Effects of a rumen-protected form of methionine and a methionine analogue on the lactation performance of dairy cows

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ABSTRACT: The objective the present study was to determine the influence of a supplemental methionine analogue, the isopropyl ester of 2-hydroxy-4-(methylthio)-butanoic acid, commercially available as MetaSmart^{TM}, on lactation performance, particularly milk protein production. The effects of this preparation were compared with those of a rumen-protected form of methionine, marketed as Smartamine^{TM} M. Experiments were conducted according to a 3 × 3 Latin square design and included 30 high-yielding dairy cows (22 Holstein and 8 Czech Fleckvieh) randomly allocated to three balanced groups. Cows were fed a basal diet based on maize silage, lucerne silage, lucerne hay, fresh brewer's grains, and a concentrate mixture in the form of a total mixed ration *ad libitum*. The diet M was supplemented with MetaSmart^{TM} (42.5 g/day) and diet S was supplemented with Smartamine^{TM} M (19 g/day), while control diet C contained solvent-extracted soybean meal, which was added to achieve required levels of dietary protein. Each period lasted four weeks in total, including three preliminary weeks and one experimental week during which samples of milk and tail vein blood were taken. Supplementation of MetaSmart^{TM} decreased dry matter intake of cows (18.96 kg) in contrast to the diet containing Smartamine^{TM} M, for which dry matter intake was the highest (20.48 kg; $P < 0.001$). Despite decreased dry matter intake, the highest average milk yields were recorded for cows supplemented with MetaSmart^{TM} (31.34 kg), which produced by approximately 1.14 kg ($P < 0.001$) and 0.78 kg ($P < 0.01$) more milk than cows fed diets C and S, respectively. As expressed by greater ratios milk/DMI, FCM/DMI, and ECM/DMI, the feed efficiency was improved in cows supplemented with MetaSmart^{TM}. Both MetaSmart^{TM} and Smartamine^{TM} M dietary supplementation increased milk yield, milk protein concentrations, and yields and increased the prevalence of β-casein fraction in milk protein.

Keywords: nutrition; amino acids; milk production; milk protein; casein

Diets for high-yielding dairy cows should be formulated to supply sufficient amount of crude protein for maintenance, growth of a possible foetus, optimal growth of rumen microorganisms, and desired milk protein synthesis. Excess nitrogenous substances in a cow's diet are degraded to ammonia, and any ammonia that is not used by rumen bacteria must be detoxified in the liver. Moreover, excess N excreted in faeces can have a negative impact on the environment. Reduction of dietary protein associated with increased N utilisation may decrease NH_3 volatilisation from manure (Dinn et al., 1998; Kröber et al., 2000). In addition to the quantity, the quality of protein consumed, in terms of its degradability and amino acid (AA) composition, is an important factor in determining N utilisation and excretion. For lactating dairy cows lysine (Lys) and methionine (Met) have been identified as the two most common limiting AA in diets based on maize silage (Schwab et al., 1992a, b; NRC, 2001; Třináctý et al., 2006), and the percentage of protein in milk has been shown to increase after dietary supplementation with these amino acids (Třináctý et al., 2009). This is likely because these amino acids are present at relatively low concentrations in feeds, compared with their concentrations in milk

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and in ruminally-synthesised bacterial protein. According to NRC (2001) the optimal fractions of Lys and Met in total metabolizable protein (MP) for maximal milk protein production are 7.2 and 2.4%, respectively. However, these concentrations are difficult to be achieved under typical circumstances. Therefore, minimum fractions of approximately 6.6% for Lys and 2.2% for Met in MP have been recommended (Schwab et al., 2003; Piepenbrink et al., 2004). However, the amino acid balance can be an even more important factor than total rumen-undegradable protein (RUP) supplementation for improving the efficiency of N utilisation, achieving maximal milk yields and increasing milk protein production (Noftsger and St-Pierre, 2003). The desired Lys : Met ratio in MP for optimal microbial protein synthesis is 3 : 1, a ratio that makes it possible to lower the concentration of crude protein in feed rations from 18–19% to only 15–16% of dry matter (DM), thus improving reproduction efficiency and lowering the amount of N excreted in faeces (NRC, 2001; Schwab et al., 2003). When feed rations are balanced with respect to Lys and Met, overall feed efficiency and, in particular, energy status are also improved (Broderick and Muck, 2009), suggesting that Met plays other roles in metabolism beyond simply serving as a building block for milk protein synthesis. Data collected by Baudet (1995) show that most of the common ruminant feeds contain sufficient levels of Lys but insufficient levels of Met. This means that the diets of most high-yielding dairy cows, particularly at the early lactation stage, are deficient in Met, making this amino acid the limiting factor in determining milk protein synthesis and milk yields. Therefore, new forms of Met and new methods of protecting Met against ruminal degradation have been developed and tested. One example of rumen-protected Met is Smartamine^{TM} M. However, as stated by the producer (Adisseo France SAS, Antony Cedex, France), this product is quite susceptible to mechanical disturbance of its protective coating which leads to its degradation in the rumen. Therefore this product is less suitable for mixing into feed mixtures and unsuitable for grinding and granulation. More recently the analogues and derivatives of Met have also been used in dairy nutrition. One newly-developed analogue of Met is the isopropyl ester of 2-hydroxy-4-(methylthio)-butanoic acid (HMBi), marketed as MetaSmart^{TM} (Adisseo France SAS, Antony Cedex, France). It has been reported that approximately one-half of the HMBi ingested by the dairy cow is absorbed across the rumen wall, hydrolyzed to 2-hydroxy-4-(methylthio)-butanoic acid (HMB) and then converted to Met (Robert et al., 2000; Graulet et al., 2004, 2005). This metabolizable Met serves for milk protein synthesis and other functions in the cow.

The objective of this experiment was to determine the effects of the supplemental preparations MetaSmart^{TM} and Smartamine^{TM} M on lactation performance, particularly on the production of milk protein, and on the physiological status of dairy cows.

MATERIAL AND METHODS

Experimental design, animals, and diets

Experiments were conducted according to a 3 × 3 Latin square design. The scheme of the experiment is depicted in Table 1. Each of three periods lasted four weeks including three preliminary weeks and one experimental week during which samples for laboratory analyses were taken. The experiment included 30 high-yielding dairy cows – 22 Holsteins and 8 Czech Fleckviehs, seven primiparous and 23 older cows (mean parity for all cows 2.61 ± 1.43), 87 ± 25 days in milk (DIM), mean milk yield 40 ± 9 kg. Cows were blocked into three groups according to breed, number of calving, previous lactation performance, and live body weight. Initially the total of 33 dairy cows was chosen for the experiment, but three cows were displaced due to various reasons (e.g. difficulty to learn eating from the automatic troughs, health disorders) before the start of the trial. Therefore there were 11 (Group 1), 10 (Group 2), and 9 (Group 3) cows in the groups. Cows were housed in the free-stall stable and milked two times daily in a parlour at regular intervals. Milk yields were recorded after each milking. The groups of cows were subsequently placed on the following one of three types of diets: control

Table 1. Scheme of the experiment

Period	Group 1	Group 2	Group 3
I	M	C	S
II	S	M	C
III	C	S	M

C = control diet, S = diet supplemented with Smartamine^{TM} M, M = diet supplemented with Metasmart^{TM}

(C), Smartamine^{TM} M (S), and MetaSmart^{TM} (M). Diets were formulated according to INRA (2007) and NRC (2001), and all diets were nutritionally equal except for Met content. The compositions of the diets are reported in Table 2. Concentrations of Lys in all diets fulfilled minimum recommended levels of 6.6–6.7% in MP (Baudet, 1995; Schwab et al., 2003), but the concentration of Met in the control diet (C) was lower than the recommended level (2–2.2% PDI), and the Lys : Met ratio in the control diet was higher than the recommended ratio 3 : 1 (NRC, 2001; Schwab et al., 2003). The diets S and M were adjusted to achieve minimum Met concentrations of at least 2% PDIE and the desired Lys : Met ratio of 3 : 1. The diet M was supplemented with Methipass Meta (170 g as-fed) mixed in the concentrate mixture. Methipass Meta contained 25% of MetaSmart^{TM} and 75% of solvent-extracted soybean meal, thus providing approximately 42.5 g/day of MetaSmart^{TM} in the diet. The diet S was supplemented with Methipass Smart (190 g as-fed) containing 10% of Smartamine^{TM} M and 90% of solvent-extracted soybean meal, thus providing approximately 19 g of Smartamine^{TM} M per day in the diet. Methipass Smart was added directly into the horizontal mixing wagon due to susceptibility of Smartamine^{TM} M to mechanical disturbance of its protective coating. The control diet C contained solvent-extracted soybean meal (160 g as fed), which was added in the concentrate mixture to achieve required levels of crude protein (17.8% of DM). Cows were fed a total mixed ration (TMR) *ad libitum*. TMRs were prepared twice daily and orts were removed before each feeding. Cows were provided with identification chips allowing automatic measurement of their feed intake which was recorded electronically by software using the automatic feeding system INSENTEC (Marknesse, the Netherlands) with troughs on tensometric scales.

Samples collection and analyses

Samples of feeds (both TMR and ingredients) were taken once a fortnight. Blended milk samples for the analysis of milk components (fat, protein, lactose, and total casein) and urea were collected three times after both the morning and evening milkings from each cow. The milk was preserved with 2-bromo-2-nitropropane-1,3-diol and cooled to 6°C. In addition, milk samples for analysis of protein fractions (κ-casein, β-casein, α-lactalbumin, β-lactglobulin A, and β-lactglobulin B) were collected from all cows once at each experimental week of each period, and milk samples for determination of Met concentration were collected once at each experimental week from four cows randomly chosen from each group (always the same four cows from each group). Samples of blood were taken into heparinised tubes from the tail vein (*vena caudalis mediana*) of each animal once during each experimental week. Tubes with blood were centrifuged at 3000 rpm and blood plasma was stored in a freezer (−18°C) until analysis of plasma glucose, total protein, urea, and non-esterified fatty acids (NEFA). During the third period, blood samples were also collected from all cows for the determination of plasma amino acid concentrations.

Dry matter was determined by drying the samples of feed at 105°C to a constant weight immediately after their collection. The AOAC (2005) procedures were used to determine the content of crude protein (954.01), starch (920.40), and ash (942.05) in the feed. Ether extracts were analyzed according to procedure 920.39 (AOAC, 1995). Crude protein content (6.25 × N) and ether extracts of the feed and faeces were determined/analyzed using a Kjeltec 1030 Auto Analyser and a Soxtec 1043, respectively (FOSS Tecator AB, Höganäs, Sweden). Acid detergent fibre (ADF) was determined according to AOAC procedure 973.18 (AOAC, 2000) and expressed without residual ash. Neutral detergent fibre (NDF), exclusive of residual ash, was assayed using a heat-stable amylase (Mertens, 2002). Gross energy was measured using an adiabatic calorimeter (C5000 control, IKA-Werke, Staufen, Germany). Mineral elements (e.g. Ca, Mg, Na) were determined by atomic absorption spectrometry (Solaar M-6, TJA Solutions, Winsford, UK), and P was determined photometrically (Spekol 11, ISO 17025).

Milk components were analyzed in a commercial laboratory using a Milkoscan FT2 (Foss Electric, Hillerød, Denmark). Milk protein fractions were determined by RP-HPLC method (Agilent 1100 Series, Agilent, Germany) for resolution of the casein in quanidine hydrochloride. The content of amino acids (e.g. Lys and Met) in feed and milk was determined after hydrolysis using an AAA 400 analyser (INGOS, Prague, Czech Republic). Plasma glucose was assayed enzymatically with the aid of a kit supplied by Pliva-Lachema (Brno, Czech Republic). Plasma urea was determined colorimetrically using diacetylmonoxim reagent and NEFA as copper complexes by means of colorimetric Cu determination. Concentrations of Met in blood plasma were determined using the AAA 400 analyser (INGOS, Prague, Czech Republic).

Variables measured and values calculated

The following variables were measured: daily consumption of feed, daily milk yield, milk composition (fat, protein, lactose, urea, total casein, protein fractions, and methionine), concentrations of plasma metabolites (glucose, lipids, total protein, urea, and methionine).

Dry matter intake and production (kg) of milk fat, milk protein, lactose, ECM, and FCM were calculated.

Data analyses and calculations

Data were recorded and calculated using the Microsoft Excel and Quattro (Corel Wordperfect Office) software packages.

FCM (fat-corrected milk containing 4% fat) and ECM (energy-corrected milk containing 4% fat and 3.4% protein) were calculated according to the following formulae:

FCM (kg/day) = milk yield in kg × (0.4 + 0.15 × % fat)

ECM (kg/day) = milk yield in kg × (0.25 + 0.122 × % fat + 0.077 × % protein)

Production and intake data were statistically analyzed using the general linear mixed model (PROC MIXED of SAS, version 9.1). The model is in the following form:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_{ij} + d_k + e_{ijkl}$$

where:

Y_{ijkl} = the l-th observation of considered variable for production and intake

μ = so-called grand mean (mean value of all observations depending on given fixed effects in the model)

α_i = fixed effect of the i-th treatment (i = 1, 2, 3)

β_j = fixed effect of the j-th period (j = 1, 2, 3)

λ_{ij} = fixed effect of interaction between the i-th treatment and the j-th period

d_k = random effect of the k-th cow (k = 1, 2, 3,...,30)

e_{ijkl} = random error related with observation Y_{ijkl}

In this model, the random effect of cow was used in order to model repeated measurements on a given cow. For these measurements we supposed that they are positively correlated and the correlation is the same for two different observations on the same cow (so-called exchangeable correlation structure). Correlation between two different observations given on different cows is zero. Thus, cows were supposed to be independent subjects. The significance level of 5% for the statistical tests was chosen.

RESULTS AND DISCUSSION

Dry matter intake and milk production

The average rates of dry matter intake (DMI) and milk production data are presented in Table 2. DMI and hence also consumption of nutrients were the lowest in the diet containing MetaSmart^{TM} (18.96 kg; M) in contrast to the diet containing Smartamine^{TM} M, for which DMI was the highest (20.48 kg; S; $P < 0.001$). Several previous studies have failed to show significant effects of Met supplementation on DMI (Lara et al., 2006; Phipps et al., 2008; Ordway et al., 2009). However, some studies reported decreased feed intake (Johnson et al., 1999), in contrast with the findings of Xu et al. (1998), who observed an increase in DMI for cows supplemented with Lys and Met. Ordway et al. (2009) reported greater postpartum DMI, higher body condition scores, and improved feed efficiency for cows supplemented with MetaSmart^{TM} than for cows fed control or Smartamine^{TM} M diets. Reviewed literature noted various and inconsistent responses in milk yields and milk composition. The addition of Met in a diet appeared to be the most effective during early lactation (Socha et al., 2005; Lara et al., 2006). In the current experiment, which used cows shifting from the first to the second lactation periods, the highest average milk yields were recorded for cows supplemented with MetaSmart^{TM} (31.34 kg), which produced approximately by 1.14 kg ($P < 0.001$) and 0.78 kg ($P < 0.01$) more milk than cows fed diets C and S, respectively. The highest concentrations of milk protein (3.42%; $P < 0.001$) were detected in cows fed diet S, followed by those fed diet M (3.39%; $P < 0.05$) to compare with the control diet C (3.34%). Due to their greater milk yield and increased protein content in milk, cows in both experimental groups S and M yielded more kg of milk protein ($P < 0.001$) than cows fed

Table 2. Composition of diets

	Diet[1]		
	C	M	S
Ingredients (g/kg DM)			
Maize silage	288.16	288.06	287.85
Lucerne silage	163.74	163.68	163.56
Ensiled maize cobs (LKS)	115.60	115.56	115.48
Lucerne hay	53.33	53.31	53.28
Brewery grain	56.92	56.90	56.86
Wheat	63.31	63.29	63.24
Barley	62.23	62.21	62.16
Rapeseed meal	44.54	44.52	44.49
Soybean meal	82.35	76.47	76.42
Methipass Meta[2]	0.00	6.20	0.00
Methipass Smart[3]	0.00	0.00	6.93
Soypass[4]	31.06	31.05	31.03
Lactoplus[5]	17.78	17.77	17.76
Limestone	3.91	3.91	3.91
Bicarbonate	2.83	2.83	2.83
Vitamin-mineral concentrate[6]	14.22	14.22	14.21
Chemical composition			
DM (kg)[7]	23.40	23.40	23.40
Crude protein (g/kg DM)	178.00	179.00	180.00
NE_L (MJ/kg DM)	6.98	6.98	6.98
ADF (g/kg DM)	173.00	172.00	172.00
NDF (g/kg DM)	325.00	320.00	320.00
PDIN (g/kg DM)	123.00	126.00	126.00
PDIE (g/kg DM)	109.00	112.00	110.00
Ca (g/kg DM)	9.90	10.30	10.30
P (g/kg DM)	4.50	4.50	4.50
Mg (g/kg DM)	2.30	2.40	2.40
Na (g/kg DM)	4.00	4.00	4.00
Vit. A (IU/kg DM)	14.50	14.50	14.50
Vit. D (IU/kg DM)	3.20	3.20	3.20
Vit. E (IU/kg DM)	60.00	60.00	60.00
Lysin (% of PDIE)	6.72	6.72	6.70
Methionin (% of PDIE)	1.75	2.23	2.23
Lys : Met ratio	3.84	3.01	3.00

[1]C = control diet, S = diet supplemented with Smartamine™ M, M = diet supplemented with Metasmart™

[2]Adisseo France SAS (Antony Cedex, France) contained: 25% MetaSmartTM and 75% solvent-extracted soybean meal (42.5 g of MetaSmartTM per day)

[3]Adisseo France SAS (Antony Cedex, France) contained: 10% SmartamineTM M and 90% solvent-extracted soybean meal (19 g of SmartamineTM M per day)

[4]Garant-Tiernahrung Gesellschaft m.b.H. (Pöchlarn, Austria)

[5]Premium Vegetable Oils Sdn. Bhd. (Pasir Gudang, Malaysia)

[6]VK-DRCMAN (Němčice, Czech Republic) contained per 1 kg: Ca 250 g, P 25 g, Mg 30 g, Na 80 g, Cu 1200 mg, Zn 5000 mg, Mn 3000 mg, Co 22 mg, I 130 mg, Se 30 mg, vitamin A 1 000 000 M.J., vitamin D3 225 M.J., vitamin E 4000 M.J.

[7]DM = estimated dry matter intake (kg) per cow and day

the diet C. There was no effect of the type of diet on fat and lactose content in milk. Lactose production, however, was higher in the diet M ($P < 0.001$) and diet S ($P < 0.01$) to compare with C. These differences together with lower DMI of cows on diet M are reflected in increased feed efficiency. The milk/DMI ratio was higher for cows on diet M to compare with both diet C ($P < 0.001$) and diet S ($P < 0.001$), respectively. Similarly, the FCM/DMI ratio was higher for cows on diet M to compare with diet C ($P < 0.01$) and diet S ($P < 0.001$), and ECM/DMI ratio was higher for M to compare with diets C ($P < 0.001$) and S ($P < 0.001$), respectively. Previous data reported by Pisulewski and Kowalski (1999) and Broderick et al. (2008) have failed to show significant effects of Met supplementation on milk yields and concentrations of milk components. The most consistent response to feeding cows on diets supplemented with HMB or rumen-protected methionine observed in previous research was an increase in milk fat concentrations (Huber et al., 1984; Lundquist et al., 1985; Yang et al., 2010). On the contrary, Stokes et al. (1981) observed no effect of supplemental Met on milk fat concentrations, similarly to our results in the current study. Noftsger et al. (2005) and St-Pierre and Sylvester (2005) observed an increase in milk protein concentrations and yields when HMBi was fed. Similarly, a metaanalysis by Kudrna et al. (2009) and Patton (2010) reported increased milk protein concentrations and yields after dietary supplementation with rumen-protected methionine. The concentrations of milk urea (Table 2) in all types of diets were rather higher than these referred to in literature (Homolka and Vencl, 1993; Jílek et al., 2006), in spite of the fact that the concentrations of milk urea in both experimental diets were nearly equal, and slightly higher than those in the control diet. St-Pierre and Sylvester (2005) observed decreased milk urea N

Table 3. Average dry matter intake (DMI) and milk production per cow and day

Item	Diet[1]			SEM	Contrast[2]			SEM
	C	M	S		C-M	C-S	M-S	
DMI (kg)	19.88	18.96	20.48	0.952	0.91*	–0.60ns	–1.51***	0.318
Milk (kg)	30.19	31.34	30.56	2.057	–1.14***	–0.36ns	0.78**	0.264
Protein (%)	3.34	3.39	3.42	0.741	–0.05*	–0.08***	–0.03ns	0.020
Fat (%)	3.80	3.75	3.75	0.135	0.05ns	0.05ns	–0.01ns	0.070
Lactose (%)	4.79	4.80	4.78	0.049	–0.01ns	0.01ns	0.02ns	0.017
Total casein (%)	2.60	2.64	2.64	0.030	–0.03*	–0.03*	0.00ns	0.013
Fat (kg)	1.14	1.16	1.14	0.093	–0.02ns	0.00ns	0.02ns	0.013
Protein (kg)	1.00	1.05	1.03	0.634	–0.05***	–0.03***	0.0ns	0.009
Lactose (kg)	1.45	1.51	1.47	0.099	–0.06***	–0.02ns	0.04**	0.013
Total casein (kg)	0.78	0.82	0.80	0.051	–0.04***	–0.02*	0.02*	0.007
FCM (kg)[3]	29.21	29.98	29.36	2.155	–0.77*	–0.15ns	0.62ns	0.282
ECM (kg)[4]	29.16	30.11	29.52	2.088	–0.95**	–0.36ns	0.59ns	0.274
Milk/DMI	1.61	1.77	1.61	0.096	–0.17***	0.00ns	0.16***	0.040
FCM/DMI	1.56	1.70	1.54	0.141	–0.14**	0.02ns	0.16***	0.040
ECM/DMI	1.56	1.71	1.55	0.140	–0.15***	0.01ns	0.15***	0.039
Total urea (mmol/l)	7.03	7.14	7.15	0.112	–0.12ns	–0.13ns	–0.01ns	0.155
Methionine (g/kg)	0.07	0.07	0.07	0.004	0.00ns	0.00ns	0.00ns	0.004

***very highly significant effect of diet ($P < 0.001$)

**highly significant effect of diet ($P < 0.01$)

*moderately significant effect of diet ($P < 0.05$)

nsnon-significant effect of diet ($P > 0.05$)

1C = control diet, M = diet supplemented with Metasmart™, S = diet supplemented with Smartamine™ M

2C-M = C vs. M, C-S = C vs. S, M-S = M vs. S

3FCM (fat-corrected milk containing 4% fat) calculated as: FCM (kg/day) = milk yield in kg × (0.4 + 0.15 × % fat)

4ECM (energy-corrected milk containing 4% fat and 3.4% protein) calculated as: ECM (kg/day) = milk yield in kg × (0.25 + 0.122 × % fat + 0.077 × % protein) concentration, and improved N efficiency by dietary HMBi supplementation. Recent research by Chen et al. (2011) reported improved apparent N efficiency when rumen-protected Met (Smartamine M) was supplemented. Concentrations of Met in the milk (Table 2) were not statistically different between all the three diets.

Concentrations of milk protein fractions

Milk protein is composed of two fractions – casein and whey protein. The casein fraction as well as the quantitative proportions of individual casein fractions (α_{s1}-, α_{s2}-, β-, and κ-fractions) influence the properties of raw milk. Increased casein levels in the milk (particularly the β- and κ-forms) are associated with improved cheese making properties, e.g. better coagulation and greater cheese yield (Wedholm et al., 2006). Supplementation with both Smartamine™ M ($P < 0.05$) and MetaSmart™ ($P < 0.01$) increased the prevalence of β-casein and there was a tendency ($P = 0.06$) for increased concentration of α-casein in milk in the diet M compared to the control diet (Table 3). The yield of κ-casein was not affected by the treatment which is similar to data reported by Třináctý et al. (2009).

Plasma metabolites

Measured concentrations of blood plasma metabolites are presented in Table 4. Concentrations of plasma glucose and NEFA were within the physio-

Table 4. Average parameters of blood plasma

Item	Diet[1]			SEM	Contrast[2]			SEM
	C	M	S		C-M	C-S	M-S	
Glucose (mmol/l)	3.59	3.59	3.55	0.077	0.00[ns]	0.05[ns]	0.05[ns]	0.090
Protein (g/l)	88.68	90.92	88.59	1.001	–2.24[ns]	0.09[ns]	2.33[ns]	1.119
Urea (mmol/l)	6.51	7.02	7.73	0.195	–0.51[ns]	–1.22***	–0.71*	0.235
NEFA (mmol/l)	0.12	0.11	0.12	0.005	0.01[ns]	0.00[ns]	0.00[ns]	0.007
Methionine[3] (mmol/l)	19.47	19.64	26.83	–	–	–	–	–

***very highly significant effect of diet ($P < 0.001$)
*moderately significant effect of diet ($P < 0.05$)
[ns]non-significant effect of diet ($P > 0.05$)
[1]C = control diet, M = diet supplemented with Metasmart™, S = diet supplemented with Smartamine™ M
[2]C-M = C vs. M, C-S = C vs. S, M-S = M vs. S
[3]methionine concentrations were determined just once in samples taken during the third experimental period

Table 5. Concentrations of milk protein fractions (mg/ml of skimmed milk)

Protein fraction	Diet[1]			SEM	Contrast[2]			SEM
	C	M	S		C-M	C-S	M-S	
κ-Casein	1.38	1.42	1.33	0.192	–0.04[ns]	0.05[ns]	0.09[ns]	0.050
α-Casein	7.39	8.04	7.88	0.344	–0.66[ns]	–0.50[ns]	0.16[ns]	0.279
β-Casein	5.90	6.58	6.44	0.364	–0.68**	–0.54*	0.14[ns]	0.207
α-Lactalbumin	0.99	1.01	1.02	0.024	–0.02[ns]	–0.03[ns]	–0.01[ns]	0.022
β-Lactglobulin A	1.01	1.01	1.07	0.121	0.00[ns]	–0.06[ns]	–0.06[ns]	0.026
β-Lactglobulin B	1.38	1.42	1.33	0.192	–0.04[ns]	0.05[ns]	0.09[ns]	0.050

**highly significant effect of diet ($P < 0.01$)
*moderately significant effect of diet ($P < 0.05$)
[ns]non-significant effect of diet ($P > 0.05$)
[1]C = control diet, M = diet supplemented with Metasmart™, S = diet supplemented with Smartamine™ M
[2]C-M = C vs. M, C-S = C vs. S, M-S = M vs. S

logical limits, while concentrations of plasma protein were slightly higher. Concentrations of plasma urea were rather high for all the three diets. This might be due to the feeding of cows on the same diet throughout the entire experimental period, although the milk production of cows in the second part of the experiment was lower than the rate for which the nutrient composition of the diet was designed. Cows fed on diet S had higher concentrations of plasma urea (7.73 mmol/l) compared to diets C and M (6.51 mmol/l, $P < 0.001$ and 7.02 mmol/l, $P < 0.05$, respectively). Previous research by Piepenbrink et al. (1998) and Chen et al. (2011) has failed to find significant effect of Met supplementation on concentrations of urea N in the blood. The addition of Smartamine^{TM} M increased the Met concentration in blood plasma (26.83 μmol/l), while the addition of MetaSmart^{TM} (19.64 μmol/l), probably due to its composition and degradation in the rumen, had no effect compared with diet C (19.47 μmol/l). These data should be assessed with caution because the concentrations of Met in the blood plasma were determined only once in samples taken during the third experimental period. Similarly to our results, Třináctý et al. (2009) reported increased plasma Met concentrations after supplementation of rumen-protected Met in the diet of high-yielding dairy cows. In contrary, Piepenbrink et al. (1998) did not observe any increase of serum Met after dietary supplementation of rumen-protected Met.

CONCLUSION

The inclusion of both MetaSmart^{TM} and Smartamine^{TM} M in optimal amounts (2.23% Met of PDIE), and at an optimal Lys : Met ratio of 3 : 1, increased milk yields, milk protein concentrations, and milk protein yields. DMI of cows fed MetaSmart^{TM} decreased, resulting in improved feed efficiency expressed as milk/DMI, ECM/DMI, and FCM/DMI ratios.

Supplementation with both Smartamine^{TM} M and MetaSmart^{TM} increased the prevalence of β-casein fraction in milk protein.

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