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Effect of ruminally-protected leucine supplement on milk yield and plasma amino acids in dairy cows

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The aim of this study was to determine the influence of leucine supplement in the form of rumen-protected tablets on milk yield and composition and plasma amino acids in four high-yielding lactating Holstein cows. The experiment was carried out as a cross-over procedure and was divided into 4 periods of 14 d (10 d preliminary period and 4 d experimental period). Cows were fed ad libitum a diet based on maize silage, lucerne hay and a supplemental mixture. The diet, defficient in methionine, lysine, and leucine, was supplemented with methionine+lysine (Control) or methionine+lysine+leucine (Leu) in rumen protected form. The dry matter intake, milk yield and milk yield expressed in energy corrected milk did not differ significantly between the treatments. Milk protein content and yield did not show statistically significant variation. The contents and yield of casein, fat, lactose and urea were unaffected by the treatment. Blood metabolites did not vary between the treatments. The introduction of Leu resulted in higher plasma levels of proline ($p<0.05$) in comparison to Control. The calculated leucine defficiency in maize silage-concentrate diet used in the present experiment was approx 5.5%. The results of this experiment suggest that under the given conditions, leucine was not a limiting amino acid.

Key-words: leucine, rumen protection, dairy cow, milk, plasma amino acids

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Introduction

Results of several experiments comprising postruminal infusion of amino acids (AA) suggest that AA supply in the feed of lactating cows is qualitatively or quantitatively suboptimal. Providing an adequate pattern of AA for absorption in the small intestine may facilitate achieving optimum productivity in high-yield dairy cows by increasing the content and yield of milk protein (Rulquin and Vérité 1993). The knowledge of limitations in individual essential AA allows supplementing these AA with minimum additional nitrogen (N). Therefore, it can be inferred that altering either the quantity (Lapierre et al. 2000) or composition (Blouin et al. 2002) of diet can vary the pattern and amount of absorbed AA. Schwab et al. (1993) emphasized that optimizing intestinal AA balance is more important in improving milk protein production than crude protein (CP) content of the ration. A potential consequence of an unbalanced AA profile entering the duodenum is that an excess of certain AA may hamper absorption of other AA, since AA are absorbed across the intestinal wall through an active transport mechanism (Schwab et al. 1976).

Besides methionine (Met), lysine (Lys) and eventually histidine (His), considered as the most critical AA for milk protein synthesis particularly when high dietary proportions of maize products rich in leucine (Leu) are fed (Rulquin and Vérité 1996), the branch-chained AA (BCAA) play an important role in mammary gland metabolism. As potentially limiting, BCAA (especially Leu) have recently attracted some attention pursuant to the hypothesis that Leu is likely to be the most limiting AA from the BCAA (e. g., Varvikko et al. 1999, Vanhatalo et al. 1999 or Miettinen and Huhtanen 1997). Brandt et al. (1987) suggest that under different feeding conditions such as forage-based grass/grass silage rations instead of concentrate-based rations, Leu might be the first or second limiting AA in dairy cows. According to Kröber et al. (2001) even mixed forage rations containing maize silage fed in combination with concentrate may be deficient in Leu depending on the proportion of concentrate and the type of concentrate ingredients used. Rulquin et al. (2001a) proposed Leu as a limiting AA for diets with low contents of maize. Although in recent studies of Korhonen et al. (2002) and Huhtanen et al. (2002), nonsignificant or inconsistent production responses to supplemental Leu were obtained in experiments with cows fed on grass/grass silage based diets, the Leu limitation in maize silage-concentrate diets is unclear (Kröber et al. 2001, Rulquin et al. 2001a). The objective of the present study was to establish, based on changes in milk yield and composition and plasma AA, the role of Leu given in the form of rumen protected tablets as a possible third-limiting AA in milk production of cows fed a maize silage-concentrate based diet.

Material and methods

Animals and procedures

Four high-yield lactating Holstein cows (lactation 2–5, 18–26 week of lactation) with similar milk production (30.8 kg, SEM = 3.9) were used in the experiment. The cows were housed in individual tie stalls bedded with sawdust. The experiment was conducted in the form of cross-over design whereby 2 received a control diet (Control) and the remaining 2 cows were fed the experimental diet supplemented with leucine (Leu). In the subsequent period the cows were switched to the other treatment. The experiment was divided into 4 periods. Each period (14 d) consisted of a 10-day preliminary period and a 4-day experimental period.

Cows were fed individually twice daily (0700 h and 1700 h) *ad libitum* with the diet based on maize silage (345 g/kg), lucerne hay (114 g/kg) and a supplemental mixture (541 g/kg). Supplemental mixture contained (g/kg): barley 350; oat 250; wheat 80; sugarbeet chippings 150; flax seed 50; soybean meal 70; sodium chloride (NaCl) 5; dicalcium phosphate ($CaHPO_4$) 15; limestone ($CaCO_3$) 15; sodium bicarbonate ($NaHCO_3$) 1; monosodium phosphate (NaH_2PO_4), 2; magnesium phosphate ($Mg_3(PO_4)_2$) 2; microelements and

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vitamin mixture 10 (1 kg of the mixture contains: vit A 27 mil IU; Vit D3 4 mil IU; Vit E 100 000 IU; Fe 1 mg; Co 1.1 g; I 2.5 g; Mn 140 g; Cu 35 g; K 2 g; Zn 150 g; Se 0.5 g). The diets were calculated to meet 100% NEL (net energy of lactation) requirements and 95% PDI (protein digestible in the intestine) requirements (Sommer et al. 1994) for better manifestation of the response to the experimental treatment. Based on the tables of AADI (digestible AA in the intestine) values of feedstuffs (Rulquin et al. 2001b), the formulated diets were ascertained as deficient in Met (approx 26%), Lys (approx 5%) and Leu (approx 5.5%). The amount of mentioned AA needed to settle the difference was calculated to meet the amino acid requirement (Rulquin et al. 2001), set for MetDI as 2.5%, for LysDI 7.3% and for LeuDI as 8.8%. The basal diet was supplemented with Met+Lys (Control) or with Met+Lys+Leu (Leu) in rumen protected form. In Control, intake of Met and Lys was 14.4 and 10.0 g/d and in Leu, intake of Met, Lys and Leu was 14.4, 10.0 and 6.5 g/d respectively. AA were supplied in the form of Smartamine M ™ (Met) and rumen protected tablets (Lys and Leu). The tablets (diameter 6.5 mm, lenticular in shape) were produced by the authors according to Ardaillon et al. (1989), were coated by a copolymer according to patent No. US 4,877,621. Assumed loss of tablets by rumination was compensated by 30% increase in the amount of tablets supplied (Třináctý et al. 2000). Smartamine M ™ and tablets were supplied during the whole period (14 d) twice a day by mixing these components into part of the supplemental mixture immediately before feeding. Refusals were monitored daily and analysed.

Analytical procedures

In feed and feed refusals the following parameters were estimated according to AOAC (1984): CP (No 7021), ash (No 7009), and fat (No 7060). Dry matter (DM) was determined by drying at 103ºC for 4 h. Neutral detergent fibre (NDF, with α-amylase) and acid detergent fibre (ADF) without residual ash were estimated according to Van Soest et al. (1991) and Goering and Van Soest (1970), respectively.

Cows were milked twice daily at 0715 h and 1715 h. During the experimental period milk yield was monitored and milk samples were taken at each milking, conserved by 2-bromo-2-nitropropane-1.3-diol (Bronopol) and cooled to 6°C. Milk composition was analysed by 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). Casein content was measured on Kjeltec auto, 1030 Analyser (Tecator AB, Höganäs, Sweden) after precipitation with 10% acetic acid.

On the last day of each experimental period, blood samples were collected into heparinised tubes from the jugular vein at four times (0630 h, 0830 h (collected 2 samples), 1030 h, and 1230 h) for determination of AA profile and blood parameters. Immediately after obtaining blood, the samples were centrifuged for 15 min at 1500 x g. Blood parameters, i. e. total protein, glucose, urea, NEFA (nonesterified fatty acids), β-hydroxybutyrate, alanine aminotransferase and aspartate aminotransferase, were analysed from samples taken at 8:30 using standard enzymatic methods kits (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 sulfosalicylic acid and centrifuged for 10 min at 3000 x g. The supernatant was stored at –80 °C until the AA profile was determined on Automatic Aminoanalyser AAA 400 (Ingos, Praha, Czech Republic).

Statistical analysis

Data obtained in the experiment were analysed using GLM procedure of the Statgraphics 7.0 package (Manugistics Inc., and Statistical Graphics Corporation, Rockville, Maryland, USA) according to the following model: $Y_{ijk} = \mu + T_i + C_j + P_k + \varepsilon_{ijk}$ where μ = general mean, T_i = treatment effect (i =

2), C_j = cow effect (j = 4), P_k = period effect (k = 4) and ε_{ijk} = error term. For all statistical evaluations period means were used (n=8). Values of $p<0.05$ were considered to be significant.

Results

Intake of nutrients and milk yield and composition

Intakes of DM and other nutrients are presented in Table 1. No significant differences between the treatments were determined. Milk yield and composition is given in Table 2, while the daily yields of milk constituents are shown in Table 3. No significant effect of Leu on milk yield was established; similarly milk yield expressed in ECM (energy corrected milk) did not differ significantly between treatments. Milk protein and yield did not show statistically significant variation. Supplementing with Leu did not affect casein content and yield which was similar in both groups. The contents of fat, lactose and urea were within normal physiological range and were not affected by the treatment. Yields of fat and lactose were also not affected by treatment.

Blood parameters and plasma AA

The blood metabolite means are given in Table 4. Leu supplement did not have any effect on plasma total protein, NEFA, glucose or urea concentrations. Changes in blood plasma concentrations

Table 1. Effect of ruminally-protected leucine on nutrient intake in lactating dairy cows

Nutrient	Unit	Control[1]	Leu[2]	SEM
Dry matter	kg/d	19.38	19.44	0.174
Organic matter	kg/d	17.56	17.62	0.161
Crude protein	kg/d	2.78	2.79	0.032
Fat	g/d	523.3	521.0	0.009
NDF[3]	kg/d	6.58	6.59	0.054
ADF[4]	kg/d	3.59	3.60	0.029
PDIN[5]	kg/d	1.83	1.84	0.021
PDIE[5]	kg/d	1.80	1.82	0.019
NEL[6]	MJ/d	130.6	131.0	1.324
LysDI[7]	% PDI	7.23	7.17	0.003
MetDI[7]	% PDI	2.44	2.39	0.006
LeuDI[7]	% PDI	8.32	8.82	0.015
HisDI[7]	% PDI	2.05	2.04	0.001
PheDI[7]	% PDI	5.02	5.00	0.001
ThrDI[7]	% PDI	5.04	5.01	0.002
IleDI[7]	% PDI	5.27	5.24	0.002
ValDI[7]	% PDI	5.78	5.75	0.001
ArgDI[7]	% PDI	4.72	4.69	0.003

[1] control group, supplemented with ruminally-protected Met (14.4 g/d) and Lys (10.0 g/d), [2] experimental group, supplemented with ruminally-protected Met (14.4 g/d), Lys (10.0 g/d) and Leu (6.5 g/d), [3] neutral detergent fibre, [4] acid detergent fibre, [5] digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively, [6] net energy of lactation, [7] digestible AA in the intestine

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of the amino acids pursuant to the experimental treatments are shown in Table 5. The supply of Leu was reflected in the different ($p<0.05$) plasma level of proline.

Table 2. Effect of ruminally-protected leucine on yield and composition of milk

Component	Unit	Control[1]	Leu[2]	SEM	*p*
Milk yield	kg/d	28.83	28.62	0.25	0.572
ECM[3]	kg/d	27.91	27.92	0.29	0.981
Dry matter	g/kg	127.3	127.6	0.88	0.826
Non-fat dry matter	g/kg	39.1	39.3	1.08	0.894
Fat	g/kg	38.3	38.6	0.99	0.806
Protein	g/kg	32.9	33.3	0.23	0.270
Casein	g/kg	26.4	26.5	0.16	0.637
Lactose	g/kg	49.3	49.3	0.15	0.862
Urea	mg/100 ml	28.24	28.94	0.51	0.370

[1] control group, supplemented with ruminally-protected Met (14.4 g/d) and Lys (10.0 g/d), [2] experimental group, supplemented with ruminally-protected Met (14.4 g/d), Lys (10.0 g/d) and Leu (6.5 g/d), [3] energy corrected milk calculated according to Sjaunja et al. (1991)

Table 3. Effect of ruminally-protected leucine on daily yield of milk components

Component	Unit	Control[1]	Leu[2]	SEM	*p*
Dry matter	kg/d	3.63	3.61	0.028	0.611
Non-fat dry matter	kg/d	1.09	1.08	0.030	0.881
Fat	kg/d	1.06	1.07	0.027	0.915
Protein	kg/d	0.95	0.95	0.011	0.812
Casein	kg/d	0.76	0.76	0.010	0.900
Lactose	kg/d	1.42	1.41	0.013	0.558

[1] control group, supplemented with ruminally-protected Met (14.4 g/d) and Lys (10.0 g/d)
[2] experimental group, supplemented with ruminally-protected Met (14.4 g/d), Lys (10.0 g/d) and Leu (6.5 g/d)

Table 4. Effect of ruminally-protected leucine on blood parameters of lactating dairy cows

Component	Unit	Control[1]	Leu[2]	SEM	*p*
Total protein	g/l	70.50	72.12	1.206	0.378
Glucose	mmol/l	3.53	3.62	0.066	0.361
Urea	mmol/l	6.07	6.03	0.214	0.899
NEFA[3]	mmol/l	0.14	0.19	0.038	0.421
BHB[4]	mmol/l	0.59	0.57	0.054	0.768
ALT[5]	U/l	19.05	19.28	1.573	0.923
AST[6]	U/l	69.75	70.35	3.597	0.910

[1] control group, supplemented with ruminally-protected Met (14.4 g/d) and Lys (10.0 g/d), [2] experimental group, supplemented with ruminally-protected Met (14.4 g/d), Lys (10.0 g/d) and Leu (6.5 g/d), [3] nonesterified fatty acids, [4] β-hydroxybutyrate, [5] alanine aminotransferase, [6] aspartate aminotransferase

Table 5. Effect of ruminally-protected leucine on plasma concentrations of free individual amino acids (in µg/g of plasma)

Component	Unit	Control[1]	Leu[2]	SEM	*p*
Lysine[3]	µg/g	12.32	12.69	0.565	0.657
Methionine	µg/g	4.24	4.59	0.261	0.375
Histidine	µg/g	4.67	5.21	0.348	0.302
Leucine	µg/g	10.16	11.21	0.428	0.119
Isoleucine	µg/g	11.22	11.87	0.421	0.305
Valine	µg/g	21.65	22.48	0.593	0.363
Arginine	µg/g	10.17	10.03	0.853	0.913
Threonine	µg/g	7.79	8.71	0.346	0.097
Serine	µg/g	7.33	7.96	0.625	0.501
Proline	µg/g	4.19[a]	5.31[b]	0.251	0.014
Glycine	µg/g	25.28	29.70	2.420	0.233
Asparagine	µg/g	3.38	3.90	0.349	0.317
Glutamic acid	µg/g	6.09	5.81	0.353	0.589
Glutamine	µg/g	42.48	42.82	2.565	0.930
Phenylalanine	µg/g	5.33	5.36	0.232	0.939
Tyrosine	µg/g	6.28	6.53	0.253	0.504
Alanine	µg/g	17.45	19.64	1.387	0.295

[1] control group, supplemented with ruminally-protected Met (14.4 g/d) and Lys (10.0 g/d), [2] experimental group, supplemented with ruminally-protected Met (14.4 g/d), Lys (10.0 g/d) and Leu (6.5 g/d), [3] determined as LysHCl, [a,b] means in the same row followed by the different superscripts differ significantly ($p<0.05$)

Discussion

The response to feeding protected AA reported in the literature is variable depending on the source of protein in the basal diet. Nevertheless, results of our previous work confirmed the functionality of produced copolymer-coated rumen protected tablets that were used in the present experiment by significantly increasing duodenal flow of supplemented AA (Křížová et al. 2007) as well as by increase in milk yield and milk protein yield (Třináctý et al. 2006).

Intake of nutrients and milk yield and composition

In the present experiment the average DM intake of cows in both experimental groups was almost identical. Similar responses to Leu supplement have been presented in other studies (e. g. Huhtanen et al. 2002, Korhonen et al. 2002, Kröber et al. 2001). In our study, we found that the effects of additional Leu on milk yield and composition were minor. Similarly, Korhonen et al. (2002), Kröber et al. (2001) and Mackle et al. (1999) did not report any positive milk production responses to either Leu or BCAA infusions with cows fed lucerne and maize based diets.

Although Leu has been proposed as a limiting AA for diets with low contents of maize (Rulquin et al. 2001a), there is no direct demonstration of this limitation (Kröber et al. 2001). Furthermore, according to Schwab et al. (1976), it is possible that with the typical mixed diets, the margin between the second- and even third-limiting AA can be very small. Therefore, the increase in milk protein yield by supplying the next-limiting AA, would be very small under practical feeding conditions. Possible

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explanation for the ambiguous response to Leu supplementation can be the fact that Leu is removed by the mammary gland in large excess relative to its output (Guinard and Rulquin 1994; Mackle et al. 2000) and is taken up in agreement with milk output only when Leu is limiting (Rulquin and Pisulewski 2000, Bequette et al. 1996). Furthermore, in the state of subclinical deficiency, changes in the plasma concentrations of supplemented AA closely reflect their metabolic availability, even when no immediate effects on milk protein synthesis occur. This could be different when the AA are supplied in clear excess of requirements for milk protein synthesis or in an unbalanced manner (Colin-Schoellen et al. 1995).

Blood parameters and plasma AA

Blood parameters determined in the experiment were not affected by the treatment. In the present study we found that plasma concentration of Leu tended to be higher (p=0.12) after supplementation of Leu in the diet. This fact conforms with the findings of other studies which also described an elevated plasma concentration of AA when they were supplied in a rumen protected form (e. g. Donkin et al. 1989, Rogers et al. 1987). The response in term of blood plasma concentrations of other AA to Leu supplementation is scarce and inconsistent. Our study found that Leu increased the plasma concentration of proline (p<0.05). Huhtanen et al. (2002) found that the only effect of Leu infusion on plasma concentrations of other AA was the tendency for increasing levels of phenylalanine and decreasing levels of valine. Compared with maize or lucerne silage-based diets (Pisulewski et al. 1996; Erasmus et al. 1994; Schwab et al. 1992a; 1992b), the supply of Leu seems to be lower in grass silage-based diet (Huhtanen et al. 2002; Korhonen et al. 2000, 2002; Chamberlain et al. 1986) and these differences were also obvious in plasma AA concentrations in the mentioned studies. Furthermore, basal diet seems to be the main factor in determining AA supply, which is in accordance with various responses to AA supplementation on grass silage and maize based diets (Vanhatalo et al. 1999; Varvikko et al. 1999; Schwab et al. 1992a, 1992b).

Conclusion

Rumen-protected individual AA, instead of whole protein sources, offer the potential for balancing diets in order to meet the AA requirements of high-yield, lactating dairy cows without resultant excess of undesirable AA. The calculated Leu deficiency in maize silage-concentrate diet used in the present experiment was approx 5.5%, inferring that the amount of Leu supplemented in the diet was relatively low. The results of this experiment suggest that under the given conditions, Leu was not a limiting AA.

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