Effects of the Energy Balance of Dairy Cows on Lactational Responses to Rumen-Protected Methionine

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ABSTRACT

This trial was designed to investigate the interactions between level of dietary energy and the response of cows after supplementation of rumen-protected Met. We examined this interaction for dairy cows fed a diet that was deficient in Met. Two percentages of energy (87 or 100% of requirements) were supplied with two concentrations of rumen-protected Met (0 or 21 g/d). Twenty-four Holstein cows (58 d in milk) were assigned to an experiment with a split-plot design including five periods of 3 wk each. The lower energy level was obtained by limiting the amount of feed offered (18.4 vs. 20.1 kg of dry matter intake). Diets characterized by low or normal amounts of energy were composed of corn silage (69.4% vs. 69.7%), energy concentrate (18.5% vs. 22.1%), oil meals treated with formaldehyde (11.5% vs. 7.4%), and urea (0.8% vs. 1.0%), respectively. The interaction between energy level and Met supplementation did not affect yield or composition of milk. An increase in the supply of both energy and Met increased the true protein content of milk by 0.11 percentage units. The effects of the level of dietary energy on the protein content of milk augmented the effects caused by Met supplementation. The main practical conclusion was that rumen-protected Met can be used with diets based on corn silage and soybean meal to increase the protein content of milk, even for dairy cows that are in a negative energy balance.

(**Key words**: rumen-protected Met, energy balance, milk proteins, lactating dairy cows)

Abbreviation key: **PDI** = protein truly digested in the small intestine.

INTRODUCTION

The best known way to increase the protein content of milk is to increase the amount of energy in the diet (6, 8, 13, 26, 27), regardless of the consequences to rumen fermentation (20). Another, more recently discovered method is to rebalance Lys, or Met, or both in the diet (22).

For cows fed a diet based on corn silage and soybean meal, the improvement of the protein content of milk and protein production following postruminal supplementation of Met has been clearly established by trials that implemented duodenal infusions of Met (18, 24, 25) and by two trials that used rumen-protected Met (19). Using comparable doses of Met, the responses were similar in the two trials of Rulquin and Delaby (19); however, energy balances were –0.85 and +1.19 Mcal of NE_L, respectively. Comparison of these results indicated, although without definite certainty, that interactions did not occur between energy intake and the response to Met supplementation. If verified, this hypothesis would have major practical implications because the energy contents of the diets used on dairy farms is often too low to meet the requirements of the cow. This trial was designed to investigate the interactions between the level of dietary energy and the response of cows after dietary supplementation with rumen-protected Met.

Performance responses and some metabolic indicators of cows that were in a negative energy balance were compared with those of cows that were in a neutral energy balance. To distinguish between the effects of the energy balance and of Met supplementation, comparisons were made with a difference of 4.25 Mcal of NE_L but with the same baseline concentrations of protein truly digested in the small intestine (**PDI**) (15), Lys truly digested in the small intestine, and Met truly digested in the small intestine (21). To prevent the possible effects of a change in rumen fermentations, particularly of propionic acid, on the protein content of milk (27), the difference in energy balance was not induced, as is most often done, by altering the amount of concentrate in the diet (6, 26) but was induced by reducing the total amount of feed offered.

MATERIALS AND METHODS

Cows

Holstein cows, 16 multiparous and 8 primiparous, were managed in free stalls. Individual feed intake

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was regulated using troughs with electronically controlled gates (Institut National de la Recherche Agronomique, St-Gilles, France). The trial was initiated immediately after calving, beginning with a conditioning phase (56 d) and followed by an experimental phase (15 wk). The cows were divided into four homogeneous groups on the basis of performance data (4% FCM, protein content of milk, DMI, and BW) that had been recorded during the 7th and 8th wk of lactation.

Treatments and Experimental Design

During the experimental phase, the effects of supplementation of rumen-protected Met (0 or 21 g/d of Smartamine M™; Rhône-Poulenc Animal Nutrition, Commentry, France) were assessed for cows fed according to their energy requirements, at 4.25 Mcal of NE_L below their requirements, and at up to 105% of protein recommendations (15). The following 3-wk treatments were tested according to a 2 × 2 split-plot factorial design; low energy plus 0 g/d of Met, low energy plus 21 g/d of Met, normal energy plus 0 g/d of Met, and normal energy plus 21 g/d of Met. An additional 3/wk period between periods 2 and 3 allowed the amount of energy to change gradually (Table 1).

The supplementation of rumen-protected Met was computed to increase the amount of Met that was truly digested in the small intestine from 1.8 to 2.3% of PDI (the Lys that was truly digested in the small intestine was 6.9% of PDI). These values, although below those recommended by Rulquin et al. (21), should have produced a response comparable with that recorded by Rulquin and Delaby (about a 0.1 percentage unit increase in the true protein content of milk) (19).

Diets and Feeding

The diet was composed of corn silage (45.6% grain as determined at harvest), energy concentrate, oil meal [80% soybean (48% CP) and 20% rapeseed], urea, and mineral and vitamin supplements (Boviphos™; Coopagri, Landernau, France). The composition of the feeds and diets is presented in Table 2.

Diets were determined and fed based on the theoretical requirements of each individual cow as computed by applying a weekly reduction of 2% to the reference milk yield for multiparous cows and a reduction of 1.5% to the reference milk yield for primiparous cows. The reference milk yield was the mean of yields recorded during the 7th and 8th wk for periods 1 and 2. References used for periods 3 and 4 were the mean of milk yields recorded during the 13th and 14 wk, corrected for the effect of energy recorded during the first part of the trial (periods 1 and 2).

The mean composition of the diets for each energy concentration were 69.4% corn silage, 8.5% energy concentrate, 11.5% oil meal, and 0.8% urea (low energy level) and 69.7% corn silage, 22.1% energy concentrate, 7.4% oil meal, and 1.0% urea (normal energy level).

Silage was offered in restricted amounts, and the mineral and vitamin supplement totaled 300 g/d. Urea was supplied at 200 g/d. Depending on the expected yield of each cow, the silage and concentrate supplements were computed to reach the predetermined energy requirements (neutral or 4.25 Mcal of NE_L below requirements). The ratio of energy concentrate to oil meal was adjusted to reach a value for PDI at 105% of requirements. Diets were readjusted every 3 wk. The feeds were provided as two equal parts and mixed in the troughs at 0900 and 1630 h. Protected amino acids were mixed with the minerals and deposited on top of the ration in two equal amounts each day.

Measurements and Sample Collection

The amount of feed offered and orts were measured daily. Feed samples were taken daily for corn silage and weekly for other feeds. The BW were measured once a week. Milk yield was measured every day at 0630 and 1630 h with Metatron 12 automatic samplers (Wesfalia Separator, Château Thierry, France). A milk sample was taken by machine at each milking over 4 d each week for analysis of the fat and protein contents of milk. One hundred fifty milliliters of milk were sampled at the morning milking on the 5th d of the last week of each period for analysis of fatty acid and casein.

A blood sample (20 ml) was drawn before the morning meal by caudal venipuncture on the 5th d of the last week of each period using heparinized Vacutainers® (Beckton Dickinson, Meylan, France).

Chemical Analysis

Dry matter was determined every day from samples of corn silage by drying at 80°C for 48 h. Determination of the nutrients was performed for ash by heating to 500°C for 5 h; for N by the Kjeldahl procedure; for ADF, NDF, and ADL according to the

TABLE 1. Experimental design.

	Period							
	1		2[1]		3		4	
Group	Energy	Met[2]	Energy	Met	Energy	Met	Energy	Met
		(g/d)		(g/d)		(g/d)		(g/d)
1	L[3]	0	L	21	N	21	N	0
2	L	21	L	0	N	0	N	21
3	N	21	N	0	L	21	L	0
4	N	0	N	21	L	0	L	21

[1] A transition period followed period 2.

[2] Smartamine M™ (Rhône-Poulenc Animal Nutrition, Commentry, France).

[3] L = low energy; N = normal energy.

methods of Van Soest (28) and Van Soest and Wine (29); for crude cellulose by standard NFV03040 (2); and for ether extract by standard NFV18104 (2). The theoretical degradability of the feed proteins (15) was assessed by measuring N clearance after feed incubation in nylon bags in the rumen according to the method described by Michalet-Doreau and Ould-Bah (16). This degradability was used to calculate the PDI values of feed (Table 2) according to equations of the Institut National de la Recherche Agronomique (15).

Fat and protein contents of milk were determined by infrared analysis (Milkoscan; Foss Electric, Hillerød, Denmark). Milk casein content and fatty acid composition were analyzed as described by Hurtaud et al. (14). Blood was prepared and analyzed for blood concentrations of glucose, NEFA, urea, and amino acids as described by Guinard et al. (12).

TABLE 2. Chemical composition and nutritive values of feedstuffs and consumed diets.[1]

				Diet	
	Concentrate[2]	Oil meal[3]	Corn silage	Low energy	Normal energy
DM, g/kg (as-fed basis)	875	870	343	514	510
	(g/kg of DM)				
OM	922	927	957	931	931
CP	124	458	84	163	143
Crude fiber	84	90	197	159	160
NDF	273	235	421	361	365
ADF	100	115	229	187	187
ADL	16	24	19	19	18
Ether extract	34	23	33	31	32
NE_L[4]	1.30	1.96	1.58	1.61	1.60
PDIN,[5] g/d	82	366	52	108	93
PDIE,[5] g/d	96	341	70	104	93

[1] Diets were supplemented with 300 g/d per cow of urea and 200 g/d per cow of a trace mineral and vitamin premix (Boviphos™; Coopagri, Landernau, France), which contained 5% P/kg, 25% Ca/kg, 5% Mg/kg, 2% Na/kg, 1000 mg/kg of Cu, 1500 mg/kg of Fe, 5000 mg/kg of Zn, 4000 mg/kg of Mn, 20 mg/kg of Co, 6.7 g/kg of Se, 100 mg/kg of I, 300,000 IU/kg of vitamin A, 80,000 IU/kg of vitamin D_3, and 200 mg/kg of vitamin E.

[2] Dry matter contained 14.8% ground corn grain, 14.9% ground barley, 14.8% ground wheat, 19.9% fine wheat bran, 25.5% dehydrated beet pulp, 4.4% beet molasses, 1.1% tallow, 1.1% limestone, 1.7% sodium bicarbonate, 1.1% dicalcium phosphate, and 0.6% salt.

[3] Mixture of 80% soybean meal and 20% rapeseed meal [spray-treated with 1% formaldehyde (30%) on raw matter basis].

[4] Estimated using the equations of Giger-Reverdin et al. (10) and expressed as megacalories per kilogram of DM.

[5] Proteins truly digested in the small intestine allowed by N (PDIN) or energy (PDIE) and fermented in the rumen. Estimated using equations of the Institut National de la Recherche Agronomique (15).

Statistical Analysis

Statistical analysis of the results was performed according to SAS (23):

$$Y = E_m + M_n + SE_i + SM_k + E_m \times M_n + SM_k \times M_n + V_j(SE_i) + P_l(SM_k) + SM_k \times V_j(SE_i) + e$$

where

E_m = energy level (m = 1 or 2),
M_n = Met supplementation (n = 1 or 2),
SE_i = energy level sequence (low and then normal or normal and then low; i = 1 or 2),
SM_k = Met supplementation sequence (0 g/d and then 21 g/d or 21 g/d and then 0 g/d; k = 1 or 2),
V_j = cow (j = 1, 2, . . . , 24),
P_l = period (l = 1 through 4), and
e = residue.

The effects of Met supplementation and the interaction between energy level and Met supplementation were tested in relation to the residual mean square. The effect of energy level was tested by using the mean square of the effect the interaction of Met supplementation sequence and cow within energy level sequence as the residual error. Significance was declared at $P < 0.05$ unless otherwise specified.

RESULTS

Effects of Level of Dietary Energy

The dietary restriction induced was reflected in a difference of 1.7 kg of DMI/d and 2.55 Mcal of NE_L/d between the two energy levels (Table 3). These differences were slightly smaller than expected because the cow fed with the normal energy diet did not eat the amount of corn silage initially presumed. Nonetheless, the difference in energy was obtained without any significant difference in the PDI supply, which was the most limiting (i.e., value for PDI allowed by energy in Table 3). The difference in energy level did not have an effect on milk, 4% FCM, or fat yield (Table 4). In contrast, the increase in energy increased protein production by 43 g/d. The 8-kg difference in BW between cows fed diets containing the two levels of energy was at the significance threshold and probably was partially due to the differences in weight of the gastrointestinal contents that resulted from the differences in DMI. The increase in energy did not modify milk fat content or the proportion of casein in milk proteins. Conversely, protein and casein contents were increased by 0.11 and 0.08 percentage units by the energy supplementation (Table 4).

Because of the lower intake of corn silage, cows fed the diet with the normal amount of energy were calculated to be in a slightly negative energy balance (–1.60 Mcal of NE_L/d) instead of the expected neutral energy balance. Nevertheless, cows fed the low energy diet had a significantly lower energy balance (–2.13 Mcal of NE_L/d) than did cows fed the normal energy diet (Table 4). All cows had a positive PDI balance. Because of the reduced production of milk proteins, cows that received the low energy diet had a significantly higher PDI balance (+104 g/d) than did cows that received the normal energy diet.

The differences in energy balance between the two diets were reflected by increased mobilization of lipid stores in cows fed the low energy diet, as was indicated by a greater percentage of $C_{18:1}$ in milk fat (16.5% vs. 15.4%) (Table 5) and by a greater concentration of NEFA in plasma (85.5 vs. 54.0 μmol/L) (Table 6). With regard to milk fat, the increased $C_{18:1}$ was totally compensated by a significant decrease in C_{16} (39.0% vs. 40.1%) (Table 5). The decrease in energy supply significantly reduced blood glucose concentrations (69.7 vs. 72.0 mg/100 ml) without altering BHBA concentrations and increased blood urea concentrations (31.8 vs. 25.1 mg/100 ml) (Table 6). This increase in uremia was accompanied by an increase in plasma amino acids of the urea cycle (Table 7); the sum of Arg plus Cit plus Orn increased (3.22 vs. 3.66 mg/100 ml; $P = 0.002$). The Tau and Thr contents decreased, and Val content increased, as the energy supply decreased (Table 7); other amino acids were not affected by the energy variations.

Effects of Met Supplementation

Supplementation of Met did not affect nutrient intake (Table 3). Energy consumption was the same for cows fed both Met concentrations. The slight differences in N intake and PDI were entirely accounted for by Met supplementation.

The supplementation of Met induced a slight reduction in milk yield (–0.7 kg/d; Table 4). Fat yield was not altered by Met supplementation, although protein yield increased significantly (908 vs. 927 g/d). This increase was due to a significant increase in the protein content of milk (3.13% vs. 3.24%), resulting from a significant increase in casein content (2.55% vs. 2.67%). The proportion of casein in proteins tended to increase ($P < 0.08$) as Met supplementation increased (81.0% vs. 82.3%) (Table 4). Supplementation of Met did not affect the PDI balance of the cow but slightly improved the energy

TABLE 3. Effects of level of dietary energy on responses of feed and nutrient intake to rumen-protected Met supplementation.

	Diet								
	Low energy		Normal energy		P				
	0 g/d of Met	21 g/d of Met	0 g/d of Met	21 g/d of Met	Energy	Met	Int.[1]	RSD1[2]	RSD2[3]
DMI, kg/d									
Corn silage	12.6	12.5	13.8	13.8	**	NS[4]	NS	0.4	1.3
Concentrate	0.32	0.32	0.44	0.44	**	NS	NS	0.14	0.43
Oil meal	0.21	0.21	0.14	0.14	**	NS	NS	0.10	0.17
Urea	0.20	0.20	0.19	0.19	**	NS	NS	0.01	0.001
Minerals	0.30	0.30	0.28	0.28	**	NS	NS	0.01	0.01
Total	18.4	18.4	20.1	20.1	**	NS	NS	0.001	0.002
Nutrient intake									
NE_L, Mcal/d	27.8	27.8	30.3	30.4	**	NS	NS	0.7	2.2
CP, g/d	3009	3028	2894	2937	†	NS	NS	79	259
PDIE,[5] g/d	1919	1926	1871	1895	NS	NS	NS	55	187
PDIN,[5] g/d	1994	2004	1871	1897	**	NS	NS	56	174

[1] Interaction between Met supplementation and energy level.

[2] Residual standard deviation used to test effects of Met supplementation and the interaction between Met supplementation and energy level.

[3] Residual standard deviation used to test effects of energy supply.

[4] $P > 0.10$.

[5] Protein truly digested in the small intestine allowed by N (PDIN) or energy (PDIE) and fermented in the rumen. Estimated using equation of the Institut National de la Recherche Agronomique (15).

†$P \leq 0.10$.

**$P \leq 0.01$.

TABLE 4. Effects of level of dietary energy on responses of lactational performances and nutrient balances to rumen-protected Met supplementation.

	Diet								
	Low energy		Normal energy		P				
	0 g/d of Met	21 g/d of Met	0 g/d of Met	21 g/d of Met	Energy	Met	Int.[1]	RSD1[2]	RSD2[3]
Milk yield, kg/d	29.2	28.2	29.4	29.1	NS[4]	**	NS	0.7	3.1
4% FCM Yield, kg/d	30.4	30.1	30.9	30.7	NS	NS	NS	1.1	3.3
Fat yield, kg/d	0.12	0.12	0.13	0.13	NS	NS	NS	0.06	0.14
True protein production, kg/d	0.89	0.91	0.93	0.95	*	**	NS	0.03	0.09
BW, kg	631^{b}	627^{bc}	635^{b}	639^{a}	†	NS	*	8	19
Milk composition									
Fat, %	4.29	4.38	4.41	4.44	NS	NS	NS	0.18	0.31
True protein, %	3.07	3.19	3.19	3.28	**	**	NS	0.07	0.13
Casein, %	2.51	2.62	2.58	2.71	**	**	NS	0.08	0.13
Casein:protein, %	81.9	82.1	80.9	82.5	NS	†	NS	2.2	3.3
Nutrient balance									
NE_L, Mcal/d	–3.77	–3.69	–1.72	–1.47	**	NS	NS	0.75	1.25
PDI,[5] g/d	119	99	10	0	**	NS	NS	55	90

[a,b,c] When interaction is significant, means within the same row with different superscripts differ ($P < 0.05$).

[1] Interaction between Met supplementation and energy level.

[2] Residual standard deviation used to test effects of Met supplementation and interaction between Met supplementation and energy level.

[3] Residual standard deviation used to test effects of energy level.

[4] $P > 0.10$.

[5] Protein truly digested in the small intestine estimated using the equation of the Institut National de la Recherche Agronomique (15).

†$P \leq 0.10$.

*$P \leq 0.05$.

**$P \leq 0.01$.

balance (–2.75 vs. –2.35 Mcal of $NE_{L/d}$). The milk fat composition was only slightly modified by Met supplementation, including a decrease in C_4 (2.99% vs. 2.90%) and $C_{18:2}$ (1.76% vs. 1.66%) and a tendency ($P < 0.06$) for a decrease in C_6 (2.26% vs. 2.20%; Table 5). These changes in milk fatty acid composition can hardly be explained by the significant decrease in plasma NEFA (78.6 vs. 60.9 μmol/L; Table 6).

Supplementation of rumen-protected Met did not affect BHBA or urea concentrations but tended ($P < 0.06$) to increase blood glucose (69.8 vs. 71.3 mg/100 ml; Table 6). Supplementation of Met was reflected by an increase in plasma Met and Met metabolic products, such as Tau, Cys, and cystathionine (Table 7). Parallel with this increase, the Ser and Gly contents tended to decrease ($P < 0.10$).

Interactions Between Level of Dietary Energy and Met Supplementation

No interactions were observed between energy level and supplementation of rumen-protected Met on the quantities ingested (Table 3) or on most of the dairy performance values (Table 4). The interaction of energy level and Met supplementation affected BW of cows; when combined with low energy, Met supplementation reduced BW by 4 kg, but BW increased by 4 kg when the energy level was normal (Table 4). Although this change was not significant, the decrease in milk yield following Met supplementation was higher for cows fed the diet with the low energy (–1 kg/d) than for cows fed the diet with a normal amount of energy (–0.3 kg/d), which partly explains the higher increase in the protein content of milk that was induced by Met supplementation for cows fed the low energy diet (+0.12%) than those fed the normal energy diet (+0.09%). Interactions neared significance for milk C_{15} and $C_{18:2}$ contents (Table 5). These interactions cannot be explained by the variations of the metabolites tested because only glucose was affected by the interaction of energy level and Met supplementation. Supplementation of Met increased blood glucose (+3.1 mg/100 ml) for cows fed the low energy diet but had no effect on cows fed the

TABLE 5. Effects of level of dietary energy on responses of the composition of milk fatty acids to rumen-protected Met supplementation.

	Diet								
	Low energy		Normal energy		P				
Fatty acid	0 g/d of Met	21 g/d of Met	0 g/d of Met	21 g/d of Met	Energy	Met	Int.[1]	RSD1[2]	RSD2[3]
	(g/100 g)								
C_4	3.04	2.91	2.94	2.89	NS[4]	*	NS	0.04	0.11
C_6	2.26	2.22	2.26	2.17	NS	†	NS	0.05	0.04
C_8	1.47	1.47	1.51	1.42	NS	NS	NS	0.04	0.04
C_{10}	3.37	3.51	3.65	3.41	NS	NS	NS	0.18	0.16
C_{12}	4.17	4.35	4.56	4.32	NS	NS	NS	0.23	0.31
$C_{14:1}$	1.34	1.41	1.30	1.41	NS	NS	NS	0.08	0.07
C_{14}	12.83	13.04	12.83	12.84	NS	NS	NS	0.12	0.08
iso-C_{15}	0.20	0.21	0.25	0.20	NS	NS	NS	0.06	0.06
$C_{15:1}$	0.40	0.41	0.42	0.42	†	NS	NS	0.02	0.01
C_{15}	1.06[c]	1.15[ab]	1.25[a]	1.18[a]	NS	NS	†	0.05	0.13
$C_{16:1}$	1.78	1.90	1.76	1.83	NS	NS	NS	0.14	0.09
C_{16}	39.19	38.88	39.58	40.60	**	NS	NS	0.63	0.16
iso-C_{17}	0.27	0.27	0.28	0.26	NS	NS	NS	0.01	0.01
$C_{17:1}$	0.68	0.68	0.66	0.66	NS	NS	NS	0.02	0.02
C_{17}	0.50	0.50	0.51	0.50	NS	NS	NS	0.04	0.01
$C_{18:2}$	1.75[ab]	1.70[b]	1.76[a]	1.62[c]	NS	*	†	0.03	0.09
$C_{18:1}$	16.60	16.40	15.50	15.38	*	NS	NS	0.65	0.42
C_{18}	8.93	9.04	9.08	8.89	NS	NS	NS	0.36	0.34

[a,b,c] When interaction is significant, means within the same row with different superscripts differ ($P < 0.05$).

[1] Interaction between Met supplementation and energy level.

[2] Residual standard deviation used to test effects of Met supplementation and interaction between Met supplementation and energy level.

[3] Residual standard deviation used to test effects of the energy level.

[4] $P > 0.10$.

† $P \leq 0.10$.

* $P \leq 0.05$.

** $P \leq 0.01$.

normal energy diet (Table 6). No interactions of energy level and Met supplementation on plasma amino acid concentrations were observed (Table 7).

DISCUSSION

Effects of Level of Dietary Energy

A reduction in dietary energy of 2.55 Mcal of NE_L/d did not decrease milk yield, which is in contrast to expectations (5, 6). For cows that received the N requirements in the diet, as was the case in this trial, milk yield should have been reduced by –0.9 kg/d according to the equations established by Coulon and Rémond (6). The duration of energy underfeeding (6 vs. 8 wk) and the type of forage used (corn silage vs. hay or grass silage) could have possibly accounted for the differences between our findings and those of Coulon and Rémond (6). Indeed, over short periods (3 wk) using a diet based on corn silage, Faverdin et al. (9) did not record variation in milk yield if intake differed by 2.72 Mcal of NE_L/d. Body reserve mobilization, indicated by BW loss, the increase in plasma NEFA concentrations, and the enrichment of $C_{18:1}$ in milk fatty acids, could also explain the persistency of milk yield.

Energy underfeeding historically has reduced glycemia (1). The decrease in Thr and the increase in Val are not easily interpretable. The increase in uremia and the plasma concentrations of urea cycle acids resulted mainly from the deamination of the excess amino acids, as reflected by the positive PDI balance observed for cows fed the diet with the low energy supply. This difference in PDI balance resulted, to a large extent, from the decrease in the protein content of milk. This decrease in the protein content of milk with the reduction of energy supply is consistent with the literature. The supplementation of 2.55 Mcal of NE_L induced a 0.11 ± 0.03 percentage units increase in the protein content of milk, which was very close to that computed (0.096 ± 0.05 percentage units) by the equation defined by Coulon and Rémond (6).

Effects of Met Supplementation

The decrease in milk yield that followed Met supplementation was not typical of changes observed after peak lactation (22). This decrease occurred mainly when the cows were in a marked energy deficiency. Using similar doses of Smartamine M™ did not induce a decrease in milk yield of cows fed according to requirements but did induce a nonsignificant decrease in the milk yield of cows that were in a slightly negative energy balance (19). In midlactation cows that were infused with Met, Pizulewski et al. (18) did not report decreased milk yield after increased doses of Met to cows that were in a neutral energy balance (+0.68 Mcal of NE_L). In contrast, in a similar Met infusion experiment, Socha et al. (25) reported that milk yield decreased linearly as Met supplementation increased; unfortunately, the energy balance of the cow is not documented. All of these observations suggest that Met supplementation in midlactation cows may induce a decrease in milk yield that would be more marked as energy balance decreased. The mechanisms by which such effects occur remain to be elucidated.

The increase in the protein content of milk (0.11 percentage units) following Met supplementation was

TABLE 6. Effects of level of dietary energy on responses of some metabolites of plasma to rumen-protected Met supplementation.

	Diet								
	Low energy		Normal energy		*P*				
Concentration	0 g/d of Met	21 g/d of Met	0 g/d of Met	21 g/d of Met	Energy	Met	Int.[1]	RSD1[2]	RSD2[3]
Glucose, mg/100 ml	67.6[b]	70.7[a]	72.0[a]	71.9[a]	**	†	*	3.8	4.5
BHBA, mg/100 ml	5.59	5.54	5.69	6.14	NS[4]	NS	NS	1.02	1.18
NEFA, μmol/L	96.2	74.7	60.9	47.0	**	**	NS	19.6	40.3
Urea, mg/100 ml	32.1	31.4	25.2	25.0	**	NS	NS	2.9	3.2

[a,b] When interaction is significant, means within the same row with different superscripts differ ($P < 0.05$).

[1] Interaction between Met supplementation and energy supply.

[2] Residual standard deviation used to test effects of Met supplementation and interaction between Met supplementation and energy level.

[3] Residual standard deviation used to test effects of energy level.

[4] $P > 0.10$.

†$P \le 0.10$.

*$P \le 0.05$.

**$P \le 0.01$.

identical to those obtained with similar doses of rumen-protected Met (+0.11 and +0.09 percentage units) in trials conducted by Rulquin and Delaby (19). The increase in the proportion of casein in true proteins confirmed the results obtained when cows were administered rumen-protected Met (19) and duodenal infusions of Met (18).

Supplementation of rumen-protected Met induced a decrease in plasma NEFA concentrations, as was observed in the jugular blood of lactating cows (18) and in the mesenteric arterial blood of preruminant calves (3) after duodenal infusion of Met. That decrease had no effect on the composition of milk fatty acids, which suggests that it did not correspond to a reduced mobilization of long-chain fatty acids from body reserves. According to studies involving the hepatic system of calves (3) and dairy cows (7), the decrease in NEFA concentrations that follows Met or Met and Lys supplementation reflects an increase in the export of these amino acids into the triglycerides of very low density lipoproteins. That these amino acids are involved in the synthesis of lecithin or apolipoprotein B has yet to be proven (17). Increased glycemia cannot be a direct effect of Met. This amino acid is not considered to be a major gluconeogenic agent (4). Furthermore, postruminal Met supplements (24 to 30 g/d) did not increase blood glucose in cows that were in positive energy balance (11, 18). According to Armentano et al. (1), gluconeogenesis from propionate is reduced by fatty build-up in the liver. The

TABLE 7. Effects of level of dietary energy on responses of the composition of plasma amino acids to rumen-protected Met supplementation.

	Diet								
	Low energy		Normal energy		*P*				
	0 g/d of Met	21 g/d of Met	0 g/d of Met	21 g/d of Met	Energy	Met	Int.[1]	RSD1[2]	RSD2[3]
EAA[4]									
Thr	1.09	1.13	1.19	1.22	**	NS[5]	NS	0.11	0.01
Val	2.43	2.42	2.30	2.34	†	NS	NS	0.22	0.06
Met	0.26	0.63	0.27	0.64	NS	**	NS	0.03	0.03
Ile	1.40	1.46	1.39	1.44	NS	NS	NS	0.15	0.08
Leu	1.68	1.73	1.61	1.64	NS	NS	NS	0.15	0.07
Phe	0.69	0.70	0.69	0.74	NS	NS	NS	0.05	0.04
Arg	1.38	1.52	1.21	1.30	**	NS	NS	0.12	0.03
Lys	1.29	1.45	1.25	1.31	NS	NS	NS	0.11	0.10
His	0.95	0.88	0.79	0.81	NS	NS	NS	0.07	0.15
Total	11.2	11.9	10.7	11.4	NS	NS	NS	0.90	0.40
NEAA[4]									
Tau	0.47	0.63	0.48	0.68	*	**	NS	0.05	0.02
Asp + Asn	1.06	1.08	1.10	1.13	NS	NS	NS	0.08	0.06
Ser	1.27	1.15	1.26	1.15	NS	†	NS	0.10	0.03
Glu + Gln	4.74	4.74	4.91	4.89	NS	NS	NS	0.23	0.37
Gly	3.37	3.12	3.35	3.13	NS	†	NS	0.17	0.27
Ala	2.33	2.40	2.37	2.40	NS	NS	NS	0.15	0.14
Cit	1.59	1.59	1.47	1.40	*	NS	NS	0.08	0.07
Cys	0.47	0.56	0.41	0.54	NS	**	NS	0.03	0.03
Hcy	0.05	0.09	0.05	0.09	NS	**	NS	0.01	0.01
Tyr	0.74	0.80	0.83	0.86	NS	NS	NS	0.08	0.07
Pro	1.18	1.20	1.11	1.11	NS	NS	NS	0.12	0.07
Orn	0.58	0.66	0.51	0.55	†	NS	NS	0.08	0.06
Total	17.8	18.0	17.8	17.8	NS	NS	NS	1.00	0.71
Total	29.0	29.9	28.5	29.3	NS	NS	NS	1.89	0.48

[1] Interaction between Met supplementation and energy level.

[2] Residual standard deviation used to test effects of Met supplementation and interaction between Met supplementation and energy level.

[3] Residual standard deviation used to test effects of energy level.

[4] EAA = Essential amino acids; NEAA = nonessential amino acids. Expressed in milligrams per 100 ml of plasma.

[5] $P > 0.10$.

† $P \le 0.10$.

* $P \le 0.05$.

** $P \le 0.01$.

effect of Met on the increased exports of liver triglycerides could account for the increased glycemia that was observed in this trial.

The increase in blood Met proves that the Met supplied by Smartamine M™ was very well absorbed. The increase in Met metabolites, such as Tau, Cys, and cystathionine, and the decrease in the amino acids consumed by that catabolism, such as Ser and Gly, corresponded with the variations observed during trials involving the postruminal infusion of Met (11, 18).

Interaction Between Level of Dietary Energy and Met Supplementation

As expected, there were no interactions of production responses to energy level and Met supplementation. The response of milk protein content to these two factors was additive. However, it appears that, in addition to its direct effect on protein synthesis, Met may play a role in liver gluconeogenesis, especially when cows are in a highly negative energy balance. Effectively, the increase in blood glucose was much higher for cows fed the low energy diet than for cows fed the diet with normal energy supply. This observation was consistent with the explanation for the increased gluconeogenesis after an increase in hepatic secretion of very low density lipoproteins, because Auboiron et al. (3) observed that supplemental Met had a beneficial effect on the secretion of very low density lipoproteins in underfed calves but not in calves that were fed according to requirements. The effect on BW of the interaction energy level and Met supplementation remains difficult to explain because it contradicts the variations in milk yield and concentrations of blood glucose and NEFA. The BW variations considered in that interaction were only recorded over 3 wk, which was too short a time for the BW variations to have physiological significance.

CONCLUSIONS

This trial confirmed the effects of energy level and protected Met supplementation on the protein content of milk. The study showed that these effects were additive and that the use of protected amino acids to increase protein content of milk was possible, even in cows that were in a negative energy balance. The hypothetical effect of Met on the liver should be verified by a similar trial, including longer experimental periods and tests to assess lipid build-up in the liver and liver function in general.

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