Effect of rumen-protected methionine, lysine or both on milk production and plasma amino acids of high-yielding dairy cows

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ABSTRACT: The objective of this study was to determine the effect of supplemental lysine (Lys), methionine (Met) or both amino acids added in the form of rumen-protected (RP) tablets with copolymer coating to a diet of dairy cows on yield and composition of milk and concentration of plasma amino acids (AA). The experiment was carried out on four high-yielding lactating Holstein cows with average milk production of 33.5 kg/day in the form of Latin square design. The four treatments were as follows: C – control without AA supplementation, L – control plus supplement of RP Lys, M – control plus supplement of RP Met and ML – control plus supplement of RP Met and Lys. The experiment was divided into 4 periods. Each period (14 days) consisted of 10-day preliminary period and 4-day experimental period. Cows were fed a diet based on maize silage, lucerne hay and supplemental mixture. Average milk yield recorded in ML was 34.18 kg and was higher than that recorded in L or M (32.46 kg and 32.13 kg, respectively $P < 0.05$) and tended to be higher than in C (33.33 kg, $P > 0.05$). The content of protein and casein was higher in L and ML in comparison with C ($P < 0.05$) and tended to be higher than in M ($P > 0.05$). Protein yield in ML (1 054 g/day) was higher than that found in C, L or M (990, 998 or 968 g/day, respectively, $P < 0.05$). The same response was found for casein yield ($P < 0.05$). Although the proportion of individual casein fractions was not affected by the treatment, the yield of α- and β-casein differed ($P < 0.05$) while the yield of κ-casein was not affected by the treatment. Concentrations of blood metabolites, except for betahydroxybutyrate (BHB), were not changed. Plasma Met concentrations were increased ($P < 0.05$) in the M and ML group. Similar, but insignificant increases ($P > 0.05$) were also observed in plasma Lys in the L and ML group.

Keywords: amino acids; rumen protection; dairy cow; lactation performance; plasma amino acids

To allow dairy cows to meet their metabolic requirements for intestinally absorbable protein, it is necessary to provide postruminally delivered protein with an amino acid (AA) profile that is consistent with AA requirements (Robinson et al., 1999). Because the amounts and proportions of AA in duodenal digesta vary when different diets are fed, it is difficult to determine which AA are limiting. The most limiting AA for the synthesis of milk and milk protein have been reported to be methionine (Met) and lysine (Lys) (Schwab et al., 1992). To supply additional Met and Lys, methods have been developed to protect these amino acids from microbial degradation resulting in the rumen-protected (RP) AA passing to the abomasum and small intestine where they are released and absorbed. However, increases in milk production have been variable. These responses are typically

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interpreted according to the limiting AA theory in which there is but one AA under a given set of dietary and physiological conditions whose absorptive supply can influence the milk protein yield (Weekes et al., 2006). A limiting AA phenomenon allows for efficient manipulation of milk protein yield by supplementing only one of many AA. Nevertheless, based on a large number of studies determining production responses to an additional single AA, the uncertainty still exists whether the supplement of the particular AA has corrected a deficiency or induced an imbalance (Weekes et al., 2006). Harper et al. (1970) defined the imbalance as arising from a surplus of essential AA other than the one in limiting supply. Deficiencies of any required AA would be expected to depress performance, although the exact nature of the depression could vary with the deficient AA (Robinson, 1996).

The objective of this study was to determine the effect of supplemental Lys, Met or both amino acids added to a diet of dairy cows in the form of RP tablets on milk yield and composition and concentration of plasma AA.

MATERIAL AND METHODS

Animals and procedures

Four multiparous (2^{nd}–5^{th} lactation) high-yielding lactating Holstein cows in their week 7–15 of lactation with average milk production of 33.5 kg per day (SEM = 3.08) were used in the experiment. Cows were housed in individual tie stalls bedded with sawdust. The experiment was carried out in the form of Latin square design. The four treatments were as follows: C – control without AA supplementation, L – control plus supplement of RP Lys, M – control plus supplement of RP Met and ML – control plus supplement of RP Met and Lys. The experiment was divided into 4 periods. Each period (14 days) consisted of 10-day preliminary period and 4-day experimental period.

Cows were fed individually twice daily (07:00 and 17:00 h) *ad libitum* the diet based on maize silage (346 g/kg), lucerne hay (86 g/kg) and supplemental mixture (568 g/kg, containing (in g/kg): barley 350; oats 250; wheat 80; sugar beet chippings 150; flax seed 50; soybean meal 70; sodium chloride (NaCl) 5; dicalcium phosphate (DCP) 15; limestone ($CaCO_3$) 15; sodium bicarbonate ($NaHCO_3$) 1; monosodium phosphate (MSP) 2; magnesium phosphate (MgP) 2; microelements and vitamin mixture 10). The diets were balanced to meet 100% of NEL (net energy of lactation) requirement (Sommer, 1994) and 95% of PDI (protein digestible in intestine) requirement by reason of a better manifestation of experimental treatment. Based on the tables of AADI (AA digestible in the intestine) values of feedstuffs (Rulquin et al., 2001a) the formulated diets were found to be deficient in Met (ca. 26%) and Lys (ca. 5%). The amount of the above-mentioned AA needed to settle the difference was calculated so as to meet the AA requirement (Rulquin et al., 2001b) being 2.5% for MetDI (Met digestible in the intestine) and 7.3% for LysDI (Lys digestible in the intestine). AA were applied in the form of RP tablets prepared by the authors (6.5 mm in diameter, lenticular in shape) and coated with a polymeric material on the basis of vinyl-pyridine/styrene copolymer (Ardaillon et al., 1989). Each tablet was composed of the respective AA (51%) and tablet additives (binding materials, modifiers of specific gravity etc.) and protective layer (49%). Assumed losses of tablets by rumination were compensated by a 30% increase in the amount of tablets applied (Třináctý et al., 2000). Thus, in M intake of Met it was 18.2 g/day (236 tablets/day), in L intake of Lys it was 11.7 g per day (194 tablets/day) and in ML intake of Met and Lys it was 18.2 and 11.7 g/day, respectively (236 and 194 tablets/day, respectively). Tablets were applied during the whole period (14 days) twice a day. Immediately before feeding the tablets were mixed into approximately 0.5 kg of supplemental mixture and given to animals. After their consumption the rest of the diet was fed in the form of mixed ratio. Refusals were monitored daily; an aliquot of them was analysed.

Analytical procedures

In feed and feed refusals the following parameters were estimated according to AOAC (1984): crude protein (CP, No. 7021), ash (No. 7009) and fat (No. 7060). Dry matter (DM) was determined by drying at 103°C for 4 h. Neutral detergent fibre (NDF, with α-amylase) and acid detergent fibre (ADF) were estimated according to Van Soest et al. (1991).

Cows were milked twice daily at 07:15 and 17:15 h. Samples of milk were taken at each milking, conserved by 2-bromo-2-nitropropane-1.3-diol (Bronopol, D & F Control Systems, Inc. USA) and cooled to 6°C. The composition of milk was ana-

lysed with an infrared analyser (Bentley Instruments 2000, Bentley Instruments Inc., USA). The urea content was determined using an UREAKVANT apparatus (AGROSLUŽBY Olomouc, s.r.o., Czech Republic). This method is based on the monitoring of the rate of change in conductance of the sample during the decomposition of urea with the enzyme urease. The casein content was measured on Kjeltec auto, 1030 (Tecator AB, Höganäs, Sweden) after the precipitation with 10% acetic acid. Casein fractions were determined electrophoretically as described earlier (Hadrová et al., 2007).

On the last day of each experimental period, blood samples were taken into heparinised tubes from the jugular vein for determination of AA profile and blood parameters. Immediately after blood collection, the samples were centrifuged at 1 500 g for 15 min. Blood parameters were analysed using kits for standard enzymatic methods (Biovendor – Laboratorní medicína, a.s. Modřice, Czech Republic) adapted to the COBAS MIRA autoanalyser (Roche diagnostics, Basle, Switzerland). For the determination of AA profile the heparinised blood plasma was deproteinised with sulphosalicylic acid and centrifuged at 3 000 g for 10 min. The supernatant was stored at –80°C until the AA profile was determined on an automatic amino acid analyser AAA 400 (Ingos, Prague, Czech Republic).

Statistical analysis

Data acquired in the experiment except of blood metabolites were analysed using the GLM (General Linear Models) procedure of Statgraphics 7.0 package (Manugistics Inc., and Statistical Graphics Corporation, Rockville, Maryland, USA) according to the following model:

$$Y_{ijkl} = \mu + T_i + C_j + P_k + D_l + \varepsilon_{ijkl}$$

where:

μ = general mean
T_i = treatment effect (i = 4)
C_j = cow effect (j = 4)
P_k = period effect (k = 4)
D_l = day of sampling effect (l = 4)
ε_{ijkl} = error term

For statistical evaluation of blood metabolites and AA the following model was used:

Table 1. Average daily nutrient intake of dairy cows fed basal diet supplemented with rumen-protected lysine, methionine or both amino acids in comparison with control diet

Nutrient	C[1]	L[1]	M[1]	ML[1]	SE
Dry matter (kg/day)	20.83[a]	20.73[a]	21.39[b]	21.64[b]	0.262
Crude protein (kg/day)	3.00[a]	3.00[a]	3.09[b]	3.16[c]	0.039
Fat (kg/day)	0.75[a]	0.75[a]	0.77[b]	0.78[b]	0.010
Ash (kg/day)	1.82[a]	1.82[a]	1.87[b]	1.87[b]	0.025
NDF (kg/day)	3.93[a]	3.96[a]	4.10[b]	4.08[b]	0.054
ADF (kg/day)	7.35[a]	7.37[a]	7.56[b]	7.61[b]	0.096
PDIN[2] (kg/day)	1.96[a]	1.96[a]	2.03[b]	2.08[c]	0.021
PDIE[3] (kg/day)	1.96[a]	1.96[a]	2.02[b]	2.07[c]	0.023
LysDI (% PDIE)	6.96[a]	7.46[b]	6.93[a]	7.25[c]	0.011
MetDI (% PDIE)	1.85[a]	1.84[a]	2.25[b]	2.39[c]	0.013
NEL (MJ/day)	148.60[a]	147.50[a]	152.40[b]	155.10[c]	1.937

[a,b]means in the same row followed by different superscripts differ ($P < 0.05$)

[1]treatments were as follows: C – control; L – control + supplemental Lys (11.7 g/day); M – control + supplemental Met (18.2 g/day); ML – control + supplemental Met and Lys (18.2 and 11.7 g/day, respectively)

[2]digestible protein in the intestine when the rumen fermentable N supply is limiting

[3]digestible protein in the intestine when the rumen energy supply is limiting

$$Y_{ijk} = \mu + T_i + C_j + P_k + \varepsilon_{ijk}$$

where:

μ = general mean
T_i = treatment effect (i = 4)
C_j = cow effect (j = 4)
P_k = period effect (k = 4)
ε_{ijk} = error term

RESULTS

The consumption of nutrients and calculated content of essential AADI in dependence on experimental treatments are presented in Table 1. Total dry matter intake (DMI) was lower in C and in L compared to M or ML ($P < 0.05$). Differences in DMI resulted in significantly higher intake of other nutrients (CP, fat, ash, NDF, ADF, PDI and NEL) in M or ML in comparison with C or L ($P < 0.05$). The content of LysDI increased in L and ML significantly ($P < 0.05$) when RP Lys was added, and similarly the content of MetDI was increased ($P < 0.05$) in M and ML after RP Met supplementation.

Milk production responses to supplemental AA are presented in Table 2. In ML there was a significantly increased milk yield ($P < 0.05$) compared with L and M and a tendency to increased milk production compared with C ($P > 0.05$). Milk production expressed in 4% FCM (fat corrected milk) was similar in C and ML and was significantly higher ($P < 0.05$) than in M or L.

The content of milk protein was lowest in C (29.7 g/kg), intermediate in M (30.1 g/kg) and highest in L and ML (30.8 and 30.7 g/kg, respectively)

Table 2. Effect of supplemental rumen-protected lysine, methionine or both amino acids on average milk yield and content and yield of milk components

Item	C[1]	L[1]	M[1]	ML[1]	SE
Milk yield (kg/day)	33.33^{a,b}	32.46^{a}	32.13^{a}	34.18^{b}	0.880
4% FCM[2] (kg/day)	30.76^{a}	27.43^{b}	27.32^{b}	30.32^{a}	1.265
FCM/DMI	1.48^{a}	1.33^{bc}	1.28^{b}	1.41^{ac}	0.065
Fat (g/kg)	35.90^{a}	30.65^{b}	31.91^{b}	33.72^{a,b}	2.522
Protein (g/kg)	29.71^{a}	30.81^{c}	30.12^{a,b}	30.78^{b,c}	0.478
Casein (g/kg)	24.83^{a}	25.45^{b}	25.15^{a,b}	25.43^{b}	0.395
α-casein (% total casein)	57.37	57.69	56.91	57.03	1.388
β-casein (% total casein)	35.75	35.50	36.81	36.47	1.102
κ-casein (% total casein)	6.88	6.81	6.28	6.50	0.730
Lactose (g/kg)	50.28	50.02	49.96	49.91	0.289
Urea (mg/100 ml)	23.30	22.91	23.05	23.46	1.114
Fat (g/day)	1 162^{a}	963^{b}	965^{b}	1 110^{a}	78.32
Protein (g/day)	990^{a}	998^{a}	968^{a}	1 054^{b}	30.76
Casein (g/day)	827^{a}	827^{a}	807^{a}	868^{b}	24.42
α-casein (g/day)	473^{ab}	475^{a,b}	458^{a}	494^{b}	16.97
β-casein (g/day)	297^{a}	294^{a}	297^{a}	317^{b}	13.83
κ-casein (g/day)	57	58	52	57	7.13
Lactose (g/day)	1 672^{a,c}	1 622^{a,b}	1 603^{b}	1 701^{c}	41.68

^{a,b}means in the same row followed by different superscripts differ ($P < 0.05$)

[1]treatments were as follows: C – control; L – control + supplemental Lys (11.7 g/day); M – control + supplemental Met (18.2 g/day); ML – control + supplemental Met and Lys (18.2 and 11.7 g/day, respectively)

[2]4% FCM – 4% fat corrected milk

but only the protein content in C differed significantly compared to L and ML ($P < 0.05$). Because of differences in milk production, the resulting milk protein yields in C, L or M were similar (990, 998 or 968 g/day, respectively) and were significantly lower ($P < 0.05$) than the protein yield found in ML (1 054 g/day). The content of casein in milk in L and ML was higher compared with C ($P < 0.05$). However, the resulting casein yields in C, L or M were significantly lower than that observed in ML ($P < 0.05$). Although the proportion of individual casein fractions was not affected by the treatment, the yield of α-casein in ML was significantly higher than in M ($P < 0.05$) and tended to be higher compared with C or L where it was almost identical. Similarly, the yield of β-casein in ML differed significantly from the yield found in C, M or in L ($P < 0.05$). The yield of κ-casein was not affected by the treatment.

The milk fat percentage in C was significantly higher ($P < 0.05$) than that in L or M. However, none of the percentages was significantly different from the milk fat content determined after ML supplementation ($P > 0.05$). The supplementation of ML to the diet resulted in similar milk fat production like that found in C ($P > 0.05$) and was higher compared with supplementation of either L or M ($P < 0.05$). Although the lactose and urea content in milk was not affected by the treatment, yields of lactose varied significantly ($P < 0.05$) in dependence on the applied treatment.

Concentrations of blood metabolites and plasma AA are presented in Tables 3 and 4, respectively. Concentrations of determined blood plasma parameters were not affected by treatments ($P > 0.05$) except the betahydroxybutyrate (BHB) values that differed significantly among treatments ($P < 0.05$). The plasma urea concentration in ML was higher than in C, L or M ($P < 0.05$). Plasma Met concentrations were increased ($P < 0.05$) in the M and ML group. Similar, but insignificant increases ($P > 0.05$) were observed in the case of plasma Lys concentrations in L and ML group. Dietary supplementation of RP Met (M), Lys (L) or both amino acids (ML) resulted in a tendency to increased concentrations of plasma histidine (His), leucine (Leu), isoleucine (Ile), valine (Val), arginine (Arg), threonine (Thr), asparagine (Asn), glutamic acid (Glu), phenylalanine (Phe) and tyrosine (Tyr), the results were not statistically significant ($P > 0.05$). The concentration of serine (Ser) and glycine (Gly) tended to be higher in L and lower in M and ML in comparison with C ($P > 0.05$). Concentration of plasma proline (Pro) tended to be higher in L and M and concentration of alanine (Ala) tended to be lower in L and M and was slightly increased in ML, none of the results was statistically significant ($P > 0.05$). Concentrations of glutamine (Gln)

Table 3. Effect of supplemental rumen-protected lysine, methionine or both amino acids added to the diet of lactating dairy cows on average means of blood plasma metabolites

Item	C[1]	L[1]	M[1]	ML[1]	SE
Total protein (g/l)	75.22	78.66	76.98	76.90	2.963
Glucose (mmol/l)	3.32	3.62	3.55	3.43	0.140
NEFA[2] (mmol/l)	0.32	0.36	0.08	0.09	0.200
BHB[3] (mmol/l)	1.01[a]	0.69[b]	0.68[b]	0.72[a,b]	0.089
ALT[4] (μkat/l)	0.17	0.27	0.18	0.27	0.035
AST[5] (μkat/l)	0.79	0.94	0.81	0.79	0.052

[a,b]means in the same row followed by different superscripts differ ($P < 0.05$)

[1]treatments were as follows: C – control; L – control + supplemental Lys (11.7 g/day); M – control + supplemental Met (18.2 g/day); ML – control + supplemental Met and Lys (18.2 and 11.7 g/day, respectively)

[2]nonesterified fatty acids

[3]betahydroxybutyrate

[4]alanine aminotransferase

[5]aspartate aminotransferase

Table 4. Effect of supplemental rumen-protected lysine, methionine or both amino acids added to the diet of lactating dairy cows on the average plasma concentration of urea and free amino acids

Item	C[1]	L[1]	M[1]	ML[1]	SE
Urea (μg/g)	20.00[a]	21.87[a]	16.69[a]	32.57[b]	2.758
Lysine[2] (μg/g)	8.74[a]	10.75[a,b]	11.91[b]	11.28[a,b]	0.899
Methionine (μg/g)	2.01[a]	2.59[a,b]	3.45[b]	5.07[c]	0.259
Histidine (μg/g)	3.45	5.01	4.58	4.89	0.705
Leucine (μg/g)	7.89	9.93	8.86	9.64	0.752
Isoleucine (μg/g)	10.39	11.98	10.66	12.20	0.798
Valine (μg/g)	19.41	21.07	19.92	24.11	2.272
Arginine (μg/g)	8.08	10.62	10.13	10.31	1.160
Threonine (μg/g)	7.50	9.39	7.66	9.49	0.845
Serine (μg/g)	9.37	9.82	8.80	8.64	0.810
Proline (μg/g)	4.48	5.39	5.16	4.24	0.602
Glycine (μg/g)	26.78	30.75	23.52	26.42	2.121
Asparagine (μg/g)	2.73	3.50	3.10	4.17	0.660
Glutamic acid (μg/g)	8.24	9.23	8.60	8.88	0.553
Glutamine (μg/g)	58.24	40.87	43.63	53.58	8.506
Phenylalanine (μg/g)	4.77	5.62	5.48	5.47	0.367
Tyrosine (μg/g)	5.24	6.74	5.25	6.28	0.538
Alanine (μg/g)	18.09	17.72	15.95	18.33	1.271

[a,b]means in the same row followed by different superscripts differ ($P < 0.05$)

[1]treatments were as follows: C – control; L – control + supplemental Lys (11.7 g/day); M – control + supplemental Met (18.2 g/day); ML – control + supplemental Met and Lys (18.2 and 11.7 g/day, respectively)

[2]Lys determined as LysHCl

tended to decline after supplementation of RP Met, Lys or both amino acids ($P > 0.05$).

DISCUSSION

Based on the results of previous studies (Třináctý et al., 2006; Hadrová et al., 2007) the physical form of rumen protection (tablets of large diameter with copolymer coating produced by the authors) was used in the present experiment. The functionality of the tablets in providing an effective postruminal delivery of AA was confirmed in the paper of Křížova et al. (2007) by significant increases in duodenal flows of supplemented AA. Production responses of lactating dairy cows to supplemental Lys and Met, or to supplementation of either Lys or Met, fed in a ruminally protected form or infused to the intestine, have been reported in numerous studies. Although the postruminal supplementation of AA to the dairy cow increases the AA supply by a known amount, the milk protein response to such supplement is often variable (Robinson, 1996; Doepel et al., 2004).

Nutrient intake

In the present experiment DMI was higher in M and ML than in C or L ($P < 0.05$). These results are consistent with the widely observed phenomenon that feed intake usually increases as increasing amounts of a limiting nutrient or nutrients are absorbed (e.g. Schwab et al., 1992). In contrast, Blum et al. (1999) or Robinson et al. (1999) did not find any effect of RP Met and Lys supplementation on the intake of dry matter and its components.

Milk yield and composition

In the present experiment the milk yield was increased ($P < 0.05$) after supplementation of ML in comparison with supplementation of either L or M. Milk yield and 4% FCM yield of cows fed C, unsupplemented diet, did not differ significantly ($P > 0.05$) from the animals with additional RP Met + Lys (ML). This is in agreement with the study of Donkin et al. (1989), who reported that the addition of RP Met + RP Lys did not alter milk production or 4% FCM yield. Similarly, Blum et al. (1999) or Kröber et al. (2001) found only minor effects of additional Met on milk yield. This contradicts findings of an elevated milk yield with supplementary Met (e.g. Robinson et al., 1992 or Kudrna et al., 1998). However, in their review Rulquin and Vérité (1996) mentioned the high variability in the lactational response of dairy cows to additional RP Met.

Although not always statistically significant, the milk protein yield has been influenced positively in all studies when both Lys and Met were infused into the abomasum or duodenum (e.g. Seymour et al., 1990; Kudrna et al., 1998; Robinson et al., 1999). Similarly in our study, the milk protein content and yield were significantly higher when both Met and Lys were supplemented compared to the control ($P < 0.05$). Recent study of Weekes et al. (2006) proved that the milk protein percentage was not affected by AA imbalance caused by a 50% decrease in the concentration of one essential AA (Lys, Met, His or branch-chained AA) and thus attested the great flexibility of the lactating cow to maintain milk production under widely non-ideal nutritional conditions.

Although the supplementation of Met, Lys or both amino acids increased the percentage of casein in milk in comparison with the control diet without AA supplementation ($P < 0.05$), the resulting casein yields in C, L or M were significantly lower than that observed in ML ($P < 0.05$). Similar results were reported e.g. by Donkin et al. (1989), Chow et al. (1990) or Armentano and Bertics (1993). On the other hand, Bateman et al. (1999) reported that the mean casein percentage and yield were not affected by the addition of RP AA. Our findings concerning changes in casein fractions are in discrepancy with Donkin et al. (1989), who reported that RP Met and Lys increased the concentration of α-casein and β-casein and decreased κ-casein in milk. On the other hand, tendencies of changes in individual casein fractions determined in our experiment after the addition of RP Met are in agreement with Pisulewski et al. (1996), who found out that the infusion of Met decreased ($P < 0.05$) the relative proportions of α-casein and tended to increase β-casein while the proportion of κ-casein was not affected by the treatment. Guinard and Rulquin (1994) found that the duodenal infusion of L-LysHCl influenced slightly the content of individual casein fractions, namely α-casein, similar tendencies were also observed in our study.

The milk fat percentage in C was higher ($P < 0.05$) than in L or M, however, none of the means differed significantly from the milk fat content determined in ML ($P > 0.05$). This is in accordance with e.g. Donkin et al. (1989) or Kröber et al. (2001).

Plasma metabolites and AA

Concentrations of plasma metabolites except BHB, determined in our study, were not affected by treatments ($P > 0.05$). This is in accordance with the other studies (e.g. Blum et al., 1999; Weekes et al., 2006). As expected, plasma Met concentrations were increased ($P < 0.05$) in the M and ML group. Similar, but insignificant increases ($P > 0.05$) were also observed in plasma Lys concentrations in the L and ML group. This is in agreement with findings of other studies that also described an elevated plasma concentration of AA when they were supplied in a RP form (e.g. Blum et al., 1999; Kröber et al., 2001) but not in all instances (e.g. Xu et al., 1998). According to Kröber et al. (2001) changes in blood plasma levels of other AA would reflect interactions with the supplemented AA which, in the case of antagonism, could indicate the necessity to supply not only the primarily limiting AA but also others. In the present experiment, in M none of the other AA in plasma except Met and Lys was affected by the treatment. The same findings were reported by Rogers et al. (1987) or Blum et al. (1999). In L, no effect of RP Lys on the concentrations of other AA was observed. This is in disagreement with Rogers et al. (1987), who found that cows fed increasing amounts of RP Lys had decreased plasma Met concentrations, however, this decrease was probably caused by increased yields of milk protein by these cows. Similarly, in the present experiment no effect of RP Met and Lys (ML) on plasma AA was determined. This is in disagreement with Christensen et al. (1994), who described that supplemental RP Met + Lys significantly decreased

the concentrations of His, Asn, Gly, Pro, Ser, and Tyr while concentrations of the other AA in plasma were not altered by feeding RP AA. Differences in plasma AA were also observed by Piepenbrink et al. (1996), who found that concentrations of Arg, Gln, Glu, ornithine, and Thr in plasma were higher when RP Met and Lys were fed to the cows in comparison with unsupplemented diet. Nevertheless, except for Met ($P < 0.05$) and Lys (insignificant), the effects on other AA as reported in the above-mentioned studies were not observed in our experiment.

The plasma urea concentration in ML was higher than in C, M or L suggesting that the degradation of AA increased. This is in accordance with the findings of Christensen et al. (1994), who reported that concentrations of urea nitrogen in plasma increased linearly as increased amounts of RP AA were fed to the cows. This increase probably occurred because of deamination and oxidation of dietary AA. On the other hand, in the study of Schwab et al. (1992) concentrations of urea in plasma were not different for infusions of Met, Lys, or Met plus Lys. Similar responses were also reported by Blum et al. (1999) after feeding RP Met.

AA imbalances

Lys and Met have often been considered as the most limiting or co-limiting AA for milk protein synthesis when a variety of rations is fed (e.g. Schwab et al., 1992). According to Harper et al. (1970) imbalances are concerned with the effects of surpluses of essential AA other than the limiting one. In the study of Rulquin and Vérité (1993), where Lys was the first-limiting AA and an extra Met was supplemented, the milk protein production was actually reduced. Similar findings were described by Robinson et al. (2000), who found that the negative effects of oversupplied Met on animal performance were much higher than those of Lys, whereas the abomasal infusion of both Met and Lys had a small influence on animal performance. Similar tendencies that were observed in our experiment suggest that Lys and Met were co-limiting under the given feeding conditions. This is in agreement with the Doepel et al. (2004), who in their calculations regressed the protein yield individually against Lys, Met and His to determine whether any of the mentioned AA has a dominant role in determining the protein yield. Based on the results of these calculations they indicated that the protein yield increased as the supply of these AA increased and further that the protein yield was not dependent on only one AA but that the AA were highly interrelated (Doepel et al., 2004).

CONCLUSION

The production responses found in this experiment suggested that Lys and Met were co-limiting under described feeding conditions resulting in significant increases ($P < 0.05$) in milk yield and protein percentage and yield after the supplementation of both amino acids in a rumen-protected form to the diet. Under such conditions the supplementation of either rumen-protected Met or rumen-protected Lys resulted in decreases in lactational performance in comparison with the diet supplemented with both rumen-protected Met and Lys. RP tablets of large diameter (6.5 mm) with copolymer coating used in the present experiment provide an effective way of postruminal delivery of AA.

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