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The effect of administration of soya-protein with Lys, Met, and His in two forms on casein yield and composition and AA profile in milk*

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ABSTRACT

The aim of this study was to determine the effect of soya-protein enriched with amino acids: lysine (Lys), methionine (Met) and histidine (His) added either as a powder or in a form of rumen-protected tablets into rumen of dairy cows on the percentage and yield of casein, changes in the proportion of casein fractions resulting in variations in amino acid profile of milk, casein and non-casein protein. The experiment was carried out on three lactating Holstein cows of average weight of 523 kg fitted with ruminal and duodenal cannulas. The experiment was divided into 4 periods of 14 d (10 d preliminary period and a 4 d experimental period). In the first period one cow received the tablets (T group) and the other two received the non-tableted mixture (C group, control) of the same composition. In the subsequent period the rate of animals was antipodal. Cows were fed on diet based on a maize silage, lucerne hay and a supplemental mixture. Tablets or mixture consisted of purified soya-protein HP 300, Lys, Met and His. The casein content and yield was higher in the group T in comparison to the group C (2.68% and 476.55 g vs 2.46% and 408.43 g; $P<0.05$). Content of β- and κ-casein was unaffected by the treatment while content of α-casein was lower in the T group (54.10 vs 55.98%; $P<0.05$). Yield of every casein fraction was significantly higher ($P<0.05$) in the T group than in the C. The increases in the casein yield resulted in significantly higher ($P<0.05$) yields of individual amino acids in milk and casein in the T group compared to the control. In the experiment we found out that the concentration of Thr in milk and Thr, Pro and Met in casein was

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significantly different ($P<0.05$) in the T group compared to the C. Duodenal flows of individual amino acids through the duodenum were determined.

KEY WORDS: rumen protection, amino acids, milk, protein, casein, dairy cow

INTRODUCTION

During lactation, the mammary gland needs large amounts of amino acids to synthesize milk protein. Most of the amino acids absorbed by the mammary gland are used to synthesize milk proteins. Major proteins synthesized from amino acids in the mammary gland of cows are caseins (α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein) and whey proteins (β-lactoglobulin, α-lactalbumin and proteose-peptones), representing approximately 92% of bovine milk proteins. Remaining proteins found in the milk (bovine serum albumin and immunoglobulins) are absorbed directly from the blood and are not synthesized within the mammary gland (Bequette et al., 1998).

Approximately 76 to 86% of the total protein in milk is casein which contributes to the high nutritive value of many dairy products. Average concentration of individual fractions in bovine milk is approximately 50% of α_s-caseins, 36% of β-casein and 14% of κ-casein (Farrell et al., 2004). Most of the study proved that postruminally supplemented essential AA positively influence casein synthesis, particularly in early lactation. The casein fraction is increased by effective rumen-protected amino acids supplementation, while the whey and non-protein fractions are not (Schwab, 1993).

Feeding supplemental amino acids in an unprotected form to dairy cows results in their degradation by microbes in the rumen before they pass to the absorption sites in the small intestine. One of the most effective ways is to provide the deficient AA in a ruminally protected form. While results of administration of various analogues of amino acids have been variable, supplementing the diet with polymerically encapsulated amino acids consistently increased protein production and milk protein concentration. However, increases in milk production have been variable. Polymers that are pH-sensitive have been commertially used to encapsulate Met and Lys. Examples of such products are Smartamine™ M (70% methionine) and ML (15% methionine, 50% lysine) with release of 90% of Met after 2 h incubation in pH 2 buffer as reported by Smartamine™ M producers or 75.0 to 97.1% as presented by Robert and Williams (1997).

The aim of this study was to determine the effect of supplemental soya-protein enriched with amino acids: lysine (Lys), methionine (Met) and histidine (His) added either as a powder or in a form of rumen-protected tablets into rumen of dairy cows on the casein content and yield and changes in the proportion of casein fractions resulting in variations in amino acid profile of milk, casein and non-casein protein.

MATERIAL AND METHODS

Treatment and experimental design

Cows, treatments and feeding regiment have been described previously (Třináctý et al., 2006). Briefly, treatment consisted of administrations of either rumen-protected tablets (T) or powder (C) containing purified soya-protein HP 300 and amino acids Lys, Met and His into the rumen of three lactating Holstein cows (1-3 lactation, 17-35 week of lactation) of mean liveweight 523 kg. Animals were fitted with duodenal and ruminal cannulas. The experiment was divided into 4 periods. Each period (14 d) consisted of 10 d preliminary period and 4 d experimental period. In the first period one cow received the tablets (T group) and the other two received the non-tableted mixture (C group, control) with the same composition. In the subsequent period the rate of animals was antipodal so each animal received both variants in two replications.

Cows were fed individually twice daily (7.00 and 16.35 h) *ad libitum* a mixed diet based on a maize silage, lucerne hay and a supplemental mixture (Table 1).

Table 1. Composition of diet, in % of DM

Component	%
Maize silage	54.7
Lucerne hay	15.0
Supplemental mixture[1]	30.3

[1] supplemental mixture contains, %: barley 35.0; oat 30.0; wheat 10.0; sugar beet chippings 15.0; linseed 5.0; sodium chloride (NaCl) 0.5; dicalcium phosphate (DCP) 1.5; limestone ($CaCO_3$) 1.5; sodium bicarbonate ($NaHCO_3$) 0.1; monosodium phosphate (MSP) 0.2; magnesium phosphate (MgP) 0.2; microelements and vitamin mixture 1.0

Basal diets were formulated to meet energy and protein requirements (Sommer et al., 1994), the amino acid requirement was calculated according to Rulquin et al. (2001). The supplement consisted, %: soya-protein HP 300 93.0, Met 2.4, Lys 1.6 and His 3.0 and was prepared in two forms, either as tablets (lenticular shape, diameter 6.5 mm, copolymer coating) or powder. Tablets or mixture were wrapped into filter paper and inserted into the bottom of the rumen *via* ruminal cannula twice daily before feeding. Daily amount of these ingredients was 306, 8.8, 5.7 and 10.6 g, respectively.

Analytical procedures

Proximal analysis of feed and orts were carried out in each period according to AOAC (1984). Refusals were daily monitored, an aliquot of them was analysed.

Cows were milked twice daily at 7.15 and 17.15 h. Milk yield was recorded and samples were taken at each milking during the experimental period. The samples of milk were conserved by 2-bromo-2-nitropropane-1.3-diol (Bronopol) and cooled to the 6°C and the basic compositions of milk were analysed by infrared analyser (Bentley Instruments 2000, Bentley Instruments Inc., USA).

Casein isolation were carried out following the conditions described by López-Fandiño et al. (1993). The lyophilized casein was dissolved in 10 ml Tris-HCl (pH 6.8) and sample buffer with 2-mercapthoethanol was added. The samples were boiled for 2 min. For separation of casein fractions there were used separation gels (15% T, 2.6% C) and focussing gels (3% T, 2.6% C) (Laemmli, 1970) using Mini-Protean III Cell Electrophoresis apparatus (Bio-Rad Laboratories, Richmond, CA). The gels were coloured with Commassie Brilliant Blue R-250. For evaluation the platform ElfoMan 2.0 (Servis Sale of Laboratory Equipment, Prague, CZ) was used. Quantification was performed on the basis of intensity of colouring and of individual areas casein fraction bands. The non-casein protein was not determined analytically but was calculated.

For AA analysis, the samples of lyofilized milk for acid hydrolysis (0.2 g) were hydrolysed with 6 $mol \cdot l^{-1}$ HCl for 24 h at 110°C. The sulphur containing amino acids, 0.5 g of samples of lyofilized milk were mixed with oxidation mixture. To the sample 1 ml concentrated HCl was added, consequently 6 $mol \cdot l^{-1}$ HCl and following hydrolysis. Sulphur amino acids were determined as cysteic acid and methionine-sulphone. All hydrolysates were separated in the automatic aminoanalyser AAA 400 (Ingos, CR) using Na citrate buffer system and quantified by reaction with ninhydrin. For determination of amino acids content the programme ChromuLan v. 0.7 was used.

Duodenal chymus (500 ml) was sampled from each animal in 6-h intervals during the whole 4-day experimental period starting on 7.00 a.m. of the first day. On each subsequent day the time of sampling was postponed by 1.5 h so that the four-day experimental period represented a set of chymus samples obtained during the day (Schwab et al., 1992). The obtained samples were immediately frozen to -20°C. After thaw, chymus samples were pooled for each dairy cow and each period. They were continuously stirred and used for the recovery of four average samples (500 ml). Chymus samples were lyophilized. For determination of free amino acids (FAA), 2 g of the sample was shaked for 30 min in 10 ml of distilled water with a supplement of 5 ml of 10% sulphosalicylic acid. After the filtration the turbidity was removed by centrifugation at 10,000 *g* for 10 min. Detectable FAA were estimated in the automatic analyser AAA 400 (Ingos, CR) using a Li citrate buffer system.

Statistical analysis

Data resulting from the experiment were analysed using the GLM procedure of Statgraphics 7.0 package according to the following model:

$$Yijk = \mu + T_i + C_j + T_iC_j + R_k + \varepsilon_{ijk}$$

where μ = general mean, T_i = treatment effect (i = 2), C_j = cow effect (j = 3), R_k = replication (k = 2), ε_{ijk} = error term. The following equation was used as a model for the comparison of concentrations and flows of nutrients in duodenum:

$$Y_{ij} = \mu + T_i + C_j + T_iC_j + \varepsilon_{ij},$$

where: μ = total average, T_i = effect of the experimental factors (i = 2), C_j = effect of the dairy cow (j = 3) and ε_{ij} = residual error.

RESULTS

Mean milk yield and the composition of cow´s milk is presented in the Table 2. The DM intake was significantly higher (P<0.05) for cows receiving supplement

Table 2. Milk yield and composition

Component	Unit	C[1](n=24) mean	SEM	T[2] (n=24) mean	SEM
Dry matter intake (DMI)*	kg	15.68[a]	0.33	16.33[b]	0.27
N intake (NI)	kg	0.30[a]	0.01	0.32[b]	0.01
Milk yield*	kg/d	16.73[a]	0.38	17.80[b]	0.28
Milk yield/DMI*	kg/kg	1.08	0.03	1.09	0.02
Protein*	%	3.17	0.09	3.21	0.06
Protein yield*	g	529.45[a]	17.24	569.27[b]	7.70
Casein*	%	2.46[a]	0.09	2.68[b]	0.05
Casein yield*	g	408.43[a]	15.47	476.55[b]	9.72
Casein yield/DMI*	g/kg	26.02[a]	0.76	29.33[b]	0.70
Casein yield/NI	g/kg	1351.53[a]	39.53	1523.80[b]	39.45
Casein fractions					
κ-casein	%	12.45	0.21	12.62	0.25
κ-casein yield	g	50.85[a]	2.10	60.32[b]	1.98
κ-casein yield/DMI	g/kg	3.25[a]	0.12	3.71[b]	0.13
κ-casein yield/NI	g/kg	168.71[a]	6.20	192.78[b]	6.84
β-casein	%	31.58	0.66	33.28	0.76
β-casein yield	g	128.81[a]	5.10	159.00[b]	5.55
β-casein yield/DMI	g/kg	8.23[a]	0.30	9.76[b]	0.33
β-casein yield/NI	g/kg	427.14[a]	15.51	506.50[b]	17.20
α-casein	%	55.98[a]	0.66	54.10[b]	0.78
α-casein yield	g	228.82[a]	9.51	257.24[b]	5.37
α-casein yield/DMI	g/kg	14.55[a]	0.45	15.86[b]	0.43
α-casein yield/NI	g/kg	755.87[a]	23.26	824.52[b]	25.45
Non-casein protein yield	g	121.02	12.94	92.71	8.73
Non-casein protein yield/DMI	g/kg	7.82[a]	0.87	5.75[b]	0.61
Non-casein protein yield/NI	g/kg	410.72[a]	47.85	301.26[b]	33.93

[a,b] means in the same row followed by the different superscripts differ (P<0.05); [1]control group; [2] experimental group; * marked results presented here for the integrity of results were published in the previous paper (Třináctý et al., 2006)

in the form of rumen-protected tablets (T group) with soya-protein and amino acids than in the control group (C group). Average milk yield of the T group was higher ($P<0.05$) than that of the C group. The protein content in milk was unaffected by the treatment ($P>0.05$), the total yield of milk protein determined in the T group was significantly higher ($P<0.05$) than in the C. Increased milk protein yield was followed closely by increases in casein content and yield in the T group ($P<0.05$). The calculated non-casein protein yield did not differ significantly ($P>0.05$) between treatments.

The content and the yield of individual casein fractions is given in Table 2. The proportions of β-casein and κ-casein were not affected by the treatment ($P>0.05$). Relative proportion of α-casein in the T group was significantly lower than that found in the C ($P<0.05$). The yield of every individual casein fraction determined in the T group was higher ($P<0.05$) than that from the C group.

With regard to differences in DM and N intake, the yield and composition of milk was expressed in dry matter intake and N intake (Table 2). Milk yield/DMI was unaffected by the treatment ($P>0.05$). Casein yield/DMI was higher ($P<0.05$) and non-casein protein yield/DMI was lower ($P<0.05$) when soya-protein with amino acids were supplemented in the form of tablets. Similarly, yields of every individual casein fractions expressed in DM intake found in group T were significantly higher ($P<0.05$) compared to the C group. Similar results were obtained when data were expressed in N intake (Table 2).

The effect of rumen-protected (RP) tablets containing soya-protein, Lys, Met, and His on the relative amino acids content in milk, casein and non-casein protein is presented in Table 3. The concentration of amino acids in milk and casein in both of the groups reached almost the same values except of Thr in milk and Thr, Pro and Met in casein that differ significantly ($P<0.05$) between groups T and C. The yield of individual amino acids in milk, casein and non-casein protein is presented in the Table 4. Administration of soya-protein enriched with AA in the form of RP tablets increased the yield of all amino acids in milk and casein ($P<0.05$) except of Met, Cys and Arg in milk that were not affected by the treatment ($P>0.05$). The yields of individual non-casein amino acids were calculated from yields of milk and casein amino acids and were not affected by the treatment ($P>0.05$) except of Glu, Tyr and Arg that differ significantly ($P<0.05$). In the Table 5, there are presented yields of amino acids in milk, casein and non-casein protein expressed in N intake, respectively. The yields of amino acids in milk, casein and non-casein protein expressed in dry matter intake are not presented in the Table. Expressed amino acids yields in casein in both cases were significantly higher ($P<0.05$) in the T group, in milk amino acids only Thr showed significant difference ($P<0.05$) between groups. Similarly, expressed yields of AA in non-casein protein were not affected by the treatment ($P>0.05$) except of Glu, Gly, Tyr and Arg that showed statistically significant differences ($P<0.05$).

Table 3. Amino acid composition of milk, casein and non-casein protein, %

Amino acid	Milk				Casein				Non-casein protein			
	C[1](n=24)		T[2](n=24)		C[1](n=24)		T[2](n=24)		C[1](n=24)		T[2](n=24)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Asp	7.29	0.06	7.36	0.04	6.53	0.03	6.57	0.04	11.20	0.74	12.33	0.63
Thr	4.07^{a}	0.08	4.25^{b}	0.03	3.86^{a}	0.03	4.01^{b}	0.02	5.69	0.58	5.88	0.29
Ser	5.20	0.08	5.27	0.04	5.38	0.03	5.43	0.02	4.73	0.44	4.31	0.36
Glu	20.92	0.29	20.69	0.24	20.79	0.22	21.29	0.20	18.12	2.05	16.26	1.64
Pro	10.30	0.18	10.30	0.13	12.40^{a}	0.11	11.92^{b}	0.11	2.16	0.54	1.39	0.48
Gly	1.80	0.02	1.77	0.01	1.62	0.01	1.60	0.01	2.73	0.20	2.84	0.19
Ala	3.14	0.02	3.11	0.02	2.68	0.02	2.68	0.02	5.29	0.30	5.68	0.26
Val	6.25	0.04	6.33	0.06	6.31	0.03	6.33	0.03	6.16	0.27	6.28	0.42
Met	2.60	0.06	2.49	0.05	2.69^{a}	0.02	2.62^{b}	0.02	2.11	0.20	1.80	0.36
Cys	0.63	0.03	0.61	0.03	0.00	0.00	0.00	0.00	3.67	0.48	4.37	0.37
Ile	4.92	0.03	4.93	0.04	4.79	0.02	4.79	0.03	5.48	0.19	5.49	0.35
Leu	9.48	0.04	9.40	0.04	8.95	0.05	8.89	0.05	11.76	0.33	11.98	0.37
Tyr	4.34	0.04	4.38	0.05	5.02	0.05	4.95	0.04	1.75	0.21	1.34	0.22
Phe	4.51	0.04	4.54	0.05	4.66	0.04	4.60	0.03	3.62	0.28	4.11	0.30
His	2.87	0.03	2.86	0.05	2.82	0.02	2.78	0.03	3.06	0.20	3.42	0.35
Lys	7.94	0.07	8.02	0.06	7.59	0.06	7.63	0.04	9.05	0.59	10.26	0.52
Arg	3.76	0.06	3.69	0.04	3.91	0.03	3.91	0.03	3.43^{a}	0.37	2.24^{b}	0.30

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

[1,2] see Table 1

Table 4. Yields of amino acids in milk, casein and non-casein protein, g/day

Amino acid	Milk				Casein				Non-casein protein			
	C[1](n=24)		T[2](n=24)		C[1](n=24)		T[2](n=24)		C[1](n=24)		T[2](n=24)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Asp	38.63[a]	1.32	41.85[b]	0.53	26.67[a]	1.02	31.31[b]	0.63	11.96	0.96	10.54	0.70
Thr	21.56[a]	0.85	24.22[b]	0.38	15.79[a]	0.66	19.09[b]	0.40	5.77	0.56	5.13	0.37
Ser	27.52[a]	1.01	30.01[b]	0.47	21.98[a]	0.88	25.89[b]	0.54	5.53	0.75	4.12	0.47
Glu	110.51[a]	3.49	117.82[b]	2.21	85.04[a]	3.43	101.41[b]	2.15	25.46[a]	3.71	16.42[b]	2.84
Pro	54.32[a]	1.72	58.59[b]	0.95	50.62[a]	1.92	56.77[b]	1.24	3.70	1.13	1.82	0.71
Gly	9.53[a]	0.31	10.06[b]	0.14	6.58[a]	0.23	7.63[b]	0.18	2.94	0.23	2.42	0.17
Ala	16.62[a]	0.56	17.73[b]	0.32	10.94[a]	0.41	12.78[b]	0.29	5.69	0.43	4.95	0.36
Val	33.08[a]	1.09	36.06[b]	0.64	25.76[a]	0.95	30.19[b]	0.67	7.32	0.80	5.87	0.62
Met	13.80	0.63	14.17	0.39	10.98[a]	0.42	12.46[b]	0.25	2.82	0.54	1.71	0.38
Cys	3.36	0.19	3.50	0.16	0.00	0.00	0.00	0.00	3.36	0.19	3.50	0.16
Ile	26.06[a]	0.89	28.06[b]	0.45	19.56[a]	0.74	22.84[b]	0.52	6.50	0.65	5.22	0.53
Leu	50.23[a]	1.73	53.49[b]	0.78	36.55[a]	1.41	42.38[b]	0.95	13.68	1.29	11.11	0.98
Tyr	23.04[a]	0.86	24.95[b]	0.42	20.51[a]	0.82	23.59[b]	0.50	2.52[a]	0.50	1.36[b]	0.29
Phe	23.93[a]	0.89	25.87[b]	0.48	19.00[a]	0.70	21.94[b]	0.51	4.92	0.67	3.92	0.43
His	15.20[a]	0.57	16.24[b]	0.29	11.50[a]	0.41	13.25[b]	0.29	3.70	0.41	3.00	0.33
Lys	42.18[a]	1.60	45.66[b]	0.70	31.02[a]	1.23	36.38[b]	0.79	11.16	1.16	9.27	0.78
Arg	19.92	0.71	20.98	0.29	15.94[a]	0.61	18.64[b]	0.43	3.98[a]	0.52	2.34[b]	0.37
Σ EAA[3]	268.98[a]	9.47	289.71[b]	4.17	206.60[a]	7.85	240.76[b]	5.15	62.38	6.63	48.95	4.49
Σ NEAA[4]	260.47[a]	7.90	279.56[b]	4.01	201.83[a]	7.73	235.79[b]	4.73	58.64[a]	6.56	43.77[b]	4.78
Total AA	529.49[a]	17.24	569.26[b]	7.70	408.43[a]	15.47	476.55[b]	9.72	121.02	12.94	92.71	8.73

[a,b] means in the same row followed by the different superscripts differ ($P<0.05$)
[1,2] see Table 1
[3] EAA = essential AA (Val, Thr, Met, Ile, Leu, Phe, His, Lys, Tyr and Arg); Trp was not determined
[4] NEAA = non-essential AA (Asp, Ser, Glu, Pro, Gly, Cys and Ala)

Table 5. Yields of amino acids of milk, casein and non-casein protein expressed in N intake, g/kg

Amino acid	Milk				Casein				Non-casein protein			
	C[1](n=24)		T[2](n=24)		C[1](n=24)		T[2](n=24)		C[1](n=24)		T[2](n=24)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Asp	128.46	4.30	134.19	3.56	88.25[a]	2.62	100.12[b]	2.60	40.22	3.46	34.07	2.78
Thr	71.23[a]	2.18	77.50[b]	1.84	52.17[a]	1.69	61.02[b]	1.59	19.06	1.81	16.48	1.31
Ser	91.06	2.75	96.03	2.31	72.70[a]	2.23	82.80[b]	2.22	18.37	2.53	13.23	1.65
Glu	369.81	14.54	379.16	13.55	281.31[a]	9.13	323.60[b]	7.53	88.50[a]	14.63	55.56[b]	11.51
Pro	180.83	5.97	187.86	5.09	167.41[a]	4.85	181.69[b]	5.17	13.42	4.18	6.17	2.55
Gly	31.73	1.10	32.22	0.82	21.84[a]	0.63	24.44[b]	0.74	9.89[a]	0.88	7.78[b]	0.62
Ala	55.32	1.86	56.91	1.76	36.22[a]	1.06	40.93[b]	1.23	19.10	1.57	15.97	1.39
Val	110.05	3.51	115.33	2.80	85.28[a]	2.44	96.66[b]	2.79	24.77	2.82	18.67	2.01
Met	45.78	1.87	45.51	1.78	36.30[a]	1.02	39.86[b]	1.05	9.47	1.70	5.65	1.40
Cys	11.25	0.75	11.26	0.62	0.00	0.00	0.00	0.00	11.50	0.73	11.61	0.66
Ile	86.60	2.79	89.80	2.19	64.71[a]	1.94	73.13[b]	2.14	21.86	2.30	16.68	1.80
Leu	166.95	5.44	171.35	4.37	120.94[a]	3.62	135.54[b]	3.74	46.01	4.59	35.80	3.52
Tyr	76.44	2.48	79.76	1.83	67.94[a]	2.19	75.40[b]	1.93	8.50[a]	1.64	4.36[b]	0.93
Phe	79.75	3.08	82.85	2.27	63.00[a]	1.90	70.25[b]	2.07	16.75	2.40	12.62	1.47
His	50.54	1.83	51.90	1.20	38.14[a]	1.12	42.43[b]	1.25	12.40	1.42	9.47	1.07
Lys	140.08	4.90	146.22	3.73	102.53[a]	3.06	116.33[b]	3.11	37.55	4.08	29.90	2.86
Arg	66.38	2.46	67.20	1.68	52.75[a]	1.58	59.60[b]	1.65	13.62[a]	1.91	7.61[b]	1.30
Σ EAA[3]	893.79	29.34	927.42	22.43	683.81[a]	20.17	770.21[b]	21.01	209.98[a]	23.19	157.21[b]	15.94
Σ NEAA[4]	868.46	29.02	897.63	26.43	667.72[a]	19.83	753.59[b]	18.79	200.99[a]	25.49	144.40[b]	19.41
Total AA	1762.25	57.77	1825.05	48.18	1351.53[a]	39.53	1523.80[b]	39.45	410.97[a]	47.78	301.61[b]	33.88

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

[1,2] see Table 1

[3] EAA = essential AA (Val, Thr, Met, Ile, Leu, Phe, His, Lys, Tyr and Arg); Trp was not determined

[4] NEAA = non-essential AA (Asp, Ser, Glu, Pro, Gly, Cys and Ala)

After the administration of the tablets the amino acids flows through the duodenum was higher (P<0.05) for the total, essential as well as non-essential amino acids compared with the control group (Table 6). The flow of His in the T group was significantly higher (P<0.05) than in the C (41.80 vs 32.63 g/d). Similarly, values of Lys, Arg, Asp, Thr, Ser, Glu, Pro flow through the duodenum were higher (P<0.05) after the tablets administration. Values of duodenal flow of Met, Ile, Leu, Phe, Val, Tyr, Ala, Gly were not affected by the treatment (P>0.05) but tended to be higher in the T group in comparison with the C group.

Table 6. Duodenal flows of individual amino acids

Item	Unit	C[1](n=24) mean	SEM	T[2](n=24) mean	SEM
Dry matter	kg/d	8.32	0.59	9.22	0.24
Asp	g/d	161.43[a]	3.30	193.14[b]	12.29
Thr	g/d	76.15[a]	2.00	89.78[b]	5.29
Ser	g/d	73.71[a]	1.72	87.08[b]	4.74
Glu	g/d	179.07[a]	4.80	215.35[b]	12.84
Pro	g/d	70.29[a]	2.42	82.54[b]	4.30
Gly	g/d	208.25	11.57	236.16	18.25
Ala	g/d	99.74	2.79	114.22	6.32
Val	g/d	91.03	2.02	103.14	5.84
Met	g/d	47.80	3.43	56.40	4.12
Ile	g/d	73.77	1.86	83.90	4.69
Leu	g/d	128.32	3.45	143.18	7.00
Tyr	g/d	61.16	1.39	67.11	3.12
Phe	g/d	71.71	1.82	79.81	3.72
His	g/d	32.63[a]	1.14	41.80[b]	1.90
Lys	g/d	105.59[a]	2.00	119.42[b]	5.24
Arg	g/d	89.55[a]	2.10	102.75[b]	4.43
Σ EAA[3]	g/d	777.73[a]	17.38	887.29[b]	42.64
Σ NEAA[4]	g/d	792.49[a]	16.91	928.49[b]	53.28
Total AA	g/d	1570.21[a]	33.15	1815.77[b]	94.68

[a,b] means in the same row followed by the different superscripts differ (P<0.05)
[1,2] see Table 1
[3] EAA = essential AA (Val, Thr, Met, Ile, Leu, Phe, His, Lys, Tyr and Arg); Trp was not determined
[4] NEAA = non-essential AA (Asp, Ser, Glu, Pro, Gly, Ala)

DISCUSSION

The experiment described herein studied the milk response to administration of soya-protein enriched with amino acids Lys, Met, and His to the rumen of lactating dairy cows in two forms, as a powder or as a RP tablets coated by copolymer.

Considering the significant differences (P<0.05) in DM and N intake between experimental groups followed by the increased consumption of other nutrients in cows receiving tablets (T), obtained results (yields of milk, milk protein, casein and its fractions and amino acids) were expressed per DM or N intake. Similar approach was presented in the other studies (e.g., Schwab et al., 1992) in which similar responses in DM intake to rumen-protected AA were observed as discussed in details in the previous paper (Třináctý et al., 2006).

The response to the protected amino acids feeding reported in the literature varies in dependence on the protein source in the basal diet because the amino acid that limits milk protein synthesis most is variable and is largely dependent on the quality and quantity of AA in the basal ration (Rulquin and Vérité, 1993). Chow et al. (1990) described that addition of RP Met and Lys resulted in greater total N and casein N contents in the diet with high fat ($P<0.05$), but it did not significantly increase total N or casein N contents with the high concentrate diet. On the other hand Christensen et al. (1994) observed that supplemental Met and Lys increased yields of milk CP, true protein and casein protein and percentages of CP, true protein and casein protein in milk when either 14.2 or 17.5% CP was in the diet.

In the presented experiment mix of soya-protein and amino acids Lys, Met, and His was encapsulated by copolymer and proved to be more efficient for milk production, protein yield and casein content and yield than the powder form of the same supplements. We found out that mean milk yield measured in the group T was higher ($P<0.05$) than that in the C. Although milk protein percentage did not differ significantly, milk protein yield from the T group was higher than that from the C ($P<0.05$). The increased milk protein yield resulted in significant increases in casein content and yield in cows receiving supplement in the form of protected tablets to the rumen. Increases in total milk protein N, milk N and casein protein after addition of RP Met and Lys to the maize-based diet were observed also by Donkin et al. (1989) and Sloan et al. (1989).

Because the amounts and proportions of amino acids in duodenal digesta vary when different diets are fed, it is difficult to determine which amino acids are limiting (Rulquin and Vérité, 1993). The most limiting amino acids for synthesis of milk and milk protein have been reported to be Met and Lys (Schwab et al., 1992). Inconsistent production responses to RP AA may be due to the possibility that several EAA are often co-limiting. In addition, some AA have several metabolic roles other than as precursors for protein synthesis. For example, Met is involved in a phospholipid biosynthesis and creatine productions, and Met is a key intermediate in the transmethylation reactions (Bequette et al., 1998). And depending on the availability of other precursors, amino acids may make a significant contribution to glucose synthesis in the liver. Furthermore response differences probably occur based on the quantity and proportion of amino acids in the microbial and dietary protein digested and absorbed from the small intestine.

Responses are often greater when mixtures of amino acids, rather than individual amino acids, are taken in beyond the rumen as proved by e.g., Schwab et al. (1992) who found out that the duodenal infusions of combination of Lys and Met increased milk protein yield more than infusions of separate AA. Furthermore in all studies when Lys and Met were infused together into the abomasum or duodenum milk protein yield has been influenced positively (e.g., Rulquin et al., 1990).

Published results from studies when Lys, Met and His have been supplemented in RP form or infused postruminally are scarce even there is no study on the effect of supplemented soya-protein with the three amino acids furthermore there is no study desribing the effect of the mixture of mentioned amino acids on the changes in proportion of casein fractions and amino acid composition of milk, casein and non-casein protein.

Addition of tablets containing soya-protein and amino acids to the basal diet increased total milk and casein protein yield but had no effect on total whey proteins. Similar results were published in most of the studies which showed that only casein fractions of milk protein increased but whey proteins and non-protein nitrogen were not influenced by supplement of Met and Lys *via* duodenal infusions or in the RP form (Donkin et al., 1989; Chow et al., 1990). On the other hand according to Liu et al. (2000) proportion of total casein tended to decrease ($P=0.08$) and whey proteins tended to increase ($P=0.08$) when lactaing dairy cows were fed the blend of protein supplements. Sloan et al. (1989) found out that feeding two experimental diets differing in CP concentrations supplemented with RP Met and Lys resulted in increased total protein and casein content in milk. On the other hand Guinard et al. (1994) found that duodenal infusion of L-LysHCl did not affect the protein and casein yield.

Concentration of α-casein decreased ($P<0.05$) and percentage of β-casein and κ-casein tended to increase in the T group. On the other hand Pisulewski et al. (1996) found out that infusion of Met linearly decreased ($P<0.05$) the relative proportions of α-casein and tended to increase the β-casein while the proportion of κ-casein was not affected by the treatment. These findings are in discrepancy with Donkin et al. (1989) who observed that concentrations of α- and β-casein were increased ($P=0.03$ or $P=0.1$, respectively) and the concentration of κ-casein was decreased ($P=0.08$) with the addition of RP Met and Lys to the diet. According to Liu et al. (2000) abomasal infusion of casein plus branched-chain AA increased ($P<0.08$) the proportion of α_s-casein. κ-casein tended to increase (8.76 vs 9.32% of total milk protein) when blend of AA + supplement of RP Lys and Met were fed to dairy cows. On the other hand Guinard et al. (1994) found that duodenal infusion of L-LysHCl influenced slightly content of individual casein fractions namely α-casein.

Yields of every casein fractions in our experiment were significantly higher ($P<0.05$) in treated cows (T). Based on the data presented by Pisulewski et al. (1996) recounted yield of individual casein fractions increased with graded amounts of Met infusions.

Several EAA (including Lys, Phe, Met, His a Thr) are transferred in amounts directly related to their amounts found in milk proteins, but the others (Arg, Val, Leu, Ile) are taken up by the gland in excess of their amounts (e.g., Mepham, 1982) and NEAA show a deficit in uptake and probably do not limit milk protein synthesis (Schwab et al., 1976). The needs of the mammary gland would appear to be greater for EAA (branched-chain AA, Arg, Lys and Thr) because their extraction exceed milk protein outputs, for Met, His, Phe, and Trp is uptake usually considerably less (10 to 70 %) than milk protein outputs (Bequette et al., 1998). In the present study we found out that the concentration of Thr in milk and Thr, Pro and Met in casein was significantly different ($P<0.05$) in the T group compared to the C group. Observed significant differences in the percentage of amino acids mentioned above will be probably connected with the polymorphism of milk proteins. Lack of literature focused on the effects of nutrition on the possible changes in amino acid composition of milk, casein and non-casein protein make us unable to explain our results. A further study is needed to explain observed phenomenon.

The variations in AA profiles flowing to the duodenum that determine quantity of individual AA available in the intestine are mainly caused by differences in the AA composition of dietary proteins and in the ratio between microbial protein and RUP (Rulquin and Vérité, 1993). Several measurements of AA flow to the duodenum in lactating dairy cows have been made studying the differences in the duodenal flow of amino acids when different sources of protein were fed (e.g., Erasmus et al., 1994), in different stages of lactation (e.g., Schwab et al., 1992). But there is no study determining the flow of AA through the duodenum when rumen-protected (encapsulated) amino acids were supplemented to the diet.

Of particular interest is the intestinal flow of specific AA, such as Met and Lys, which affect the biological value of the dietary protein. Current recommendations for Lys and Met that are considered to be first and second limiting AA for milk production, should be according to Schwab et al. (1992), 14.9 and 3.9% of EAA in duodenal protein, respectively (based on the *in vivo* measurements). In the present study, proportions of Lys (% of EAA flow) in the duodenal protein ranged from 13.6% for the C group to 13.5% for the T. Proportions of Met (% of EAA flow) varied from 6.2 and 6.4% for C and T group, respectively, and were much higher than that recommended by Schwab et al. (1992). Therefore, the differences between proportions of Lys and Met in the diet supplemented with soya-protein, Lys, Met and His either in the form of powder or in the form of tablets were negligible. Further study is needed to precise the determination of amino acids flows through the duodenum in lactating dairy cows fed diets supplemented with the different sources of RP amino acids.

CONCLUSIONS

These data show that rumen-protected tablets with soya-protein, Lys, Met, and His added to maize-based diet fed to lactating dairy cows resulted in an increase in milk protein yield and was followed closely by progressive increases in casein content and yield. Higher proportion of casein proteins in total milk protein was observed when the tablets were given to cows. Content of β- and κ-casein was unaffected by the treatment while content of α-casein was lower in the T group. Yield of every casein fraction was significantly higher after feeding the rumen-protected amino acids. The increases in the milk protein and casein yield resulted in significantly higher yields of individual amino acids in milk and casein. In the present study we found out that the concentration of Thr in milk and Thr, Pro and Met in casein was significantly different ($P<0.05$) in the T group compared to the C group. Presented results suggest that supplementation of diet with rumen-protected polymerically encapsulated tablets containing soya-protein and limiting amino acids was effective in delivering protein and amino acids Met, Lys and His postruminally and in influencing milk and casein yield and AA composition. Results from this study suggest that the duodenal flows of amino acids could be increased when rumen-protected amino acids were used.

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