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The effect of protected methionine on milk yield and its composition in lactating dairy cows fed grass silage-based diets*

P.M. Pisulewski[1] and Z.M. Kowalski[2]

[1]Department of Human Nutrition
[2]Department of Animal Nutrition
The Agricultural University of Cracow
Al. 29 Listopada 46, 31-425 Kraków, Poland

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ABSTRACT

Five multiparous Polish Red-and-White cows (in wk 8-12 of lactation) were assigned to a 5 x 5 Latin square design and fed total mixed ration TMR containing grass silage and concentrates representing 58 and 42% of dietary DM, respectively. The five treatments were dietary supplements of protected DL-methionine (Smartamine™ M) fed in the following amounts: 0, 8, 16, 24 and 32 g/d. Dry matter intake yield milk, milk protein and fat, and contents of protein, fat and lactose in milk were not affected by the treatments. At the same time, NPN (%) was significantly affected ($P<0.01$), and protein-N and NPN (% of total-N) responded in an opposite manner ($P<0.05$). Of the plasma metabolites studied (β-hydroxybutyrate, glucose, and urea), only glucose levels were significantly ($P<0.01$) decreased by methionine supplementation. Plasma free methionine was significantly elevated ($P<0.01$) over the five treatments. In conclusion, the lack of apparent changes in the studied responses could have been due either to a higher methionine supply than that predicted or to a lower methionine requirement than that assumed. Consequently, a consistent relationship between increasing amounts of Smartamine™ M (0, 8, 16, 24, and 32 g/d) and milk protein responses of the cows, could not be described.

KEY WORDS: dairy cow, grass silage, protected methionine, milk yield, milk composition

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INTRODUCTION

It has been emphasized that milk protein has replaced milk fat as the primary target of the dairy industry (Schwab et al., 1992; Murphy and O'Mara, 1993; Sloan, 1997). In this context, increasing milk protein production, particularly the casein fraction, would certainly be of great practical importance. Consequently, nutritional modification of milk protein yield and content by feeding amino acids protected from the rumen degradation, at least L-lysine and DL-methionine, have attracted particular attention (Rulquin et al., 1995; Schwab, 1995). The requirements of dairy cows for lysine and methionine, i.e. 7.3 and 2.5% PDI, respectively, have recently been established (Rulquin et al., 1993).

Since maize silage-based diets predominate in intensive dairy cow feeding, the majority of trials focused on their supplementation with protected lysine and methionine (Rulquin, 1992; Rulquin et al., 1993, 1995; Sloan, 1997). It was shown that under these conditions lysine was clearly the first limiting amino acid and methionine a colimiting one (Rulquin et al., 1993; Sloan, 1997). Conversely, a major nutritional limitation inherent in grass silage-based diets seems to be an insufficient supply of methionine (Thomas and Thomas, 1985; Thomas and Rae, 1988). Interestingly, the determined proportion of these amino acids in the sum of total amino acids determined in the small intestine of cows fed grass silage (Teller et al., 1992; Harrison et al., 1994; Holden et al., 1994), were apparently below the optimum requirement value for methionine (i.e. 2.5% PDI) and comparable or only slightly inferior to the optimum requirement value for lysine (i.e. 7.3% PDI). Therefore, methionine is most likely to limit milk protein yield and/or content, whereas the lysine supply may be sufficient in dairy cows offered grass silage-based rations (Robert et al., 1994; Chillard et al., 1995; Xu et al., 1998).

The objective of this experiment was to verify the validity of the recommended methionine requirement, i.e. 2.5% PDI (Rulquin et al.,1993), in dairy cows fed a grass silage-based diet. The effects of protected DL-methionine on milk yield and composition were used as the main criteria of the above verification.

MATERIAL AND METHODS

Design and treatments

A Latin square (5 x 5) design, with 14-d periods, was used. The treatments were dietary supplements of protected DL-methionine (Smartamine™ M, containing 70% DL-methionine; Rhône-Poulenc Animal Nutrition, Poland): 0, 8, 16, 24, and 32 g/d. Smartamine™M was mixed with a small amount of wheat bran, then top-dressed, and fed to each cow twice daily, in equal doses.

Cows and management

Five multiparous Polish White-and-Red cows (average body weight, 610 kg), in their second or third lactation, were assigned to the trial 8 to 12 week postcalving. Their average pre-experimental milk yield was 20 kg/d. The management of cows was described previously (Pisulewski and Kowalski, 1999).

Diet and feeding

The animals were fed with total mixed ration (TMR; Table 1) consisting of wilted grass silage and concentrates (58 and 42% of dietary DM, respectively). The diet was formulated to cover the INRA (1989) net energy and protein (PDI) recommendations. Concentrations of lysine and methionine in the sum of total AA passing to the small intestine were calculated using an amino acid profiling method according to Rulquin et al. (1998).

Measurements, sample collection, preparation and analytical procedures

The details of the performed measurements, sampling of feeds, refusals, milk and blood, as well as their preparation and analyses were described in a previous paper (Pisulewski and Kowalski, 1999).

TABLE 1

Ingredients, nutrients content (%DM[1]) and nutritive value

Indices			
Ingredients		Nutritive value	
grass silage	58.0	UFL/kg	1.00
concentrate[2]	42.0	PDI(E), g/kg	98
		PDI(N), g/kg	116
Chemical composition		LysDI% PDI	6.89
dry matter	37.4	MetDI% PDI	1.88
organic matter	94.1		
crude protein	19.1		
ether extract	5.6		
crude fibre	18.5		
NDF	41.7		
ADF	26.8		
Ca	0.84		
P	0.66		

[1] the basal diet was supplemented (100 g/cow/day) with a commercial mineral-vitamin mixture „Kuh-Gold – 2000" (Sano, Poland)

[2] contained (%DM): sugar beet pulp – 35, ground maize – 30, ground barley – 20, soyabean meal – 10, dicalcium phosphate – 2.7, sodium chloride – 0.3, sodium bicarbonate – 2

Statistical analysis

The data obtained were analyzed by ANOVA for a 5 x 5 Latin square design using the general linear models procedure of SAS (1985). All data are expressed and presented throughout the text as least square means. The significance of differences was accepted at $P<0.05$.

RESULTS

The average dry matter intake was 15.7 kg (Table 2) and there were no differences among treatments. The daily ration exceeded both net energy (UFL) and protein (PDI) requirements of cows (INRA, 1989), and the average excess of energy was 5.8, 6.7, 8.1, 7.2 and 3.0%, and that of protein 15.2, 16.8, 18.4, 15.4 and 13.4%, respectively. Based on the amino acid profiling method according to Rulquin et al. (1998), the available lysine concentration in the diets were fairly constant, whereas those of methionine were progressively increased over the range of Smartamine™ M supplements (Table 2).

Average milk, milk protein and fat yield, and milk protein, fat and lactose contents did not differ significantly among the treatments, and the recorded values varied inconsistently (Table 3). In contrast, milk N constituents (Table 4) varied

TABLE 2

Feed intake (DM), energy and protein supply and balance in dairy cows fed protected methionine

Indices	Smartamine™ M, g/d					SE	P[1]
	0	8	16	24	32		
DM intake, kg/d	16.0	15.6	16.1	16.0	15.0	0.38	NS
Supply							
UFL/d	14.6	14.3	14.7	14.6	13.7	0.36	NS
total protein, g/d	3047	2990	3068	3052	2860	72	NS
PDI(E), g/d	1563	1534	1575	1566	1468	37	NS
PDI(N), g/d	1851	1816	1864	1853	1737	44	NS
Balance							
UFL/d	0.8	0.9	1.1	0.8	0.4	0.3	NS
PDI, g/d	206	221	245	209	174	34	NS
Amino acids							
Lys, %PDI	6.89	6.87	6.86	6.85	6.82	–	–
Met, %PDI	1.88	2.06	2.31	2.49	2.73	–	–

[1] NS – non significant

TABLE 3

Milk yield and composition in dairy cows fed protected methionine

Indices	Smartamine™ M, g/d					SE	P[1]
	0	8	16	24	32		
Yield							
milk, kg/d	19.6	18.6	19.8	20.0	18.8	0.45	NS
fat, g/d	802	765	779	801	749	25	NS
protein, g/d	659	622	669	674	631	16	NS
Composition							
fat, g/100g	4.07	4.10	3.92	4.00	4.00	0.06	NS
protein, g/100g	3.36	3.36	3.38	3.37	3.36	0.03	NS
lactose, g/100g	4.71	4.61	4.73	4.62	4.65	0.02	NS
urea, mg/100g	31.9	33.3	38.5	37.4	37.4	2.70	NS

[1] NS – non significant

TABLE 4

Distribution of nitrogen fractions in milk from cows fed protected methionine

Indices	Smartamine™ M, g/d					SE	P[1]
	0	8	16	24	32		
N – fractions							
total – N, %	0.562	0.564	0.576	0.564	0.574	0.008	NS
protein – N, %	0.521	0.522	0.535	0.526	0.533	0.007	NS
casein – N, %	0.422	0.416	0.432	0.426	0.426	0.007	NS
whey – N, %	0.099	0.106	0.103	0.100	0.107	0.003	NS
NPN, %	0.041	0.042	0.041	0.038	0.041	0.002	**
N – fractions, % of total N							
protein – N	92.76	92.53	92.89	93.22	92.97	0.25	*
casein – N	75.14	73.72	74.80	75.64	74.18	0.61	NS
whey – N	17.58	18.76	18.04	17.54	18.74	0.51	NS
NPN	7.24	7.47	7.11	6.78	7.03	0.25	*
N – fractions, % of protein - N							
casein – N	80.98	79.67	80.51	81.13	79.80	0.57	NS
whey – N	19.02	20.33	19.49	18.87	20.20	0.57	NS

[1] NS – non-significant, * $P<0.05$, ** $P<0.01$

significantly. Of these, milk NPN (%) responded significantly ($P<0.01$). However, no consistent pattern of this response was observed. Moreover, the fraction of protein-N as % of total-N was significantly ($P<0.05$) increased and that of NPN decreased ($P<0.05$).

Concentrations of blood metabolites (glucose, β-hydroxybutyrate, and urea) were within physiological ranges. At the same time, the concentrations of glucose were significantly ($P<0.01$) decreased by increasing doses of protected methionine (Table 5). Of plasma free amino acids, concentrations of methionine were significantly ($P<0.01$) elevated over the range of treatments (Table 6). The concentrations of the remaining essential and nonessential amino acids were fairly constant. The only exception could be high concentrations of taurine, only slighty associated with increasing doses of protected methionine.

DISCUSSION

The main objective of the present study was to describe productive and some systemic responses in dairy cows fed grass silage-based diets when supplemented with increasing doses of protected methionine (Smartamine™ M). The amounts of the protected amino acid were designed to meet the methionine requirement (i.e. 2.5% PDI) proposed by Rulquin et al. (1993), and produced the following concentrations of methionine (%PDI): 1.88, 2.06, 2,31, 2.49, and 2.73, respectively.

There was no significant effect of protected methionine supplementation on DMI and milk yield (Table 2). These effects confirm earlier results of several studies in which grass silage-based diets were intravenously (Chamberlain and Thomas, 1982) or postruminally (Remond et al., 1989; Chillard et al., 1995) supplemented with methionine. Similarly, postruminal methionine did not affect DMI or milk yield of cows fed with maize silage (Pisulewski i in., 1996; Rulquin and Delaby, 1997) or lucerne-maize silage-based diets (Overton et al., 1996, 1998). However, in some conditions, supplementing grass silage-based diets with protected (lysine and methionine) amino acids increases DMI (Xu et al., 1998). Since milk yield of dairy cows may be increased by supplemental amino acids in the early stages of lactation, and not in mid- or late lactation (Rulquin, 1992) we did not expect marked changes of these measurement in our studies (Table 3).

The lack of significant effects of increasing supplements of methionine on milk protein content and yield (Table 3) contradicted the results of some previous reports. Namely, milk protein content and yield were consistently increased by postruminal supplements of methionine on both grass silage (Chillard et al., 1995; Kowalski et al., 1999), and maize silage (Pisulewski et al., 1996; Rulquin and Delaby, 1997) based diets. However, no positive responses were observed in similar experiments using intravenous (Chamberlain and Thomas, 1982) or postruminal supplements of methionine (Remond et al., 1989; Overton et al., 1996; Overton et al., 1998). To offer an explanation, it seems that in spite of the calculated deficit of intestinally available methionine (Table 2), adequate amounts of this amino acid were supplied by the basal diet and/or protein reserves of the experimental cows. Alternatively, the actual methionine requirement could be lower than that assumed. The changes induced in milk N fractions (Table 4), particularly an opposite pattern of responses noted for protein-N and NPN (% of total N), were particularly important. Similar trends have been already reported for dairy cows fed grass silage- (Chillard et al., 1995) and maize silage- (Pisulewski et al., 1996; Rulquin and Delaby, 1997) based diets. However, the results of comparable studies of Overton et al. (1996; 1998) were inconclusive. Generally, the trends noted in our studies could be indicative of a stimulating effect of the supplemental amino acids on milk true protein synthesis. These observations are of particular importance because processing characteristics of milk (i.e. cheese yielding capacity) are closely related to its casein content. This aspect of feeding protected lysine and methionine was studied by Hurtaud et al. (1995) in cows fed maize silage-based rations. Milk urea concentrations, considered to be a means of monitoring the adequacy of protein nutrition of dairy cows (Hof et al., 1997) were not affected significantly by the treatments. However, they tended to increase over the supplemental doses of protected methionine. We have no explanation for this effect.

Of particular interest are responses of milk fat content and yield. Generally, they were in line with the results of similar studies (Chillard et al., 1995), in which, feeding protected methionine did not significantly alter these parameters. Moreover, marked increases in milk fat yield might be expected only in early stages of lactation as achieved through increasing milk yield (Rulquin, 1992) or both fat content and milk yield (Xu et al., 1998). However, the effects of postruminal supplements of methionine on milk fat synthesis are a matter of controversy. In earlier (Chamberlain and Thomas, 1982) and more recent (Overton et al., 1996, 1998) studies, methionine increased both milk fat content and yield. This effect could be related to metabolic effects of choline synthesized from methionine (Sharma and Erdman, 1989).

Concentrations of plasma metabolites (Table 5) can be discussed in terms of the energy and protein status of the dairy cows. Since the net energy requirements (INRA, 1989), were met with an average excess of 6.2% (Table 2), energy was probably not a limiting factor in our studies. In spite of this evidence, plasma glucose concentrations were significantly decreased, thus indicating an insufficient energy supply. On the other hand, β-hydroxybutyrate concentrations were not affected by the treatments, implying that mobilization of body fat was not in excess of carbohydrate energy supply.

TABLE 5

Concentration of metabolites in plasma of dairy cows fed protected methionine

Metabolite	Smartamine™ M, g/d					SE	P[1]
	0	8	16	24	32		
β-hydroxybutyrate, mg/dL	8.23	8.56	11.20	8.28	7.91	0.74	NS
Glucose, mg/dL	55.1	59.0	49.0	56.9	50.8	1.26	**
Urea, mg/dL	39.4	42.6	37.9	37.3	34.8	0.99	NS

[1] NS – non-significant, ** $P<0.01$

TABLE 6

Concentrations of free amino acids ((moles/100 ml) in blood plasma of dairy cows fed protected methionine

Amino acids	Smartamine™ M, g/d					SE	P[1]
	0	8	16	24	32		
Essential							
Lys	6.99	5.88	6.08	6.89	7.61	0.38	NS
His	9.99	10.45	9.28	9.89	10.20	0.38	NS
Arg	8.89	8.14	7.62	8.54	9.22	0.26	NS
Thr	13.99	13.58	15.10	14.04	15.29	0.68	NS
Val	16.18	15.99	15.66	17.60	17.91	0.67	NS
Met	2.46	3.07	2.67	4.34	4.70	0.26	**
Cys[2]	1.74	1.31	1.22	1.20	1.47	0.13	NS
Ile	8.62	7.70	8.22	9.22	9.70	0.35	NS
Leu	11.15	10.40	8.80	11.34	12.27	0.56	NS
Phe	3.85	3.95	3.53	4.27	4.95	0.17	NS
Tyr[2]	3.74	3.85	3.71	4.48	4.43	0.20	NS
Nonessential							
Asp + Asn	1.75	1.58	1.47	1.58	1.84	0.05	NS
Ser	8.73	8.96	7.22	6.77	9.23	0.53	NS
Glu + Gln	27.02	29.37	25.49	27.30	25.83	0.94	NS
Pro	10.33	9.45	11.02	10.46	12.47	1.22	NS
Gly	36.21	34.00	33.99	39.93	37.13	3.01	NS
Ala	17.22	18.03	14.31	18.94	18.39	0.77	NS
Tau	3.29	2.80	4.29	3.66	4.02	0.30	NS

[1] NS – non-significant, ** $P<0.01$

[2] included as semiessential amino acids

The protein status of the cows resulted from the amount of protein provided by the ration and the amounts of protected lysine and methionine fed. Generally, the cows were in a positive protein balance resulting from relatively high crude protein content (19.1%DM) of the ration. In addition, the ration was imbalanced (Table 2) in terms of PDIN and PDIE. Furthermore, it supplied an excess (averaging 15.6%) of recommended PDI (INRA, 1989). Importantly, the increased protein supply should have enhanced more marked responses of the cows to the supplemental amino acids. Indeed, milk protein responses to postruminal lysine and methionine were much elevated in cows fed high protein than in cows receiving low protein diets (Rulquin et al., 1994), and could be due to sufficient supply of other amino acids limiting milk protein secretion. At the same time, excessive protein supply could have led to ammonia overload in the liver, as evidenced by elevated plasma and milk urea concentrations (Tables 3 and 5). Consequently, the ammonia excess could have affected glucose synthesis in the liver and the resulting plasma glucose concentrations.

Significant ($P<0.01$) increases of plasma free methionine (Table 6), prove that methionine was effectively protected in the rumen and then absorbed in the small intestine. This effect is a common feature of experiments involving protected forms of methionine (Overton et al., 1996, 1998; Rulquin and Delaby, 1997; Overton et al., 1998; Xu et al., 1998). Moreover, elevated levels of methionine metabolites, such as taurine, were consistent with the results of similar experiments using methionine (Overton et al., 1996; 1998; Pisulewski et al., 1996; Rulquin and Delaby, 1997).

The described pattern of responses could not be exploited further to determine the extent of methionine limitation in the cows fed grass silage-based diets. Indeed, inconsistent and insignificant milk protein yield and content responses (Table 2) could not be described in terms of graded methionine supplements and resulting postruminal methionine supply. On the other hand, feeding protected methionine significantly increased the true protein content in milk.

CONCLUSIONS

Dairy cows fed a grass silage-based ration providing adequate net energy (NE_L) and protein (PDI), and supplemented with predetermined amounts of protected methionine (Smartamine™ M) had no ability to increase milk protein content or yield. This may indicate that the diet supplied more available methionine than predicted or that the actual methionine requirement was lower than that assumed. On the other hand, the methionine supplied by Smartamine™ M was effectively absorbed, and the cows significantly increased the true protein fraction (% of total N) in milk. Further studies using high-producing dairy cows fed grass silage-based rations, are certainly needed to conclusively establish the nutritional situation and corresponding required supplemental allowances of protected methionine.

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STRESZCZENIE

Wpływ chronionej metioniny na wydajność, skład mleka i wybrane wskaźniki metaboliczne u krów żywionych kiszonką z traw

Badania przeprowadzono na 5 krowach wieloródkach rasy czerwono-białej, w 8-12 tygodniu laktacji, w układzie kwadratu łacińskiego (5 x 5). Zwierzęta żywiono dawką pokarmową zawierającą w s.m. (%): kiszonkę z traw 58 i mieszankę pasz treściwych 42 (%: wysłodki buraczane 35, kukurydza 30, jęczmień 20, poekstrakcyjna śruta sojowa 10 i mieszanka mineralna 5). Zastosowano pięć poziomów dodatku chronionej DL-metioniny (Smartamine™ M): 0, 8, 16, 24 i 32 g/d. Nie stwierdzono istotnych różnic w pobraniu s.m., dziennej wydajności mleka, białka i tłuszczu oraz zawartości białka, tłuszczu i laktozy w mleku. Stwierdzono natomiast istotny ($P<0{,}01$) wpływ chronionej metioniny na zawartość N-niebiałkowego, a ujemny ($P<0{,}05$) na względny udział N-białkowego i N-niebiałkowego (% N-ogólnego). Spośród badanych metabolitów osocza (kwas β-hydroksymasłowy, glukoza i mocznik), istotnie ($P<0{,}01$) obniżył się tylko poziom glukozy. Poziom wolnej metioniny w osoczu wzrastał istotnie ($P<0{,}01$) przy każdym dodatku metioniny.

Brak wyraźnych zmian badanych parametrów produkcyjnych wskazywałby bądź na większą podaż metioniny w stosunku do przewidywanej, bądź na mniejsze zapotrzebowanie na ten aminokwas w stosunku do zakładanej wartości. Przedstawione wyniki nie pozwalają zatem na zdefiniowanie zależności pomiędzy rosnącym poziomem dodatku Smartamine™ M (0, 8, 16, 24 i 32 g/d) oraz wydajnością i zawartością białka w mleku.