Feeding ruminally protected methionine to pre- and postpartum dairy cows: effect on milk performance, milk composition and blood parameters

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ABSTRACT: An experiment was conducted to evaluate the effect of ruminally protected methionine (Mepron®, Degussa AG, Germany) in dairy cows. Three weeks before calving 36 cows (Holstein and Czech Fleckvieh breeds) were assigned to one of the two dietary treatments (M and O), and received a total mixed ration with protected methionine at 18.2 g per head per day or without this supplement. After calving, both groups were divided into two subgroups and fed a diet for dairy cows based on ensiled feeds and concentrates for 90 days. A half of the cows received protected methionine (subgroups MM and OM), the other cows were fed the same diet without protected methionine (subgroups MO and OO). Milk yield in cows fed protected methionine for the whole experiment duration (cows MM) was higher and feed intake was lower than in cows of the other groups. The effect of protected methionine on milk yield was not, however, statistically significant ($P > 0.05$). Effects of protected methionine on milk fat and protein were small and inconsistent. Supplemental methionine significantly increased the methionine concentration in serum ($P < 0.05$) while the methionine concentration in milk was not increased quite significantly ($P < 0.10$). Concentrations of essential amino acids in milk were significantly or marginally significantly higher in cows fed protected methionine. In summary, the supplementation of ruminally protected methionine at 18.2 g per head per day had beneficial, but small and mostly statistically insignificant effects on milk performance and milk composition.

Keywords: methionine; milk yield; milk components; serum

Methionine is often the first limiting amino acid in a variety of ruminant diets. The metabolic requirement for methionine is high in dairy cows, in part because of its role as a methyl donor in transmethylation reactions in the synthesis of milk fat. Methionine supplementation of dairy diets is a common method to improve the performance of high-yielding dairy cows. Literature dealing with the use of ruminally protected methionine in dairy cows is extensive. There are reports on effects on milk performance and physiological measures (Rogers et al., 1987; Donkin et al., 1989; Blum et al., 1999; Pisulewski and Kowalski, 1999; Misciattelli et al., 2003), reports on the effect on rumen microorganisms (Noftsger et al., 2005; Karnati et al., 2007) as well as reports on effects during the periparturient period and in the course of lactation (Bach et al., 2000; Piepenbrink et al., 2004; Foroughi et al., 2005; Socha et al., 2005). Rumen protection of methionine is based on chemical derivatization or physical protection by coating. The latter method seems to be the most effective approach to date. Several commercial preparations of this type are available. The effects of various commercial preparations are not, however, predictable as dietary sources of carbohydrate and

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Table 1. Ingredients and chemical composition of diets before and after calving[1]

	Before calving	After calving
Ingredients (%DM)		
Ensiled cereals and legumes	41.30	–
Maize silage	20.20	7.60
Ensiled maize cobs	–	22.20
Lucerne silage	6.50	20.30
Lucerne hay	13.80	2.10
Sugar beet pulp, ensiled	–	5.80
Wheat, ground	4.30	10.80
Barley, ground	1.90	1.20
Maize, ground	2.50	1.50
Brewer's grain	–	4.60
Soybean meal, extracted	5.90	14.50
Rapeseed meal, extracted	1.80	4.10
Dried whey	–	1.20
Megalac	–	1.90
Limestone	0.15	0.40
Salt	0.18	0.20
Propylene glycol 65	0.55	0.30
Vitamin-mineral supplement[2]	0.92	1.30
Diet formulation (nutrients in kg per head/day)[3]		
Dry matter	12.65	22.40
Crude protein	1.84	4.18
Crude fat	0.30	0.94
NDF	3.44	3.32
ADF	2.35	2.33
Ca	0.11	0.24
Mg	0.04	0.07
P	0.05	0.10
PDIE	1.15	2.42
NE_L (MJ)	79.40	159.60

[1]experimental diets were supplemented with Mepron® at the expense of rapeseed meal
[2]supplement contained per kg: Ca – 105 g, P – 85 g, Na – 95 g, Mg – 70 g, Zn – 3.6 g, Mn – 1.5 g, Cu – 0.51 g, Se – 80 mg, vitamin A – 570 000 IU, vitamin D_3 – 68 000 IU, vitamin E – 0.65 g
[3]the rations were formulated with respect to nutrient requirements and nutritive values of feedstuffs for ruminants (Sommer et al., 1994)

protein can modulate the response of animals to an increased methionine supply from the intestine (Overton et al., 1998).

The aim of the present study was to assess the effect of Mepron® (Degussa AG, Frankfurt am Main, Germany) on milk yield, milk composition and blood parameters in high-yielding dairy cows fed a total mixed ration (TMR). Mepron® is methionine coated with a thin layer of ethyl cellulose. It is known that preparturient cows have a large requirement for methionine (Bach et al., 2000). Methionine supplementation in the last weeks of pregnancy may spare body reserves of the dam and positively influences protein metabolism and milk yield postpartum. Thus, the effect of methionine supplementation before calving was also assessed.

MATERIAL AND METHODS

Animals and diets

Thirty-six multiparous high-yielding dairy cows (Holstein and Czech Fleckvieh breeds) were divided into two groups ("M" and "O"), according to an analogue method, 21 days before their expected calving days. Cows were allocated into groups of eighteen on the basis of milk production in the previous lactation (8 259 and 8 253 kg in groups M and O, respectively) and genotype. The average body weight and parity of cows was 610 kg and 3.0, respectively. Diets of groups M and O differed in the presence of ruminally protected methionine (Mepron®). Mepron® (Degussa AG, Germany) contains 85% of methionine, 3% of crude fibre and 2% of crude fat. Cows of the M group received Mepron® at 18.2 g (i.e. 15.5 g methionine) per head per day. Diets were fed as a total mixed ration (TMR) to avoid the selection of dietary components. The diet for dry cows (Table 1) was fed until the 7th day postpartum. Immediately after calving, both groups were divided according to an analogue method into two subgroups, 9 cows each. A half of the cows received Mepron® (subgroups MM and OM), the other cows did not receive any supplement (subgroups MO and OO). The scheme of the experiment was as follows:

Groups	
prepartum	*postpartum*
M	MM
	MO
O	OM
	OO

In groups M, O, MM/OM and OO/MO Lys DI represented 6.90, 6.95, 6.69 and 6.71% PDIE. The respective values of Met DI in these groups were 2.97, 1.84, 2.25 and 1.74%. In the MM/OM diets the Lys DI/Met DI ratio of 2.97 is optimal for milk protein secretion (St-Pierre and Sylvester, 2005). Since the 28th day of experiment, i.e. from the 8th day postpartum, a diet for dairy cows with a higher concentration of dry matter and crude protein and a lower concentration of fibre (Table 1) was fed till the 90th day after calving. The rations were formulated with respect to nutrient requirements and nutritive values of feedstuffs for ruminants (Sommer et al., 1994). The animals were housed in a free-stall barn and fed using the INSENTEC system for the individual roughage intake control by Insentec B.V. (Marknesse, The Netherlands). Diets were fed *ad libitum*.

Sampling and measurements

Feed intake was recorded electronically. Samples of feeds were taken once a fortnight. Milk production was recorded at both milkings daily. Milk for determination of milk components was sampled from each cow in ten-day intervals. Blood was sampled once a week from the *vena jugularis*. Samples of milk and blood for determination of amino acids were frozen and kept at –40°C until analyzed. Milk for determination of fat, protein, lactose and urea was preserved with 2-bromo-2-nitropropane-1,3-diol. Samples of feed were analyzed immediately after sampling.

Analyses and calculations

Analyses of feeds, milk and plasma constituents were performed as described previously (Kudrna and Marounek, 2006, 2008). Amino acids in milk and methionine in serum were determined according to ISO 13903, employing the AAA 400 amino acid analyser (Ingos, Praha, Czech Republic). Met DI and Lys DI were calculated according to Rulquin and Vérité (1993) and Rulquin et al. (2001).

The t-test was used to compare data of two groups. One-way ANOVA, followed by Tukey's test, was used to determine the significance of differences between four groups (SAS Software, Version 8.2).

RESULTS

The intake of TMR in dairy cows fed diet M in the preparturient period was significantly lower

Table 2. Postpartum intake of nutrients[1] in cows fed diets with or without ruminally protected methionine

Methionine (*prepartum*/*postpartum*)	Subgroup[2]				SEM
	MM +/+	MO +/–	OM –/+	OO –/–	
Dry matter	17.89	17.77	18.83	19.04	3.77
Crude protein	3.27	3.30	3.56	3.53	0.70
Crude fat	0.86	0.87	0.94	0.93	0.18
NDF	6.52	6.79	7.12	7.30	1.40
ADF	3.43	3.68	3.82	3.96	0.76
Ca	0.138	0.146	0.150	0.156	0.031
Mg	0.038	0.039	0.042	0.043	0.008
P	0.067	0.069	0.073	0.074	0.014
PDIE	1.85	1.87	2.01	2.00	0.40
NE_L (MJ)	127	130	138	139	28

[1]kg per head/day
[2]see Material and Methods for explanation

($P < 0.05$) than the feed intake in cows fed diet O (10.69 ± 0.30 and 12.52 ± 0.31 kg DM/day, respectively; means ± SEM). Table 2 documents the postpartum intake of nutrients in cows fed diets MM, MO, OM and OO. The effect of diets on feed intake, milk yield and milk composition is shown in Table 3. There was no significant effect of treatments on nutrient intake. Milk yield in cows fed protected methionine for the whole experimental period (subgroup MM) was numerically higher than in cows of the other groups. However, the difference was not statistically significant. There

Table 3. Average milk production and milk composition in cows fed diets with or without ruminally protected methionine

Methionine (*prepartum*/*postpartum*)	Subgroup[1]				SEM
	MM +/+	MO +/–	OM –/+	OO –/–	
Milk yield (kg/day)	34.84	32.32	33.62	33.55	7.31
Fat-corrected milk (kg/day)	32.42	30.89	31.29	30.14	6.54
Milk fat (%)	3.54[ab]	3.70[a]	3.54[ab]	3.32[b]	0.76
Fat production (kg/day)	1.23	1.20	1.19	1.11	
Milk protein (%)	3.11[ab]	3.22[a]	3.12[ab]	3.04[b]	0.37
Protein production (kg/day)	1.08	1.04	1.05	1.02	
Lactose (%)	4.90	4.95	4.97	4.93	0.18
Lactose production (kg/day)	1.71	1.60	1.67	1.66	
Urea N (mg/l)	134[a]	123[b]	137[a]	129[ab]	25

[1]see Material and Methods for explanation
[a,b]means in the same row not sharing the same superscript differ significantly ($P < 0.05$)

Table 4. Amino acid and fatty acid composition of milk from cows fed diets with or without ruminally protected methionine

Methionine (*prepartum*/*postpartum*)		Subgroup[1]				SEM
		MM +/+	MO +/–	OM –/+	OO –/–	
Amino acids[2]						
Methionine		0.71	0.48	0.55	0.46	0.27
Threonine		1.38[a]	1.29[ab]	1.27[ab]	1.21[b]	0.15
Alanine		1.01[a]	0.92[ab]	0.92[ab]	0.85[b]	0.09
Valine		1.87[a]	1.78[a]	1.89[a]	1.65[b]	0.19
Leucine		3.03[a]	2.81[ab]	2.83[ab]	2.69[b]	0.25
Isoleucine		1.51[a]	1.42[ab]	1.39[ab]	1.31[b]	0.13
Tyrosine		0.46[a]	0.36[ab]	0.32[b]	0.37[ab]	0.08
Phenylalanine		0.31	0.61	0.47	0.50	0.31
Histidine		0.64	0.74	0.71	0.66	0.27
Lysine		2.58[a]	2.41[ab]	2.32[ab]	2.26[b]	0.23
Arginine		1.64	1.33	1.18	1.17	0.60
Fatty acids[3]						
Caproic	C6:0	1.36	1.27	1.29	1.28	0.20
Caprylic	C8:0	0.99	0.95	0.95	0.94	0.11
Capric	C10:0	2.68	2.46	2.60	2.18	0.38
Lauric	C12:0	3.37[a]	3.21[a]	3.42[a]	2.77[b]	0.46
Myristic	C14:0	11.89	11.28	11.95	10.77	1.14
Myristoleic	C14:1	0.79	0.97	1.18	1.13	0.36
Palmitic	C16:0	35.71	33.89	35.14	34.15	2.20
Stearic	C18:0	9.58	9.84	8.18	9.95	1.77
Oleic	C18:1	22.18	23.86	22.14	24.42	2.43
Linoleic	C18:2	3.80	3.75	3.98	3.82	0.41
CLA	C18:2	0.52	0.53	0.57	0.50	0.12
α-Linolenic	C18:3	0.39	0.46	0.41	0.40	0.05
Arachidonic	C20:4	0.20	0.20	0.20	0.18	0.03
Other acids		6.54	7.33	7.99	7.51	2.79
SFA		70.17	67.88	69.05	67.08	
MUFA		24.58	26.79	25.34	27.62	
PUFA		5.25	5.33	5.61	5.30	
C6-C14 acids		21.08	20.14	21.39	19.07	

[1]see Material and Methods for explanation
[2]expressed in g per kg of milk
[3]expressed in g per 100 g of fatty acids determined
[a,b]means in the same row not sharing the same superscript differ significantly ($P < 0.05$)

Table 5. Concentrations[1] of methionine in the blood serum of cows fed diets with or without ruminally protected methionine

Period before calving[3]	Group[2]				
	M		O		
3rd week	20.83 (0.53)		19.33 (073)		
2nd week	24.26 (0.84)[a]		19.55 (0.61)[b]		
1st week	28.17 (1.11)[a]		18.55 (0.87)[b]		
Period after calving	subgroup[2]				SEM
	MM	MO	OM	OO	
1st week	28.47[a]	21.27[b]	27.20[a]	20.58[b]	4.52
2nd week	28.42[a]	21.93[b]	29.37[a]	19.58[b]	2.85
3rd week	25.07[a]	22.50[ab]	25.08[a]	19.02[b]	3.89
4th week	28.10[a]	22.20[ab]	27.95[a]	19.02[b]	3.68

[1]in µmol/l

[2]see Material and Methods for explanation

[3]mean values and SEM in parentheses

[a,b]means in the same row not sharing the same superscript differ significantly ($P < 0.05$)

was no effect of diets on FCM yield and lactose content of milk. The lowest milk fat and protein concentrations were observed in cows that did not receive any ruminally protected methionine at all (subgroup OO). The methionine supplementation marginally ($P < 0.10$) increased methionine concentration in milk (Table 4). Concentrations of 7 out of 11 amino acids were significantly higher in milk of cows fed protected methionine for the whole experiment than in cows fed the unsupplemented diet (subgroups MM and OO, respectively). Five of these amino acids (threonine, valine, leucine, isoleucine, lysine) are generally regarded as essential for humans. Milk fat of cows fed the diet OO contained significantly less lauric acid than milk fat of other cows (Table 4). No other significant changes in the fatty acid profile of milk fat were observed. The supplementation of protected methionine significantly increased methionine concentration in serum (Table 5). On the other hand, glucose, triacylglycerols, cholesterol, total protein and urea in serum were not significantly affected (data not shown).

DISCUSSION

Reports on effects of ruminally protected methionine in dairy cows are controversial. In some experiments, high-producing dairy cows fed diets that were adequate in crude protein responded to ruminally protected methionine by increased production of milk, milk fat and protein (Robinson et al., 1995; Kudrna et al., 1998; Schmidt et al., 1999; Samuelson et al., 2001; Lara et al., 2006; Broderick et al., 2008). In our previous study the effect of Smartamine[MT] (Rhône-Polenc Animal Nutrition, France) on performance and milk composition was investigated in cows fed a maize and grass-silage based diet (Kudrna et al., 1998). The authors concluded that Smartamine[MT] at 12 g per head per day increased milk yield and milk protein production without affecting lactose and fat contents of milk. The effect of Smartamine[TM] in the first phase of lactation was higher than in the mid-lactation. In a comparative trial with steers, Smartamine[M] was the most effective source of methionine out of three ruminally protected methionine sources (Sudekum et al., 2004). The encapsulation of methionine in Smartamine[MT] and Mepron® is different. In Smartamine[MT] methionine is covered with a coat of stearic acid containing poly-2-vinylpyridine-co-styrene. In the present study and in experiments by Donkin et al. (1989), Blum et al. (1999), Pisulewski and Kowalski (1999), Izumi et al. (2000), Kowalski et al. (2003) and Broderick et al. (2005) the supplementation of ruminally protected methionine to diets for early-lactation dairy cows did not significantly increase milk yield, and it influenced the milk composition only marginally. A significant elevation of methionine concentration in serum was the most pronounced effect of protected methionine supplementation (Table 4). However, the circulating methionine did not influence the methionine concentration in milk or fatty acid composition of milk lipids very much. The proportion of saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively) was similar to that observed in other studies (e.g. Liu et al., 2008). The proportion of fatty acids synthesized *de novo* in the mammary gland (C6-C14) was higher by 10.5% in cows of the group MM than in those of the group OO. Circulating methionine concentrations were in the range of values reported by other authors (e.g. Rogers et al., 1987).

Most of the studies in the literature dealing with ruminally protected methionine in the diet start either a few weeks after parturition or during mid-lactation. Information on the effects of ruminally protected methionine before calving is insufficient. In the present experiment cows receiving ruminally protected methionine before calving (subgroups MM and MO) consumed significantly less feed than cows of the negative control (subgroup OO). Socha et al. (2005) observed an insignificantly lower feed intake in cows fed encapsulated methionine, both in the pre- and postparturient period. No negative effect of ruminally protected methionine on feed intake has been reported by other authors (Robinson et al., 1995; Blum et al., 1999; Pisulewski and Kowalski, 1999; Lara et al., 2006).

Socha et al. (2005) concluded that in early-lactation cows the response to rumen-protected methionine and lysine depended on dietary crude protein concentration, supply of metabolizable protein, and intestinal digestibility of rumen-undegradable protein. Milk and milk component yield responses to rumen-protected methionine and lysine were greater with the diet containing 18.5% of crude protein than with the diet containing 16.0% crude protein. The former crude protein concentration was similar to that in the present experiment (18.7%). In our study, the lactational response of dairy cows to ruminally protected methionine was disputable because of a lack of statistical significance. The effect of Mepron® supplementation to diets was lower than expected, in spite of the fact that the greatest effect to improved methionine nutrition should occur in early lactation when the need for absorbed amino acids is the highest.

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