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

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ABSTRACT

Five multiparous Polish Red-and-White cows, weeks 8-12 of lactation, in a 5 x 5 Latin square treatment were fed a total mixed ration with grass silage and concentrates (58 and 42% DM). The five treatments were dietary supplements of protected L-lysine and DL-methionine (Smartamine™ ML) and DL-methionine (Smartamine™ M) fed in the following amounts: I: 0 g/d + 0 g/d; II: 35 g/d + 0 g/d; III: 35 g/d + 10 g/d; IV: 35 g/d + 20 g/d; and V: 35 g/d + 30 g/d. The corresponding intestinal concentrations of lysine and methionine (% PDI) were: I: 6.89 and 1.88; II: 7.37 and 2.05; III: 7.32 and 2.29; IV: 7.31 and 2.54; V: 7.28 and 2.78, respectively. The DMI and milk yield were similar among treatments. In contrast, milk protein content was significantly increased ($P<0.05$) over the II treatment and then varied little. Milk fat content and yield varied inconsistently. Plasma metabolites such as glucose and β-hydroxybutyrate were not affected and fell within physiological limits.

Plasma free amino acids responded to the treatments, particularly lysine ($P<0.05$) and methionine ($P<0.001$), producing a pattern of responses similar to that described for milk protein. Significant increases in milk protein content in the second treatment (Smartamine™ ML, 35 g/d) as resulting from improved postruminal supply of lysine (equal to needs), confirm the validity of the assumed requirement for this amino acid (i.e. 7.3% PDI). These increases could have also been due to a higher supply of limiting methionine. However, further improvements in intestinal supply of methionine, gradually meeting the assumed requirement (i.e. 2.5% PDI), had no effect on milk protein content. This lack of responses to methionine (treatments III-V), could have resulted either from a higher postruminal supply of this amino acid than that predicted or from a lower methionine requirement than that assumed.

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

INTRODUCTION

In the last ten years, a definite trend towards decreasing milk fat consumption and increasing consumption of milk protein has become evident (Murphy and O'Mara, 1993; Sloan, 1997). This trend resulted in development of various nutritional and breeding strategies designed to alter milk protein content in order to meet current consumer needs. Indeed, the composition and quality of animal products has now become of importance and is considered to be a part of the overall assessment of the efficiency of animal production.

Generally, there are two major strategies of altering milk composition, namely genetic (through breeding and selection) and nutritional manipulation of milk protein yield and content (Murphy and O'Mara, 1993). Of the nutritional factors substantially affecting milk protein yield and/or content, feeding protected amino acids, at least L-lysine and DL-methionine, has attracted particular attention (Schwab, 1995). Furthermore, it is considered the most efficient nutritional means of maximizing milk protein production, particularly milk protein content as well as minimizing the amount of excess N that the cow must excrete (Dinn et al., 1998).

In the majority of trials confirming the beneficial effects of optimising the profile of amino acids available for absorption in the small intestine of dairy cows, mostly by feeding protected L-lysine and DL-methionine, dairy cow were fed maize silage-based diets (Rulquin et al., 1993; Sloan, 1997). In these conditions, where lysine-deficient maize protein was the major protein fraction in the small intestine, lysine was the first limiting amino acid and methionine a colimiting one (Schwab et al., 1992; Rulquin et al., 1993). In the above trials lysine and methionine were identified as limiting amino acids for milk protein production and their respective requirements, i.e. 7.3 and 2.5% PDI, were established (Rulquin et al., 1993). In contrast, only several trials have been carried out to test the effects of protected L-lysine and DL-methionine or both on milk protein yield and/or content in dairy cows fed grass silage-based diets. In the experiments reported so far, the increases in milk protein production have been more variable and evidently lower than those obtained commonly with maize silage-based diets (Hoden and Coulon, 1991; Sloan, 1997).

Since grass silage is the major winter feed for dairy cows in most parts of Poland, the subject of the present studies was to verify the validity of the estimates of lysine and methionine requirements (i.e. 7.3 and 2.5% PDI) recommended by Rulquin et al. (1993). The effects of protected L-lysine and DL-methionine on milk yield and composition of dairy cows fed grass silage-based diets were determined as the major criteria of the above recommendations.

MATERIAL AND METHODS

Design and treatments

A Latin square (5 x 5) arrangement of treatments, with 14-d periods, was used. The five treatments were dietary supplements of protected L-Lysine and DL-methionine (Smartamine™ ML) and DL-methionine (Smartamine™ M) fed in the following amounts: Treatment I: 0 g/d + 0 g/d; Treatment II: 35 g/d + 0 g/d; Treatment III: 35 g/d + 10 g/d; Treatment IV: 35 g/d + 20 g/d; and Treatment V: 35 g/d + 30 g/d. Smartamine™ ML contains 15% DL-methionine and 50% L-lysine HCl and Smartamine™ M contains 70% DL-methionine. They were commercial products obtained from Rhône-Poulenc Animal Nutrition (Poland). The designated amounts of protected amino acids were mixed with a small amount of wheat bran, then top-dressed, and fed to each cow twice daily in equal amounts.

Cows and management

Five multiparous Polish Red-and-White cows (average liveweight 600 kg), in their second or third lactation, were assigned to the trial 8 to 12 wk postcalving. Their average pre-experimental milk yields were close to 20 kg/d. The cows were fed and milked twice daily at 05.30 and 06.00 and 17.30 and 18.00 h, respectively. They were fed and housed individually.

Diet and feeding

The basal experimental diet (Table 1) was offered as TMR and consisted of wilted grass silage and concentrates (58 and 42 % DM, respectively). The diet was formulated and fed to provide 100% of net lactational energy (UFL; 1UFL = 1700 kcal NE_L) and 100% of protein, as protein truly digested in the small intestine (PDI), required for maintenance and lactation according to the INRA (1989) recommendations. Concentrations of lysine and methionine in the total AA passing to the small intestine (i.e. PDI) were calculated using an amino acid profiling method according to Rulquin et al. (1998). The diet was offered in two equal parts. Water and mineralized salt licks were available daily.

Measurements, sample collection, and preparation

For the second week of each 14-d experimental period, the amounts of feed offered and refusals were recorded daily, and their representative samples were taken for determination of DM in a air-forced oven at 80°C for 48 h. Milk yield was recorded daily at each milking, and milk samples taken during the 5-d periods were assayed for protein, fat and lactose content by infrared analysis, using a Milko-scan 133B (Foss Electric, Denmark). On d 3 of the sampling period, samples from a.m. and p.m. milkings were composited according to yield and analysed for N fractions. On the same day, 3 h after morning feeding, blood samples were taken by jugular venipuncture into heparinized tubes. The tubes were immediately centrifuged for 15 min at 3000 g and the plasma was kept frozen at –20°C until analysis.

TABLE 1

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

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

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

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

Analytical procedures

Chemical composition of feeds offered and refusals was determined on dried (100°C, 24 h) and ground (Cyclotec™; Tecator, Sweden) samples, according to the standard AOAC procedures (1995). NDF and ADF were analysed using the methods of Goering and Van Soest (1970).

Total N, NPN, and non-casein N in milk were determined by Kjeldahl procedures (Hurtaud et al., 1993). Casein N was calculated as the difference between total N and non-casein N, and true protein N as the difference between total N and NPN. Urea in milk was determined according to Roseler et al. (1993), using a colorimetric diacetyl monoxime procedure (Sigma Diagnostics, no 535; Sigma-Aldrich, Poland).

Heparinized plasma samples were analysed for glucose and ß-hydroxybutyrate using test kits (POCH, no 178165140; Poland and Sigma-Diagnostics, no 310A; Sigma-Aldrich, Poland, respectively), and for urea as for milk samples. Free amino acids were determined in plasma samples deproteinized with 5-sulphosalicylic acid (50 mg/mL plasma) and centrifuged (15 min at 2000 g). The deproteinized samples were used for derivatization with o-phtaldialdehyde (OPA), and the OPA amino acid derivatives were separated on a Waters 625LC (Waters, USA) HPLC system with fluorescence detection (Waters 474 detector), according to the procedure described by Czauderna and Kowalczyk (1998).

Statistical analysis

The data obtained were analysed by ANOVA for a 5 x 5 Latin square design using the general linear models procedure of SAS (1985). The model employed for statistical analysis was as follows: $Yijk = \mu + Ci + Pj + Tk + Eijk$, where: μ = general mean, Ci = cow effect, Pj = period effect, Tk = treatment effect, and Eijk = experimental error. All data are expressed and presented throughout the text as least square means. Differences were considered to be significant at $P<0.05$, unless otherwise indicated.

RESULTS

The dry matter intake averaged 16.0 kg (Table 2) and was similar across the treatments. According to calculations based on dry matter intake and 4% FCM yield, the daily ration fully met both energy (UFL) and protein (PDI) requirements of cows. The average excess of energy was 8.1, 5.7, 6.0, 6.8, and 6.4% and that of protein 21.2, 19.5, 18.4, 19.8, and 19.1% respectively, of the INRA recommendations (1989). Available lysine concentrations, estimated by the amino acid profiling method according to Rulquin et al. (1998), were increased over the second treatment and then remained fairly constant, whereas those of methionine were progressively increased, over the range of Smartamine^{TM} M supplements (Table 2). Lysine concentrations were close to the lysine requirement value of 7.3% PDI (treatments II-V), whereas those of methionine gradually reached and exceeded the methionine requirement value of 2.5 % PDI, at the two highest doses of Smartamine^{TM} M (treatments IV-V).

Average milk yield, milk protein and fat yield, and milk fat content did not differ significantly among treatments (Table 3). In contrast, milk protein yield tended to increase and milk protein content was elevated significantly ($P<0.05$), over the last four treatments. Although lactose concentration varied significantly ($P<0.05$), no systematic responses could be described.

TABLE 2

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

Indices	Smartamine™ ML + Smartamine™ M, g/d					SE	P[1]
	0 + 0	35 + 0	35 + 10	35 + 20	35 + 30		
DM intake, kg/d	16.1	15.4	16.3	16.1	16.1	0.6	NS
Supply							
UFL/d	14.4	13.7	14.6	14.4	14.4	0.6	NS
total protein, g/d	3073	2947	3106	3068	3076	116	NS
PDI(E), g/d	1577	1512	1594	1574	1578	60	NS
PDI(N), g/d	1866	1790	1886	1863	1868	71	NS
Balance							
UFL/d	1.08	0.74	0.82	0.92	0.86	0.34	NS
PDI, g/d	277	247	248	261	253	34	NS
Amino acids							
Lys, %PDI	6.89	7.37	7.32	7.31	7.28	–	–
Met, %PDI	1.88	2.05	2.29	2.54	2.78	–	–

[1] NS – non significant

TABLE 3

Milk yield and its composition in dairy cows fed protected lysine and methionine

Indices	Smartamine™ML + Smartamine™M, g/d					SE	P[1]
	0 + 0	35 + 0	35 + 10	35 + 20	35 + 30		
Yield							
milk, kg/d	18.8	18.6	20.0	19.1	18.8	0.76	NS
fat, g/d	750	720	790	770	770	37	NS
protein, g/d	620	630	670	650	640	26	NS
Composition							
fat, g/100g	3.99	3.87	3.95	3.97	4.13	0.05	NS
protein, g/100g	3.28	3.41	3.38	3.42	3.43	0.04	*
lactose, g/100g	4.68	4.55	4.71	4.59	4.68	0.03	*
urea, mg/100g	31.3	36.5	33.9	37.4	34.3	1.91	NS

[1] NS – non significant, * P<0.05

Generally, no significant differences between milk N constituents were detected across the treatments (Table 4). Equally, relative concentrations of the analysed components, when expressed in terms of total N and protein N, were not affected. Also, milk urea did not varied significantly (Table 3). Although no significant changes in response to treatments could be detected, the contents of total N, protein N, and casein N in milk tended to reach a maximum at the highest level of amino acid supplementation. A similar trend was observed for the proportion of casein N in total N. This also held, though non-significantly, for the proportion of casein N in protein N.

Concentrations of blood metabolites (glucose, β-hydroxybutyrate, and urea) were within physiological ranges and were not affected significantly by increasing doses of protected amino acids (Table 5). In contrast, concentrations of lysine and methionine in blood plasma responded significantly to supplementation of protected lysine and methionine (Table 6). The lysine concentration increased initially ($P<0.05$) from the second treatment, and then the elevated values varied inconsistently. Similarly, the methionine concentration was significantly increased ($P<0.001$) from the third treatment, and then remained fairly constant. Concentrations of cystine responded in a similar manner ($P<0.05$). Also taurine tended to increase in response to treatments. Although histidine concentrations varied significantly among treatments, there was no apparent trend in this response. Of the non-essential amino acids, glutamic acid ($P<0.01$) and proline showed the highest increases in response to graded doses of supplemental amino acids.

TABLE 4

Distribution of nitrogen fractions in milk from cows fed protected lysine and methionine

N fractions	Smartamine™ML + Smartamine™M, g/d					SE	P[1]
	0 + 0	35 + 0	35 + 10	35 + 20	35 + 30		
N – fractions							
total – N, %	0.564	0.566	0.574	0.564	0.582	0.010	NS
protein – N, %	0.524	0.524	0.530	0.523	0.539	0.009	NS
casein – N, %	0.422	0.414	0.424	0.420	0.438	0.008	NS
whey – N, %	0.102	0.110	0.106	0.103	0.101	0.005	NS
NPN, %	0.040	0.042	0.044	0.041	0.043	0.002	NS
N – fractions, % of total N							
protein – N	92.96	92.47	92.34	92.77	92.68	0.29	NS
casein – N	74.85	73.36	73.71	74.66	75.34	0.861	NS
whey – N	18.1	19.1	18.6	18.1	17.4	0.78	NS
NPN	7.04	7.53	7.66	7.23	7.32	0.29	NS
N-fractions, % of protein – N							
casein – N	80.5	79.4	79.8	80.5	81.3	0.86	NS
whey – N	19.5	20.6	20.2	19.5	18.7	0.86	NS

[1] NS – non significant

TABLE 5

Concentration of metabolites in plasma of dairy cows fed protected lysine and methionine

Metabolite	Smartamine™ML + Smartamine™M, g/d					SE	P[1]
	0 + 0	35 + 0	35 + 10	35 + 20	35 + 30		
β-hydroxybutyrate, mg/dl	7.12	8.45	8.26	7.12	6.76	0.38	NS
Glucose, mg/dl	58.7	58.7	59.4	67.0	57.0	1.98	NS
Urea, mg/dl	34.1	40.2	40.6	38.1	39.2	1.80	NS

[1] NS – non significant

TABLE 6

Concentration of free amino acids (μmoles/dL) in blood plasma of dairy cows fed protected lysine and methionine

Amino acid	Smartamine™ML + Smartamine™M, g/d					SE	P[1]
	0 + 0	35 + 0	35 + 10	35 + 20	35 + 30		
Essential							
Lys	6.33	7.59	8.56	7.49	7.15	0.32	*
His	8.02	9.95	10.09	8.39	8.21	0.33	*
Arg	7.53	8.65	7.12	9.02	9.18	0.41	NS
Thr	12.6	13.15	15.21	12.58	14.00	0.56	NS
Val	14.9	15.41	17.24	16.00	16.33	0.58	NS
Met	2.49	2.91	4.02	4.10	4.28	0.20	***
Cys[2]	0.86	1.86	2.00	1.72	1.33	0.18	NS
Ile	8.34	8.76	9.13	8.67	9.02	0.33	NS
Leu	9.57	10.54	10.81	10.18	10.07	0.34	NS
Phe	4.02	3.73	4.14	3.96	4.05	0.11	NS
Tyr[2]	3.72	4.17	4.06	3.71	4.23	0.17	NS
Nonessential							
Asp + Asn	1.49	1.81	1.82	1.56	1.73	0.07	NS
Ser	8.53	9.26	9.16	7.72	9.07	0.34	NS
Glu + Gln	22.54	30.42	26.40	24.14	24.17	0.86	**
Pro	9.27	8.00	8.57	12.80	16.24	1.22	NS
Gly	36.08	37.43	34.10	33.37	36.69	1.91	NS
Ala	16.33	16.62	18.95	17.51	17.49	0.47	NS
Tau	3.78	3.67	4.04	4.70	4.54	0.32	NS

[1] NS – non significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

[2] included as semiessential amino acids

DISCUSSION

The major objective of the present study was to describe productive and some systemic responses in dairy cows fed grass silage-based diets and supplemented with five doses of protected lysine and methionine (Smartamine ML) and methionine (Smartamine[TM] M): I: 0 g/d + 0 g/d; II: 35 g/d + 0 g/d; III: 35 g/d + 10 g/d; IV: 35 g/d + 20 g/d; and V: 35 g/d + 30 g/d). These amounts of protected amino acids were designed to meet the lysine and methionine requirements proposed by Rulquin et al. (1993) and were (%PDI): I: 6.89 and 1.88; II: 7.37 and 2.05; III: 7.32 and 2.29; IV: 7.31 and 2.54; V: 7.28 and 2.78, respectively.

Amino acid supplementation had no significant effect on mean dry matter intake or mean milk yield (Table 2). These effects confirm earlier findings of several studies using grass silage-based diets (Robert et al., 1994; Chillard et al., 1995; Younge et al., 1995). On the other hand, feeding protected amino acids may increase dry matter intake of grass silage diets in early lactation (Xu et al., 1998). Significant ($P<0.05$) increases in milk protein content (II: +1.3, III: +1.0, IV: +1.4, and V: +1.5 g/d, compared with I: 32.8 g/kg) and corresponding positive trends in milk protein yield (II: +10 g, III: +50 g, IV: +30 g, and V: +20 g/d, compared with I: 620 g/d) were in the range of usually reported values. Indeed, milk protein content was consistently increased by postruminal supplements of these amino acids on both maize silage and grass silage rations fed to dairy cows (Rulquin et al., 1993; Sloan, 1997). At the same time, the responses reported for milk protein yield, are dependent on the stage of lactation, and are usually less consistent than those for milk protein percentage (Robert et al., 1994; Chillard et al., 1995; Younge et al., 1995; Xu et al., 1998). Milk N fractions did not respond in a significant manner to suppplements of protected lysine and methionine (Table 4). However, the increases in the content of casein-N and in the proportion of casein-N in both total-N and protein-N, associated with the last treatment (i.e. V), could be indicative of a stimulating effect of the supplemental amino acids on milk protein synthesis. Of particular interest are also milk urea concentration as a mean of monitoring protein adequacy of protein nutrition of dairy cows (Hof et al., 1997). Although the ration provided an excess of N, as indicated by high content of PDIN compared with that of PDIE (Table 2), milk urea was uniform across treatments and similar to values for cows fed either maize silage (Pisulewski et al., 1996) or grass silage (Xu et al., 1998) rations. Inconsistent responses of milk fat content and yield were in line with comparable studies (Robert et al., 1994; Chillard et al., 1995; Younge et al., 1995; Xu et al., 1998), in which, feeding protected lysine and methionine did not significantly alter these parameters.

Concentrations of plasma metabolites (Table 5) can be discussed in terms of energy and protein, including amino acids status of the dairy cows. Since the net energy requirements (INRA, 1989), were met with an average excess of 7.6%

(Table 2), energy was probably not a limiting factor in our studies. Consequently, plasma glucose concentrations, reaching on average 60 mg/100 ml, fell within a normal physiological range, and were similar to those reported for dairy cows in positive energy balance (Rulquin and Delaby, 1997; Xu et al., 1998). β-Hydroxybutyrate concentrations were unaffected by the treatments and fairly low, implying that mobilization of body fat was not in excess of carbohydrate energy supply. Protein status of the cows resulted from the amount of protein provided by the ration and the amounts of protected lysine and methionine fed. Generally, the cows were in a positive protein balance resulting from the relatively high crude protein content (19.1%DM) of the ration. In addition, the ration was imbalanced (Table 2) in terms of PDIN and PDIE. Furthermore, it supplied an excess (averaging 19.6%) of recommended PDI (INRA, 1989). The increased protein supply could have enhanced more marked responses of the cows to the supplemental amino acids (Rulquin et al., 1994). In spite of the reported protein status, plasma urea concentrations were only slightly elevated above commonly reported values and corresponded with those for milk urea (Pisulewski et al., 1996; Rulquin and Delaby, 1997; Xu et al., 1998). Indeed, a strong correlation between plasma urea and milk urea concentrations does exist (Roseler et al., 1993). The apparent increases of plasma free lysine ($P<0.05$) and methionine ($P<0.001$) concentrations can be attributed to the imposed treatments (Table 6). Consequently, these increases prove that both amino acid preparations were effectively protected for on the rumen degradation and then absorbed in the small intestine. These effects are a common feature of experiments involving protected forms of lysine and methionine (Piepenbrink et al., 1996; Bremmer et al., 1997; Xu et al., 1998). Moreover, elevated levels of methionine metabolites, such as cystine and taurine, were consistent with the results of similar experiments using methionine (Pisulewski et al., 1996; Rulquin and Delaby, 1997; Overton et al., 1998).

The described pattern of responses can be exploited further to verify the assumed estimates of lysine and methionine requirements, i.e. 7.3 and 2.5% PDI, respectively. As can be seen (Table 2), increased postruminal supply of lysine and methionine, from the second treatment (Smartamine™ ML, 35 g/d), met the above lysine requirement, whereas that of methionine was still largely below the assumed needs. These increases in intestinal lysine and methionine supply resulted in greater milk protein responses (particularly protein content). At the same time, the above responses were limited only to the second treatment and additional supplements of Smartamine™M (10, 20, and 30 g/d), increasing postruminal methionine supply (2.29, 2.54, and 2.78% PDI, respectively) did not produce any further improvements. Therefore, the assumed lysine requirement (i.e. 7.3% PDI) can be considered valid for the cows fed grass-silage based diets. In contrast, we were not able to confirm the validity of the assumed methionine requirement (i.e. 2.5% PDI). The lack of responses to methionine (treatments III-V), could have resulted either from a higher postruminal supply of this amino acid than that predicted or from a lower methionine requirement than that assumed. However, it is possible that more acute methionine deficiency could have been produced by cows giving significantly higher milk yields than those recorded in our studies (ca 20 kg/d).

CONCLUSIONS

Dairy cows fed grass silage-based diet, providing adequate net energy (NE_L) and protein (PDI), and supplemented with protected lysine and methionine, had the ability to increase milk protein content and yield. At the same time, the above responses, resulting from increased lysine and methionine supply, were evident only from the IInd treatment. Further supplementation with protected-methionine, over the IIIrd, IVth and Vth treatment, merely sustained elevated levels of these measurements. The above results indicate that the assumed estimate of lysine requirement (i.e. 7.3% PDI) is valid, whereas that of methionine (i.e. 2.5% PDI) needs to be established conclusively in cows fed grass silage-based diets.

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STRESZCZENIE

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

Badania przeprowadzono na 5 krowach wieloródkach rasy czerwono-białej, w 8-12 tygodniu laktacji, w układzie kwadratu łacińskiego (5 x 5). Zwierzęta żywiono dawką pokarmową zawierającą w s.m. (%): kiszonkę z traw 58 i mieszankę pasz treściwych 42 (skład %: wysłodki buraczane 35, kukurydza 30, jeczmień 20, poekstrakcyjna śruta sojowa 10 i mieszanka mineralna 5). Zastosowano pięć poziomów dodatku chronionej L-lizyny i DL-metioniny (Smartamine™ ML) oraz DL-metioniny (Smartamine™ M): I – 0g/d + 0g/d; II – 35 g/d + 0 g/d; III – 35 g/d + 10 g/d; IV – 35 g/d + 20 g/d; V – 35 g/d + 30 g/d.

Uzyskane jelitowe stężenia lizyny i metioniny (% BTJ) wynosiły odpowiednio: I – 6.89 + 1.88; II – 7.37 + 2.05; III – 7.32 + 2.29; IV – 7.31 + 2.54; V – 7.28 + 2.78. Nie stwierdzono istotnych różnic w pobraniu s.m. i dziennej wydajności mleka. Zawartość białka w mleku wzrastała istotnie (P<0.05) przy II poziomie dodatku chronionych aminokwasów i nie ulegała dalszym zmianom przy wyższych poziomach. Zawartość i wydajność tłuszczu nie różniły się istotnie. Poziom glukozy i kwasu b-hydroksymasłowego w osoczu krwi był podobny i mieścił się w zakresie norm fizjologicznych, a poziom wolnych aminokwasów, przede wszystkim lizyny i metioniny zwiększał się istotnie (P<0,05 i P<0,001, odpowiednio). Przebieg tych zmian był zbliżony do zmian zawartości i wydajności białka mleka. Istotny wzrost zawartości białka w mleku, przy II poziomie jako następstwo zwiększonej (do potrzeb) podaży lizyny, potwierdza wiarygodność założonej wartości zapotrzebowania na ten aminokwas (tj. 7,3% BTJ). Wzrost ten mógł być także efektem zwiększonej podaży methioniny. Dalsza podaż tego aminokwasu, pokrywająca stopniowo założone zapotrzebowanie (tj. 2,5% BTJ), nie miała jednak wpływu na zawartość białka w mleku. Wyniki te wskazywałby bądź na wyższą podaż metioniny w stosunku do przewidywanej, bądź na niższe rzeczywiste zapotrzebowanie na ten aminokwas w stosunku do zakładanej wartości.