA is most likely low (less than 40%) at doses below 20 g/d, and the actual degradability is in dispute (Koenig et al., 1998). The two prevailing theories of action are a direct ruminal effect to increase microbial growth, increasing fiber digestibility and perhaps feed intake as a downstream effect, or by supplying methionine to tissues postruminally. These mechanisms are probably not mutually exclusive. Several studies in dairy cattle demonstrate some effect of MHA either in DMI, increased N balance, or increased milk fat synthesis (see Koenig et al., 1998; Schwab et al., 1998 for an extensive reference list).

A predictable model for the amino acid, glucose, and fat requirements in the peripartum ration of dairy cows would be useful to the industry as well as to scientists. It is no longer adequate just to study only at the level of dietary inputs and milk component outputs. Metabolic rates of body organs, primarily the viscera, muscle, and adipose tissues are affected by rates of nutrient intake and milk synthesis. Further, metabolism of nutrients in these organs then affects the supply of milk component precursors to the mammary gland, affects the metabolism in each other, and may also affect rates of feed intake. Examples would include the effect of adipose tissue release of fatty acids on feed intake and fatty liver, which may affect gluconeogenic capacity, and the effect of protein turnover in the muscle on use and release of amino acids, which can supply glucose necessary for milk lactose synthesis. During the transition period where large day-to-day changes in metabolite flux are the norm, we still have a limited body of data on these key metabolic processes. Therefore, this experiment was designed to help define the effect of variation in protein intake prepartum and use of MHA as a supplement on BP and fat use, amino acid metabolism, and production variables of dairy cows from 21 d prepartum to 120 d postpartum. In addition, this experiment was conducted to obtain a dataset with which to challenge an existing model of metabolism of lactating dairy cattle (McNamara and Baldwin, 2000), which will be reported separately. We specifically wanted to test whether or not there would be additional effects either at the animal or the organ (BP, BF) level of MHA supplementation of a ration already regarded as balanced for all components, including methionine and lysine (CNCPS; O'Connor et al., 1993; Boston et al., 2000).

MATERIALS AND METHODS

Cows and Diets

Forty-eight multiparous, high-producing Holstein cows in second through sixth parities were used in the trial. The trial period ranged from 21 d prepartum to 120 d postpartum. The animals were blocked by parity (either parity = 2 or '3 and above') allotted to four prepartum treatments, which were formulated for two levels of CP (target levels of 12 and 16%) and supplemented with 20 g/d of AT-88 (AT-88; Rhodimet AT88, a liquid MHA, Rhone-Poulenc Animal Nutrition, Atlanta, GA) or not supplemented (no. AT-88) (Table 1); the final number of cows in each group were: 11, 10, 9, and 12 for 12% CP with AT88, 12% CP without AT88, 16% CP with AT88, and 16% CP without AT88, respectively. Upon analysis of the ingredients and TMR after the trial had begun, the actual protein contents were 11.4 and 15.6% (Table 1), primarily due to the lower actual analyzed values for the alfalfa hay and haylage compared with the analyses available at the time of purchase and feed formulation. Six animals were removed without replacement because of failure to use the Calan doors (n = 4), lameness (n = 1), and displaced abomasums (n = 1).

The basal dry cow diet contained 44% (DM basis) each of alfalfa haylage and grass hay. The remaining 12% contained a concentrate mix containing primarily barley and soybean meal adjusted to accommodate the two protein levels of the treatment (Table 1). Protected soybean meal (AminoPlus, 48% RUP, Rhone-Poulenc Animal Nutrition) was added to the 16% CP diet, and SmartAmine M (a rumen-stable, solid source of methionine; Rhone-Poulenc Animal Nutrition) was added to both dry cow diets to optimize and balance the delivery of RUP and methionine according to the Cornell Net Carbohydrate and Protein system (O'Connor et al.,

Table 1. Dietary ingredients and composition fed to dairy cattle in late pregnancy and early lactation.

	Prepartum		Postpartum		
Ingredients	Low Prot.	High Prot.	Transition 1	Transition 2	
			(% of DM)		
Alfalfa haylage	24	24	28	23	19.1
Alfalfa hay	. . .	. . .	6	5	4.6
Grass hay	60	60	23	18	18.0
Whole cottonseed	. . .	. . .	9	12	12.9
Wheat mill run	. . .	. . .	9	12	12.9
Concentrate	16	16	25	30	32.4
Corn grain, ground	. . .	. . .	10	12	12.5
Barley	13	4.0	8.6	11	11.4
Soybean meal	2.0	3.0	0.6	0.8	1.0
AminoPlus[1]	. . .	8.0	2.4	3.0	3.7
Molasses, cane	0.6	0.7	1.0	1.2	1.4
Fat, animal	. . .	. . .	0.5	0.7	0.8
Vitamin and mineral mix[2]	0.4	0.3	1.2	1.4	1.6
Cellulose gum	0.009	0.009	0.01	0.02	0.01
SmartAmine M[2]	5 g/d	10 g/d	12 g/d	12 g/d	12 g/d
Chemical composition[3]					
DM, %	65.6	66.9	66.7	69.0	71.3
CP, % of DM	11.4	15.6	15.8	16.2	16.2
RUP, % of CP	34	37	27	26	25
NDF, % of DM	49.4	49.4	38.9	36.8	36.2
ADF, % of DM	36.3	36.3	28.9	26.4	25.4
Ash, % of DM	12.0	12.1	10.0	10.1	10.0
NE_L, Mcal/kg DM	1.34	1.32	1.58	1.60	1.69

[1]Rhone-Poulanc Animal Nutrition, Atlanta, GA.

[2]Prepartum diets contained 0.11% Cl, 0.07% Na, 0.033 mg/kg of Mn, 0.007 mg/kg of Mg, 0.064 mg/kg of Zn, 0.033 mg/kg of Fe, 0.006 mg/kg of Cu, 0.002 mg/kg of I, 0.002 mg/kg of Se, 0.001 mg/kg of Co, 1.4 IU of vitamin A/g, and 1.5 IU of vitamin D/g. The initial postpartum ration contained 0.14% Cl, 0.09% Na, 21.7 mg/kg of Ca, 9.1 mg/kg of Mg, 2.0 mg/kg of poloxalene (Bloatguard), 0.09 mg/kg of Zn, 0.044 mg/kg of Fe, 0.044 mg/kg of Mn, 0.008 mg/kg of Cu, 0.002 mg/kg of I, 0.002 mg/kg of Se, 0.001 mg/kg of Co, 3.2 IU of vitamin A/g, and 1.9 IU of vitamin D/g. The concentrate was changed later to increase ZN, Mn, Cu, and Co concentrations to 0.61, 0.34, 0.21, and 0.04 mg/kg.

[2]All data are analyzed except for RUP and NE_L which were calculated from NRC (2001) using measured values available or tabulated values for measurements not done.

1993; Boston et al., 2000). The cows were housed in a free-stall pen with free access to water and fed individually a totally mixed diet once daily through a Calan head gate system (American Calan, Northwood, NH) beginning at 21 d prepartum. Feed was delivered to allow 5% orts, which were collected using a feed mixer with an internal scale and vacuum assembly attached (Data Ranger, American Calan). The amount of feed offered and refused was recorded using the computer mounted on the mixer. The AT88 and SmartAmine supplements were mixed in a barley carrier and were administered as a top dress to the TMR at 285 g/d prepartum and 714 g/d postpartum.

At parturition, cows were offered a diet formulated to consist of 17% CP (Table 1) that was either supplemented with 50 g/d AT88 (Post AT-88) or not supplemented (No Post AT-88). The lactating cow diet contained alfalfa hay, alfalfa haylage, grass hay, wheat mill run, whole cottonseed, and a corn and barley concentrate (Table 1). Two transition diets were fed for each of the first 2 wk after parturition, one fed in wk 1, and one fed in wk 2. These diets were formulated with the same ingredients as the lactation diets, but with a higher ratio of forage to concentrate intended to gradually bring the cow to a 40:60 forage to concentrate ratio on a DM basis (Table 1). The cows were milked twice daily and milk weights recorded. Cows were weighed weekly and BCS were determined monthly using a 0 to 5 scale (0 = thin, 5 = fat; Wildman et al., 1982).

Sampling and Analytical Procedures

Feed ingredient samples were obtained at the start of the trial and when new batches were introduced. Orts were sampled twice weekly and composited monthly. All feed samples were dried in a forced-air oven (55°C), ground (Wiley mill, 1-mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for DM, nitrogen (Kjeldahl procedure, AOAC, 1980), NDF (Van Soest et al., 1991), ADF (method 973.18 of AOAC), and ash (2 h at 600°C in a muffle furnace). Data on diets

are presented as the average of chemical compositions of ingredients (Table 1).

Milk samples were obtained twice monthly (a.m. and p.m. mixed, weighted sample) and analyzed at the regional Dairy Herd Improvement Agency laboratory in Burlington, Washington (Milk-O-Scan, Foss North America, Eden Prairie, MN), using AOAC procedures (AOAC, 1980) for determination of CP, fat, lactose, SNF, MUN, and SCC.

Blood was collected from cows at approximately d –7, 0, –7, 14, 28, 56, 84, and 112 about calving from the coccygeal vessel. Serum was separated from the samples by centrifugation (Sorvall RT6000 B Refrigerated Centrifuge, 1900 × *g*, 30 min; Sorvall, www.sorvall.com) and frozen at –20°C for later analysis.

Serum amino acid profiles were determined by reverse-phase HPLC analysis of serum samples at Auburn University, Auburn, Alabama. The Pico-tag method (Waters Corporation, procedure WM02 Rev. 1, Waters Corp., Milford, MA) was followed, samples of 100 μl of serum were ultra filtered (Millipore Ultrafree-MC, 10,000 NMWL filter units) and centrifuged for 40 mi. A 30-μl portion of the sample (and standards; Sigma #6407 and 6282) was derivatized and then reconstituted with 100-μl sample diluent (Waters Corporation, #88119). Samples (10 μl) were then injected into a Pico-tag column for free amino acids (c-18, Reverse Phase, 3.9 × 30 cm; Waters Corporation, #10950) and analyzed at 46°C.

Serum creatinine content was determined by chemical assay (Sigma, method 555). Serum was also assayed for glucose using an enzymatic reaction with hexokinase resulting in the formation of glucose-6-phospate, followed by a reaction with glucose-6-phosphate dehydrogenase to form NADPH, which is then reacted with a dye (phenazine methosulfate) that then reduces iodonitrotetrazolium chloride, which is measured colorimetrically at 520 nm wavelength, following a reaction recipe supplied by Sigma chemical bulletin procedure No. 115 (Sigma, method 115; Bergmeyer, H. U., 1963). Nonesterified fatty acids were assayed enzymatically using acyl CoA synthetase and Acyl CoA oxidase to produce hydrogen peroxide that reacts with a dye to form a reactant absorbing at 550 nm (Wako, code 994-75409E as modified by McNamara and Hillers, 1986a). Triglyceride concentration was measured enzymatically after hydrolysis to glycerol, phosphorylation to glycerol-3-phosphate, and formation of lactate and NAD, which was determined colorimetrically (Wako, code 432-40201).

Adipose Tissue Biopsies, Fat Cell Size, and Body Fat and Protein Determination

Fat biopsies were removed aseptically from the tailhead region (3 to 4 cm lateral to coccygeal vertebrae in the depression between the ischium and vertebrae) at approximately d –14, 60, and 120 for fat cell size determination as described previously (Smith and McNamara, 1990). The fat tissue was immediately immersed in a bottle containing 200 ml of warm (37°C) physiological saline. After removal of the biopsy, the incision was sutured and the animal observed until recovery from the procedure was apparent. Samples of adipose tissue were fixed and stained in osmium tetroxide and fat cell size was determined microscopically and used along with BW to estimate BF according to the following equation developed previously in our herd: kg BF = –195.6 + 0.29 × BW, kg + 0.927 × Fat cell diameter, microns (Waltner et al., 1994). This equation uses BW and fat cell size, a direct correlate of BF content (not measured indirectly from body water). Previously, this method has been used in pregnant and lactating cattle (McNamara et al., 1995; McNamara and Baldwin, 2000), providing results that were consistent with the energy inputs and outputs measured in those studies. In addition, the statistic relating fat cell size to BF was 0.927 in our herd and 0.964 in Robelin et al. (1989), a difference of only 4%. The equation also gives a close agreement of predicted BF and actual BF with those derived using deuterium space (Brown and Taylor, 1986; Robelin et al., 1989). Body protein was calculated from BW and fat cell size using the following equation validated previously in both lactating and nonlactating Holstein cattle: BP, kg = 12.5 + 0.125 × BW, kg – 0.052 × fat cell diameter, microns (Robelin et al., 1989).

Statistical Analysis

The statistical design used for the trial was a completely random design with repeated measures (day, week, or month). The response variables were milk yield, DMI, BW and BCS, and composition of milk produced (protein, fat, lactose, SNF, MUN, and SCC). The main effects were prepartum dietary CP and AT88 supplementation prepartum (AT88) and postpartum (Post). The treatments were assigned in blocks by calving date and parity. Parity was grouped into two groups, lactation 2 or 3 and greater. Cows of similar parity, aligned in calving date sequence, were originally assigned to one of the eight treatments within the calving date group; however, due to loss of cows before treatment with no possibility of balanced replacement (within the same calving date group), the calving date group blocking factor was ignored, and we used the more conservative completely randomized design to analyze the data. The initial model used for the trial was: $Y_{ijkl} = \mu + CP_i + AT88_j + Post_k + (CP \times AT88)_{ij} + (CP \times AT88 \times Post)_{ijk}$ [main effect error]+ $Time_l + (Time \times CP)_{li} + (Time \times AT88)_{lj} + (Time \times Post)_{lk} + Error_{ijkl}$ [sub-

Table 2. Dry matter intake of cows in the transition period fed varying amounts of protein and methionine hydroxy analog.

	Prepartum			Postpartum month**				
Treatment	d –21 to d –8	d –7 to d –1	d –21 to d –1	1	2	3	4	Total
	(kg/d)							
Low CP, no AT88	13.0	12.8	12.9	19.2	25.4	28.0	27.9	25.2
Low CP, with AT88	12.9	11.7	12.5	19.6	24.7	27.4	28.6	25.0
High CP, no AT88	12.8	13.2	12.9	19.5	24.9	29.2	30.3	25.8
High CP, with AT88	13.1	14.9	13.7	20.9	25.6	27.7	28.7	25.7
No AT88 Prepartum	12.9	13.0	12.9	19.3	25.2	28.4	29.0	25.5
AT88 Prepartum	13.0	13.5	13.2	20.3	25.2	27.6	28.7	25.4
Low CP Prepartum	12.9	12.3	12.7	19.4	25.0	27.7	28.2	25.1
High CP Prepartum	12.9	14.2	13.3*	20.3	25.3	28.3	29.4	25.8
No AT88 Postpartum	13.4	14.2	13.7	20.3	25.5	27.5	27.9	25.4
AT88 Postpartum	12.5	12.3	12.4	19.4	24.9	28.4	29.0	25.6
SD	3.8	3.6	2.5	3.8	3.6	3.4	3.6	3.1

*Effect ($P = 0.12$) for high CP on DMI prepartum.

**Time was a significant effect for all treatments postpartum ($P < 0.0001$).

plot error]. Subsequently, the data from the prepartum period were then analyzed separately using this model. Other interactions were also tested but found not significant and removed from the model. Time factors were either DIM (DMI, milk yield, BP, BF, and blood variables), week (BW), or month (BCS) depending on the variable measured. For no variable was there an effect of, or interaction with, postpartum treatment with AT88; therefore, the final model removed these factors. We did decide to present some interaction means, as, in our thinking, the data will still be useful to scientists engaged in quantitative modeling, even though the interactions were statistically nonsignificant.

Statistical significance between treatment means was determined using the general linear model (GLM) protocol (SAS, 1999) and the proper error terms for a split plot in time as shown in the model above. Due to proper use of error terms and the relative lack of interactions in the model, it was not determined to be worthwhile to reanalyze the entire dataset using the PROC MIXED procedure, which was not fully recognized when this study was completed and analyzed. We did analyze the milk production data without noting a change in any statistical inference; therefore, this extensive dataset was not completely reanalyzed. Differences were considered significant at $P < 0.05$ unless otherwise noted, trends are discussed if the P value was < 0.15. In addition, regression of milk yield and postpartum DMI on prepartum protein intake was also evaluated using the general linear model protocol.

RESULTS AND DISCUSSION

Dry matter intake during the majority of the trial was not greatly affected by either varying CP intake or AT88 consumption prepartum (Table 2). However, cows consuming the higher protein ration did consume 0.6 kg/d more DMI than those consuming the lower protein, though this was statistically a trend (DMI as a percentage of BW followed the same pattern). Also, the cows on the higher CP rations also consumed 0.7 kg/d more DMI during lactation, a trend only at $P < 0.10$. Time was significant postpartum ($P < 0.001$).

An interesting finding was the lack of a significant ($P > 0.3$) decline in DMI during the last week prepartum (Bertics et al., 1992; several references in Grummer et al., 1995). The DMI means were 12.8 kg/d for –21 to –8 d and 13.3 kg/d for –7 to –1 d. Only on the low CP, AT88 treatment did DMI drop more than 0.2 kg/d during this time; on both high CP rations it increased. The authors do not have any novel explanation for this, nor are we arguing against the excellent datasets showing the typical reduction during this time period (see Grummer et al. 1995). However, this finding is in keeping with previous data from this herd in which prepartum intakes exceeded 12 kg/d and very little (less than 1 kg/d) drop in intake the last week was noted (Harrison, et al., 1995; McNamara, et al., 1995). Some recent trials reported prepartum DMI as great or greater than for this study (Overton, 2001; Park et al., 2002), so it is not unique to this herd. The relationship between prepartum intake of nutrients and postpartum intake and milk production is not a simple one. These findings and other recent findings are helping to define the range and scope of variation in intake of feed and feed components prepartum and feed intake and milk production postpartum (French, 2002; Hristov et al., 2002).

There were no effects of treatment or interactions on treatment means for milk yield during this trial. There

Table 3. Milk yield of dairy cattle fed varying amounts of protein and methionine hydroxy analog in the dry period.

Treatment	Month**				Total period
	1	2	3	4	
	kg/d				
Low CP, no AT88	32.0	42.6	42.4	39.5	39.2
Low CP, with AT88	35.0	45.1	44.7	42.9	42.2
High CP, no AT88	39.1	46.3	45.4	44.5	43.8
High CP, with AT88	35.8	43.6	43.6	41.8	41.2
No AT88 Prepartum	35.2	44.3	43.8	41.8	41.2
AT88 Prepartum	35.4	44.3	44.1	42.3	41.7
Low CP Prepartum	33.4	43.8	43.5	41.1	40.6
High CP Prepartum	37.2	44.7	44.3	43.0	42.3
No AT88 Postpartum	36.0	44.2	43.6	40.9	41.2
AT88 Postpartum	34.7	44.4	44.7	43.1	41.7
SD	7.4	6.9	6.5	7.3	6.3

**Time was a significant effect for all treatments postpartum ($P < 0.0001$).

was an effect of time ($P < 0.001$). Average milk production was over 41 kg/d for the first 120 DIM (Table 3). However, there was an increase of 1.7 kg/d for the cows consuming the higher CP diet prepartum, consistent with the 0.7 kg/d increase in DMI; again, these were not significant effects but were consistent biologically. These findings are inconsistent with some studies showing an increase in DMI and milk yield in cows fed more protein prepartum (see Schwab et al., 1992; Park et al., 2002). However, other studies have not shown an effect or have shown a decrease with increasing protein (VandeHaar et al., 1999; Greenfield et al., 2000; Hartwell et al., 2000).

In the present study, there was a positive and significant ($P < 0.05$) relationship between prepartum DMI and milk yield and between prepartum protein intake and milk yield (Figure 1). It has been well documented that increased DMI prepartum through a variety of mechanisms usually leads to increased DMI and milk yield postpartum (see Park et al., 2002 for a list of other pertinent references). Prepartum DMI or protein intake accounted for about 16 to 17% of the variation in milk yield, a reasonable figure considering all the other factors (genetic capacity for feed intake and milk yield, body size, physical, and chemical dietary factors). In this particular dataset, it is not clear whether DMI or protein intake was the most significant contributor to the increase in milk yield. Speculation based on published data (Schwab et al., 1992; NRC, 2001; French, 2002; Hristov et al., 2002) that the increased CP content prepartum increased postpartum DMI, leading to an increase in milk yield. In any case, we need to remember that it is a long list of factors that can influence the response to protein (for example, chemical and physical form of the diet, genetic capacity of the cow). In fact, a very recent summary, not available when this study

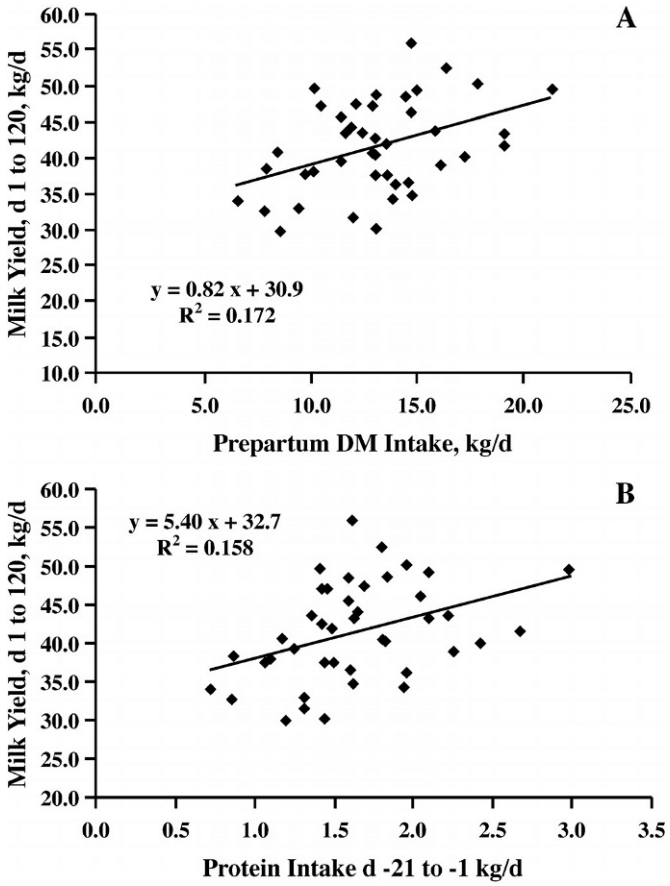

Figure 1. Regression of DMI and milk on CP intake prepartum. Dairy cattle were fed diets containing 11 or 14% CP from 21 d prepartum and milk yield from 1 to 120 d of lactation was recorded. Milk yield was regressed on either daily DMI (a) or protein intake (b) prepartum.

was designed and conducted, also found that in many different trials, prepartum intake related positively to postpartum DMI and milk yield (French, 2002; Hristov et al., 2002).

The lack of clear effect of AT88 supplement on DMI or milk production is also consistent with the experimental design: we wanted to determine whether additional methionine supplementation would have an effect on a ration already 'balanced' for protein and amino acids (O'Connor et al. 1993; Boston et al., 2000;) and, in fact, we primarily wanted to investigate the effects of amino acid intake on use of BP and BF. Thus, from this study there is no support for using additional methionine analog supplementation on a ration already balanced as adequate (even though CP may be slightly lower as a percentage of diet as recommended) strictly to improve DMI or milk production.

A simulation of these diets was made using the NRC (2001) Nutrient Requirements of Dairy Cattle model for metabolizable protein (**MP**). On the lower protein prepartum ration, animals were predicted to be in a positive balance of 440 g/d of MP, and those on the higher protein ration were in a balance of 689 g/d. Lysine as a percentage of MP was 7.13 and 7.0%, and methionine was 2.41 and 2.13% on the lower and higher protein prepartum rations. This would suggest that the rations fed in this trial prepartum were all adequate in MP, as planned. On the lactation ration, the MP balance was –304 g/d using the intake average over the trial and –59 g/d at the intake in mo 4. Lysine was 6.06 and 6.51%, and methionine was 2.41 and 2.76% of MP at the average and fourth month intakes. Cows in this study lost approximately 8 kg of BP in the first 60 d of lactation with little to no recovery by d 120 (Figure 2 and discussed below). That is an average of 133 g of BP lost in the first 60 d. Considering the known variation in this trial and the assumptions and variation inherent in the NRC model, this is pretty fair agreement. The NRC model at the fourth month intake and production rate suggests 179 g/d of MP for reserves repletion, again pretty fair agreement with the 133 g/d of BP lost in this trial. In fact, this is the first published test that these authors are aware of, of the dairy NRC to describe BP loss in early lactation. Given the NRC suggestion that lysine as a percentage of MP should be 7.2 and methionine 2.4% for production and maintenance, the loss of BP is consistent with the idea that these cows may not have been absorbing sufficient lysine to produce milk at this yield rate *and* to avoid BP loss. It is highly unlikely that very many cows producing over 40 kg of milk do not lose BP during early lactation, a statement supported by the only known two studies that have actually reported it (Komaragiri et al., 1997; this study).

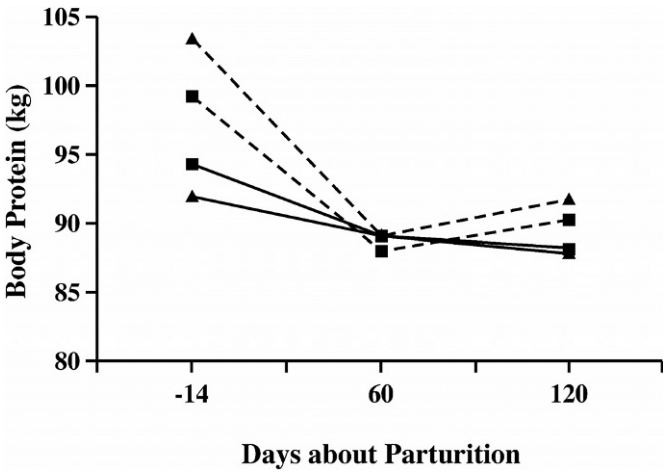

Figure 2. Body protein in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Body protein was determined from BW and fat cell diameter from a biopsy of subcutaneous body fat (Waltner et al., 1992; Robelin et al., 1989) as described in Methods. Dashed lines = no AT88, solid lines = 20 g/d prepartum AT88, ■ = 11% CP, triangles ▲ = 14% CP. There was a significant effect of DIM ($P < 0.01$) and of AT88 ($P < 0.05$) on body protein. Standard deviations were 8.0, 7.7 and 6.3 kg at –14, 60, and 120 DIM.

Some other points must be stressed: total intake of a nutrient is important, herds with lower intakes may well benefit from MHA, and that the overall health and performance of the cow is more than just DMI and milk production. From a biological standpoint it is not likely that any one factor (such as protein or fiber percentage in the prepartum ration) should or will have a predominant effect on postpartum physiology; thus, as nutritional scientists in the future we should be more concerned with compiling the total equation, so to speak, than with defining a 'major factor' or factors.

There were no treatment or interaction effects on milk composition (Table 4), BW, or change in BW (Table 5), BCS, or change in BCS (Table 6). One would probably not expect such for BW or BCS under these conditions. Some have reported changes in milk composition, especially fat, when feeding MHA (see Schwab, 1998), but as we fed it prepartum, one might not expect such an effect.

Our primary objective was to study the use of BP and BF in these cows under varying protein and methionine analog intake. Animals lost on average 8 kg of BP from d -14 to d 60 (Figure 2). Cows consuming the AT88 prepartum lost less BP ($P < 0.05$) than those consuming none, a difference of about 8 kg of BP loss (Figure 2). It is clear that the cows in the control (no AT88) groups had more BP at 14 d prepartum (Figure 2). These cows were consuming approximately 1 kg/d more food during the third week before parturition, yet it is unlikely that this intake difference resulted in an increased BP of

Table 4. Milk composition from cow fed varying amounts of protein and methionine hydroxy analog in the dry period.

Treatment	MUN (mg/dl)	Fat (%)	Prot (%)	Lact (%)	SNF (%)	SCC (1000)
Low CP, no AT88	14.32	3.76	3.23	4.87	8.75	254.48
Low CP, with AT88	13.71	3.55	3.16	4.83	8.70	281.05
High CP, no AT88	14.83	3.44	3.16	4.72	8.63	224.72
High CP, with AT88	14.71	3.44	3.16	4.78	8.64	83.05
No AT88 Prepartum	14.54	3.63	3.21	4.80	8.70	241.95
AT88 Prepartum	14.28	3.49	3.15	4.80	8.67	175.34
Low CP Prepartum	14.04	3.66	3.20	4.84	8.73	266.62
High CP Prepartum	14.76	3.44	3.16	4.75	8.64	142.70
No AT88 Postpartum	14.38	3.49	3.19	4.81	8.69	49.62
AT88 Postpartum	14.40	3.63	3.18	4.79	8.67	373.97
SD	3.24	0.73	0.27	0.23	0.42	918.54

Table 5. Body weights of cows fed varying amounts of protein and methionine hydroxy analog in the dry period.

						Change in BW
	Start*	d –4	d 7	d 120	d –4	Start d 120–7
Low CP, no AT88	715	716	605	650	1	45
Low CP, with AT88	699	718	622	650	19	28
High CP, no AT88	756	782	670	682	26	11
High CP, with AT88	691	713	622	650	22	27
No AT88 Prepartum	733	746	636	663	13	27
AT88 Prepartum	695	715	622	650	20	28
Low CP Prepartum	707	717	613	650	10	37
High CP Prepartum	720	743	645	663	23	18
No AT88 Postpartum	706	722	628	661	16	33
AT88 Postpartum	719	737	629	653	18	24
SD	63.2	62.7	63	52.7	42.3	50.5
n	34	42	34	34	36	34

*Time was a significant effect for all treatments postpartum ($P < 0.0001$).

Table 6. Body condition score of cows fed varying amounts of protein and methionine hydroxy analog prepartum.

	Month about calving*					Change	Change
Treatment	–1	1	2	3	4	(–1 to 1)	(1 to 4)
Low CP, no AT88	3.26	2.43	2.18	2.21	2.23	–0.84	–0.17
Low CP, with AT88	3.23	2.31	2.40	2.30	2.28	–0.92	–0.01
High CP, no AT88	3.44	2.52	2.11	2.22	2.27	–0.94	–0.26
High CP, with AT88	3.21	2.41	2.38	2.27	2.36	–0.79	–0.06
Low CP Prepartum	3.25	2.37	2.29	2.26	2.27	–0.88	–0.10
High CP Prepartum	3.21	2.46	2.27	2.48	2.32	–0.85	–0.14
No AT88 Prepartum	3.35	2.47	2.16	2.22	2.26	–0.88	–0.21
AT88 Prepartum	3.22	2.37	2.39	2.28	2.24	–0.85	–0.04
No AT88 Postpartum	3.25	2.41	2.34	2.32	2.32	–0.86	–0.16
AT88 Postpartum	3.30	2.42	2.22	2.22	2.27	–0.88	–0.50
SD	0.56	0.52	0.50	0.37	2.29	0.59	0.65
n	42	42	42	42	41	41	42

*Time was a significant effect for all treatments postpartum ($P < 0.0001$).

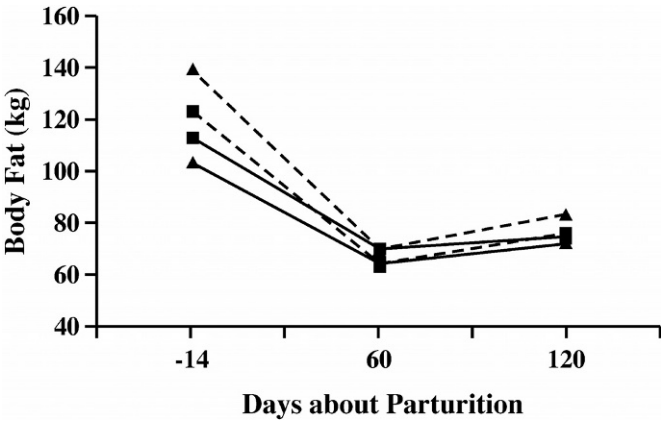

Figure 3. Body fat in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Body fat was determined from BW and fat cell diameter from a biopsy of subcutaneous body fat (Waltner et al., 1992) as described in Methods. Dashed lines = no AT88, solid lines = 20 g/d prepartum AT88, ■ = 11% CP, and triangles ▲ = 14% CP. There was a significant effect of DIM ($P < 0.01$); there were no treatment effects. Standard deviations were 23.7, 25.1, and 23.1 at –14, 60, and 120 DIM.

about 8 kg in 7 d. It simply cannot be known from this study whether that was a true effect of treatment or due to the fact that those animals had more protein to lose. Covariate analyses using initial BW did not change the statistical differences in the analysis. Yet this difference in protein loss is not trivial. To put this in a perspective, if the difference in BP (8 kg) was converted completely to milk protein, an 8 kg loss in 60 d is 133 g/d of amino acids available for milk protein. At an efficiency of 83% (Baldwin, 1968) for milk protein synthesis assuming adequately balanced amino acid supply to the mammary gland, this could supply up to 110 g of protein per day or 8 to 9% of the milk protein yield from these cows. This amount of milk protein is certainly greater than any response measured in milk protein yield due to increased CP intake at rates used in this or similar studies (Greenfield et al., 2000; Hartwell et al., 2000; Park et al., 2002, see also Schwab, 1998). If one-half of the amino acids (67 g/d) were converted to glucose and the glucose used for milk lactose, then this would supply about 52 g of lactose per day, or about enough for 1 kg of milk per day. There was no effect of prepartum CP intake on body protein loss postpartum. Thus, from one perspective, when one considers how much importance is put on an extra kilogram of milk per cow per day, one can not discount the importance of muscle amino acids in supplying the mammary gland as well as other organs in early lactation. Organs such as the liver and gastrointestinal tract have rapidly changing metabolic and growth rates in early lactation, and the amount of amino acids available from the muscle may easily be used by these other organs. Thus on one level, it is not reasonable to think that changes in body protein should be translated directly to changes in milk protein output. However, as yet no one has determined a clear quantitative relationship between the change in muscle protein content and milk protein output. A perusal of such studies (present study, Komaragiri and Erdman, 1998; Overton et al., 2001) simply demonstrates that the error of our measurements usually exceeds the difference in treatment effects, no matter how economically and biologically important these effects may be.

Cows lost on average 56 kg of BF by 60 d with no difference due to treatment. From d 60 to 120 BF increased 8.5 and 11.5 kg for low and high protein groups, and BP increased 0.5 and 1.0 kg. The cows that lost the most BP and BF (high CP, no AT88) also gave the most milk (numerically) on average DMI. This is consistent with the concept that it is milk yield that primarily drives BF loss (McNamara, 1991). We might now extend that concept to the loss of body protein as well—there is evidence that proteolytic rates are driven higher by a lack of amino acids (Lobley, 1980; Baldwin, 1995). In other studies when BP was measured or BP breakdown measured indirectly, BP loss in early lactation was not a function of intake, but might have been primarily a function of milk yield (Komaragiri and Erdman, 1997; Overton et al., 2001). We need to understand the mechanisms and practical applications of BP use in early lactation. Presently available data support the concept that the need for milk protein and lactose synthesis is the critical factor in loss of BP, consistent with current concepts concerning homeorhetic regulation (Baldwin and Elliott, 1983). This concept is embodied explicitly in the detailed mechanistic model of Baldwin and colleagues (Baldwin, 1995; McNamara and Baldwin, 2000) and helps explain the usual overprediction of BP and BF gain (and requirements thereof) with other models that do not explicitly embody this concept (Kohn et al., 1994; NRC, 2001).

We also investigated the changes in serum 3-methylhistidine and its ratio with serum creatinine as a qualitative indicator of muscle protein breakdown (Figure 4). There was a spike of 3-methyl histidine (**3MH**) concentration (Figure 4a) and an increase in the 3MH:creatinine ratio (Figure 4c) only at d 7; by d 14 this was back down to prepartum concentrations and remained there for the duration of the study. The greatest concentration was found in cows consuming the lower CP intake prepartum, regardless of AT88 treatment, although there were no significant differences other than for the main effect of DIM alone ($P < 0.05$). The rate of BP loss and 3MH:creatinine ratio were both elevated for the low CP, no AT88 group but not for the high CP, no AT88 group. However, the very small number of

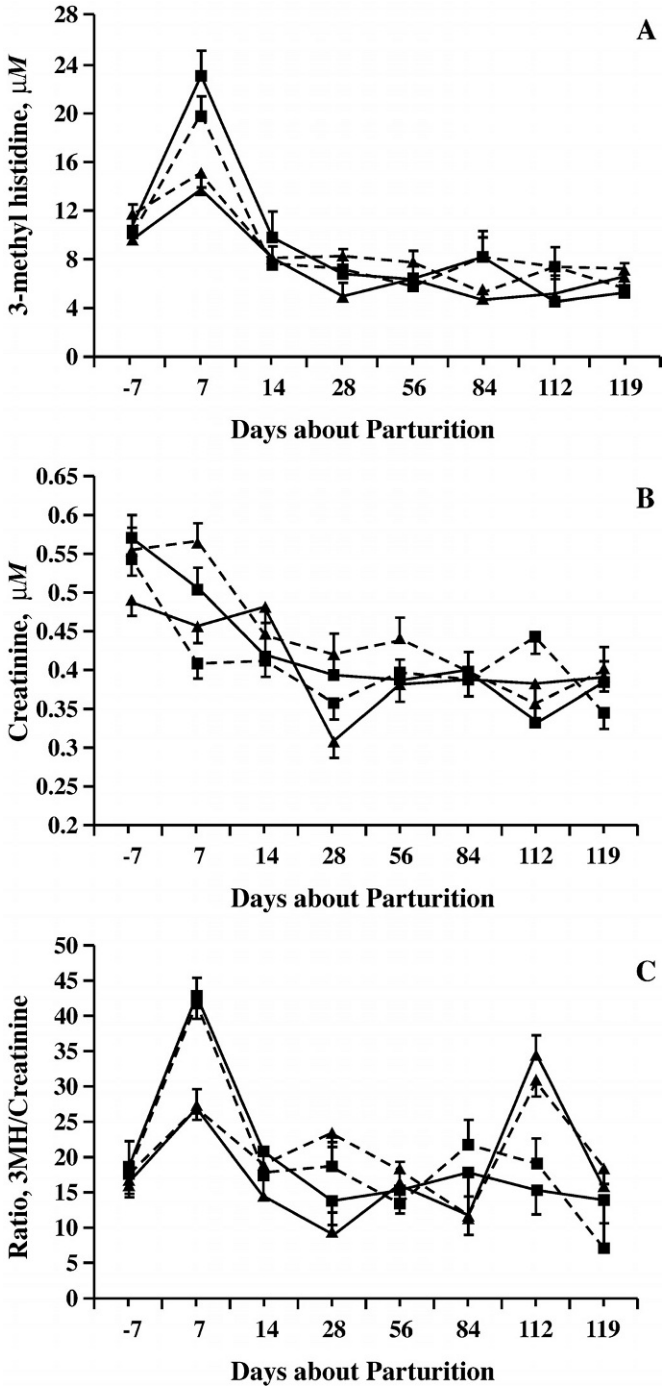

Figure 4. 3-Methyl histidine (3MH), creatinine, and 3MH:creatinine ratio in serum in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Blood was sampled and serum assayed for 3MH and creatinine. The ratio was calculated as in indicator of the rate of muscle protein breakdown. Data are in micromoles/L. Dashed lines = no AT88, solid lines = 20 g/d prepartum AT88, ◆ = 11% CP, triangles ▲ = 14% CP. A) 3MH methylhistdine, b) creatinine and c) 3MH:creatinine ratio. There was an interaction of AT88 and protein at $P = 0.10$.

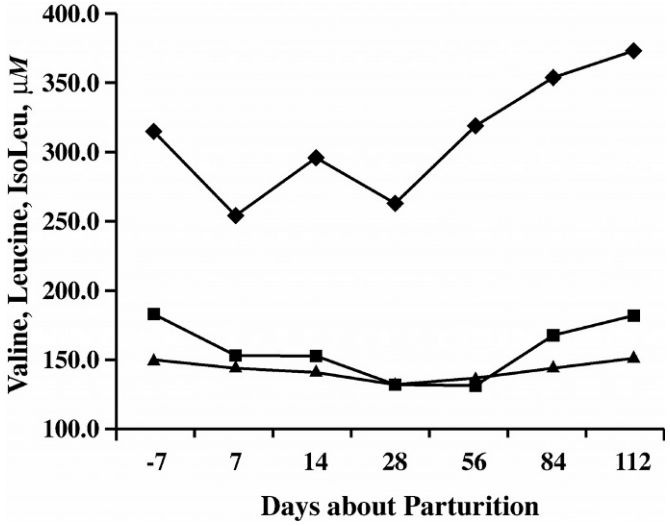

Figure 5. Branched-chain amino acids in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Data are in micromoles/L. ◆ = valine, ■ = leucine, ▲ = isoleucine. There was an effect of DIM on valine ($P < 0.001$) and leucine and isoleucine ($P < 0.05$). Standard deviations for valine were 85, 71, 60, 76, 66, 62, and 64; for leucine were 59, 85, 37, 55, 41, 38, 35, and for isoleucine were 41, 47, 32, 60, 36, 28, 27 for –7, 7, 14, 28, 56, 84, and 112 DIM.

samples for the 3MH work precludes any further inferences. This dataset can be compared with the dataset of Overton et al (1998), who found that the 3MH:creatinine ratio in urine increased in early lactation, but remained elevated for several weeks. In this study, we found a similar increase in the ratio; however, it was not sustained for as long a period of time. On the one hand, the similarity between these two datasets provides some credibility to the use of this indirect method. On the other, the difference in duration of the increase in 3MH:creatinine may be due to the greater rates of DM and protein intake in this study compared with that of Overton (1998) or it may be in differences in sample collection; they used urine and we used serum. The kinetics of 3MH and creatinine in serum related to BP breakdown may be different than that for the amount lost in urine; nevertheless, the results in this study are supportive of a rapid rate of BP breakdown in early lactation.

Serum concentrations of branched-chain amino acids fell ($P < 0.05$) 17% between 7 and 28 DIM, primarily due to a drop in valine and isoleucine (Figure 5). There was a trend to an interaction between protein and DIM ($P < 0.08$) and protein and AT88 ($P < 0.11$), but no clear pattern emerged. Lysine fell 12 to 15% at 7 DIM only, glutamate fell 12 to 17% from –7 d to 7 and 14 d, whereas histidine fell 16% at 28 d (Figure 6). There was a trend to an interaction for histidine between AT88 and protein ($P = 0.11$) and between AT88 and

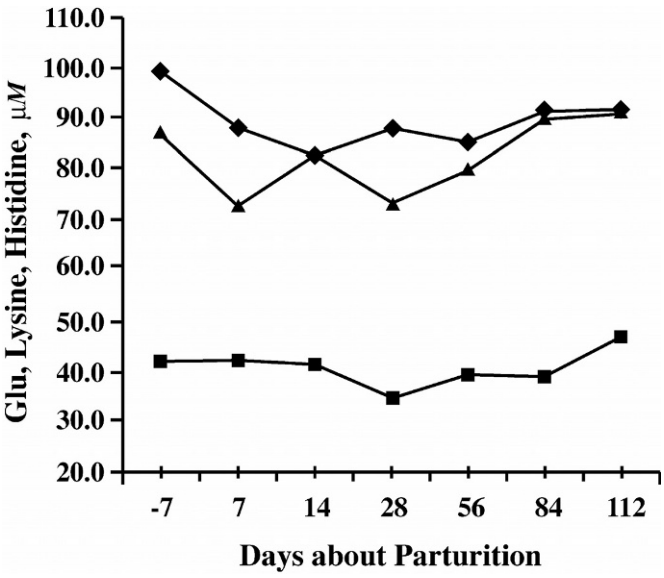

Figure 6. Glutamate, lysine, and histidine serum concentration in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Data are in micromoles/L. ◆ = glutamate; ▲ = lysine ; and ■ = histidine. There was an effect of DIM on all variables ($P < 0.05$). Standard deviations for glutamate were 23, 18, 17, 19, 14, 19, and 18; for lysine were 34, 19, 23, 29, 22, 25, and 25 and for histidine were 7, 14, 13, 10, 17, 10, 12 at –7, 7, 14, 28, 56, 84, and 112 DIM.

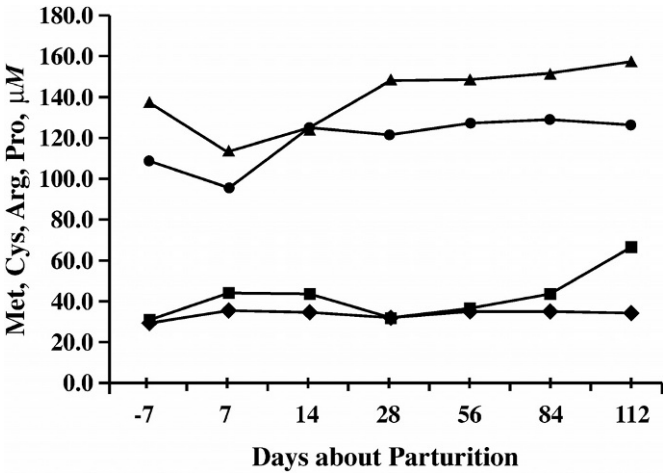

Figure 7. Methionine, cysteine, arginine, and proline serum concentration in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Data are in micromoles/L. ◆ = methionine; ▲ = arginine ; ■ = cysteine and ● = proline. Standard deviations for methionine were 8, 13, 12, 12, 7, 10, and 34; for cysteine were 29, 53, 44, 44, 26, 22, and 93; for arginine were 41, 56, 55, 41, 52, 46, and 46 and for proline were 24, 13, 30, 20, 25, 31, and 25 at –7, 7, 14, 28, 56, 84, and 112 DIM.

DIM ($P = 0.08$), and this was explained by a higher concentration of histidine at d 28 for animals fed AT88 prepartum (32.4 [no AT88] vs. 36.5 μM [AT88]). Arginine and proline also showed significant transient drops (Figure 7).

Methionine and cysteine increased 20 to 30% (Figure 7, all $P < 0.05$ for DIM). There was a trend to an effect of AT88 for methionine ($P = 0.09$) and an interaction of protein with AT88 ($P = 0.07$) and protein with DIM ($P = 0.09$; Figure 8). This was explained primarily by a greater concentration of methionine at 7 and 14 d in the cows fed low protein with AT88; methionine concentration of the cows fed high protein did not respond to AT88 during this time. It was also the cows fed AT88 that lost the least amount of BP, the animals (low protein with AT88), which lost the least BP had the greatest serum methionine concentration in early lactation, and those that lost the most (high protein, no AT88) had the lowest. The prepartum feeding of AT88 on the lower protein diet may have helped methionine balance and spared BP during early lactation. Note that methionine and cysteine did not fall but actually increased, except for the cows on the low protein with no AT88 treatment, confirming that the other rations were probably adequate in sulfur amino acids. These data confirm older datasets demonstrating a drop in key amino acids, especially BCAA and glutamate for at least some time in early lactation, stressing the quantitative importance of these to milk production (Meijer et al., 1995).

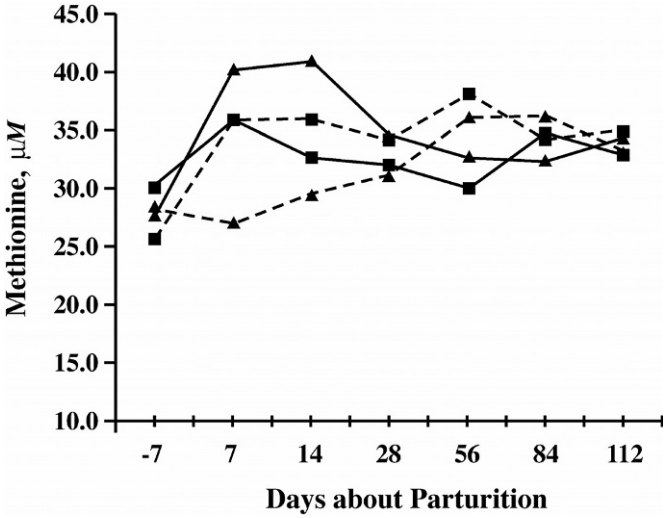

Figure 8. Methionine serum concentration by treatment in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Data are in micromole/L. Dashed lines = no AT88, solid lines = 20 g/d prepartum AT88, ■ = 11% CP, triangles ▲ = 14% CP. There was a trend to a main effect of AT88 ($P = 0.09$), where methionine was greater in cows fed AT88 prepartum; and a trend of AT88 × protein ($P = 0.07$) and DIM X protein (P = 0.09), where methionine was highest in early-lactation in cows fed AT88 and higher protein. Standard deviations for methionine were 8, 13, 12, 12, 7, 10, and 34 at –7, 7, 14, 28, 56, 84, and 112 DIM.

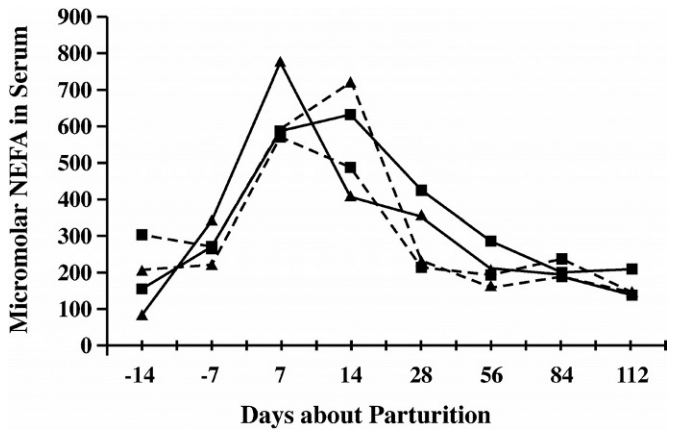

Figure 9. Nonesterified fatty acids in dairy cattle fed varying amounts of CP and methionine hydroxy analog prepartum. Data are in micromoles/L. Dashed lines = no AT88, solid lines = 20 g/d prepartum AT88, ■ = 11% CP, triangles ▲ = 14% CP. Standard deviations were 85, 176, 244, 330, 216, 88, 93, and 80 for –7, 7, 14, 28, 56, 84, and 112 DIM.

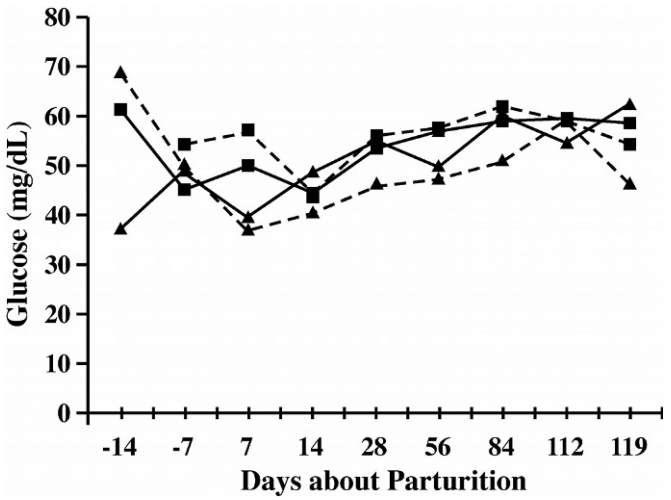

Figure 10. Glucose in dairy cattle fed varying amounts of CP and methionine hydroxy (MHA) analog prepartum. Data are in milligrams/dl. Dashed lines = no MHA, solid lines = 20 g/d prepartum MHA, ■ = 11% CP, triangles ▲ = 14% CP. Standard deviations are: 11, 13, 11, 10, 12, 14, and 12 for –7, 7, 14, 28, 56, 84, and 112 DIM.

These data support the concept that there may be additional benefit to supplementing methionine to spare BP. They also reiterate the proposals of others before these authors that the role of other amino acids than lysine and methionine, such as the branched chains and special amino acids such as histidine, arginine, and proline play important metabolic roles in the overall nutrient use of the cow, even though they may not be of the most practical economic significance (Meijer, 1995; Korhonen, 2000; Huhtanen et al., 2002). Histidine tends to be more limiting when cows are fed primarily grass-based rations (Korhonen, 2000; Huhtanen et al., 2002), but that is not to say it is not at all important on diets such as fed here—only not first-limiting.

Serum concentrations of NEFA, glucose, and triglyceride showed a usual pattern during the transition period, with NEFA increasing ($P < 0.05$) through the period from d 7 to 14 or 28 (Figure 9); whereas glucose dropped ($P < 0.05$) slightly at 7 and 14 d (Figure 10) and triglycerides dropped ($P < 0.05$) considerably at 7 d after the hyperlipidemia of pregnancy (Figure 11). There were no effects of treatments. The relatively short period of elevated NEFA concentrations in relation to other studies is in keeping with the greater rates DMI in these cows.

This dataset provides some novel information on the use of BP and BF in the transition period. The role of amino acid nutrition in sparing BP is critical to the health and longevity of dairy cattle above and beyond the importance for mammary output. Some reports find increases in DMI and milk yield (Park et al., 2002), whereas others have found a decrease or no change due to more CP prepartum (VandeHaar et al., 1999; Hartwell et al., 2000; Greenfield et al., 2002). However, these authors and others (Schwab et al., 1992) have pointed out before that it is likely the balance of RDP and RUP, the amino acid balance, and the interactions with other chemical and physical feed characteristics that are really important. This is especially true when considering the interactions among gastrointestinal organs, muscle, and mammary gland for amino acid use. The relationship of about 16% of the variation in post-

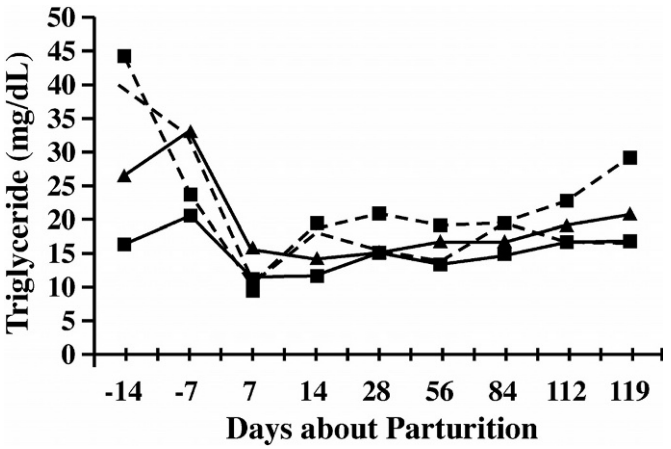

Figure 11. Triglycerides in dairy cattle fed varying amounts of CP and methionine hydroxy analog (MHA) prepartum. Data are in milligrams/dl. Dashed lines = no MHA, solid lines = 20 g/d prepartum MHA, ■ = 11% CP, triangles ▲ = 14% CP. Standard deviations are: 14, 16, 5, 9, 6, 8, 8, and 9 for –7, 7, 14, 28, 56, 84, and 112 DIM.

partum DMI and milk yield explained by prepartum protein intake, along with the size of the mean changes in these variables in previous studies supports this: no one factor such as protein content prepartum will have a large effect in and of itself on postpartum performance—although there may be small and economically important effects. The more specific message remains that if we are to predict responses in postpartum metabolism within body organs, including the mammary gland, to changes in rations prepartum, the system as a whole must be understood. In addition, our knowledge of the quantitative amino acid requirement for production and maintenance under a range of nutrient intakes and milk component outputs is still inadequate, as noted by the NRC subcommittee (NRC, 2001). The study also provides a detailed dataset for challenge and improvement of a mechanistic model of metabolism in dairy cattle (Baldwin, 1995; McNamara et al. 2001) as well as other models of nutrition in dairy cattle (CPM Dairy; Boston et al., 1999; NRC 2001).

IMPLICATIONS

Increasing DM and protein intake in the dry period increased postpartum DMI and milk production, accounting for about 16% of the variation in postpartum DMI and milk production. Use of a MHA in rations already balanced for CP and methionine spared the loss of BP and BF. Dairy cattle with the capacity for fast rates of DMI fed primarily alfalfa-based TMR may not experience as large a drop in prepartum intake as noted in other cattle consuming primarily grass and corn-based rations. The understanding of the metabolism of all major amino acids, especially branched-chain amino acids, glutamate, and histidine and not just methionine and lysine is important to improving our quantitative descriptions of nutrient use in dairy cattle.

ACKNOWLEDGMENTS

The excellent technical assistance of Jan Vierck is gratefully acknowledged.

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