Effect of duodenal infusions of leucine on milk yield and plasma amino acids in dairy cows

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ABSTRACT: Four high-yielding lactating Holstein cows fitted with duodenal cannulas were used in the experiment. Cows were divided into 2 groups – control (Control) with leucine deficiency and experimental (Leucine) with a leucine supplement. The experiment was divided into 4 periods of 7 days, each consisting of a 3-day preliminary period followed by a 4-day experimental period. In the first period, 2 cows were assigned to Control and the remaining 2 to Leucine. In the subsequent period the cows were switched to the other treatment. Cows were fed individually twice daily the basal diet based on maize silage, lucerne hay and supplemental mixture. Infusions of amino acids in Leucine consisted of methionine (12.6 g/day), lysine (20.7 g/day), histidine (10.7 g/day) and leucine (19.3 g/day). The composition of amino acid infusate in Control was the same except for leucine that was replaced with monosodium L-glutamate. The intake of dry matter was not affected by the treatment ($P > 0.05$). No effect of leucine infusion on milk yield and composition was observed ($P > 0.05$), nevertheless the concentration of protein and casein in milk tended to be higher in Leucine (38.3 and 31.3 g/kg) than in Control (37.4 and 30.4 g/kg, respectively, $P < 0.1$). The yield of milk components was not affected by the treatment ($P > 0.05$). Duodenal infusion of leucine resulted in a decreased plasma level of isoleucine in Leucine compared to Control ($P < 0.01$). Concentrations of leucine, cysteine and citrulline tended to be higher and the concentration of tyrosine tended to be lower in Leucine in comparison with Control ($P < 0.10$).

Keywords: leucine; duodenal infusion; milk; plasma amino acids; dairy cow

The major part of the amino acid (AA) requirement of lactating dairy cows is met by postruminal digestion of microbial protein. Nevertheless, diets for high-yielding cows need not meet the requirement for all AA. In general, the most limiting AA in maize silage based diets for the synthesis of milk and milk protein have been reported to be methionine (Met) and lysine (Lys) (e.g. Schwab et al., 1992a). Brandt et al. (1987) suggested that under different feeding conditions such as forage-based grass/grass silage diets instead of concentrate-based diets leucine might be the first or the second limiting AA in dairy cows. According to Kröber et al. (2001) even mixed forage rations containing maize silage fed along with concentrate might be deficient in leucine depending on the proportion of

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concentrate and the type of concentrate ingredients used. Thus leucine (Leu), which is suggested to be the most-limiting from branched chain AA (e.g. Varvikko et al., 1999), has recently received some attention (Miettinen and Huhtanen, 1997; Varvikko et al., 1999; Iburg and Lebzien, 2000; Rulquin and Pisulewski, 2006) as a potentially limiting AA for milk protein synthesis.

The objective of the present study was to determine the role of Leu as a potentially third limiting AA supplemented in the form of duodenal infusions in milk production of cows fed maize silage-concentrate based diet.

MATERIAL AND METHODS

Animals and procedure

Four high-yielding lactating Holstein cows (lactation 2–3, week 22 of lactation) with similar milk production (18.6 kg, SEM = 1.5) fitted with duodenal closed T-shaped cannulas (Bar Diamond, Inc., USA) were used in the experiment. The cows were housed in individual tie stalls bedded with sawdust and they were divided into 2 groups of 2 animals. Factor with 2 levels was applied – Leu deficiency (Control) and and Leu supplement (Leucine). The experiment was divided into 4 periods. Each period (7 days) consisted of a 3-day preliminary period followed by a 4-day experimental period. In the first period, the first group was assigned to Control and the second to Leucine. In each subsequent period the groups were switched to the other treatment according to the following scheme: the first group – Control, Leucine, Control, Leucine; the second group – Leucine, Control, Leucine, Control.

Cows were fed individually twice daily (7.00 and 17.00 h) *ad libitum* the basal diet based on maize silage, lucerne hay and supplemental mixture (Table 1). The diet was formulated to provide 100% of NEL (net energy of lactation) and 95% of PDI (protein digestible in the intestine) requirements according to the recommendations of Sommer et al. (1994). Based on Rulquin et al. (2001a) the formulated diets were considered to be deficient in Met (approx 22%), Lys (approx 7.4%), histidine (His) (approx 18.0%) and Leu (approx 3.4%). This deficit of AA was covered by duodenal infusions of an AA mixture which was composed in such a way that the respective AA requirements would be met (Rulquin et al., 2001b). Infusions of AA in Leucine consisted of the appropriate amounts of crystalline Met (12.6 g/day), Lys (20.7 g/day), His (10.7 g/day) and Leu (19.3 g/day, Ajinomoto Co., Inc. Japan). The composition of the AA infusate in Control was the same except for Leu, which was replaced with monosodium L-glutamate to ensure the isonitrogenicity. AA were dissolved in 4–5 litres of fresh tap water for each cow daily and infused continuously to the duodenum over a 24-h period using a four-channel infusion pump (Dávkovací čerpadla Ing. Kouřil, Czech Republic)

Table 1. Composition of diets

Ingredient	Content
Maize silage (g/kg)	587
Lucerne hay (g/kg)	94
Supplemental mixture[1] (g/kg)	319
PDIN (g/kg)[2]	82.5
PDIE (g/kg)[2]	88.9
NEL (MJ/kg)[3]	6.83
LysDI (% PDIE)[4]	6.76 (7.3)[5]
MetDI (% PDIE)[4]	1.95 (2.5)[5]
LeuDI (% PDIE)[4]	8.51 (8.8)[5]
HisDI (% PDIE)[4]	2.05 (2.5)[5]

[1]supplemental mixture contains (g/kg): barley 449; wheat 451; flax 45; sunflower oil meal 137; sodium chloride (NaCl) 7; dicalcium phosphate ($CaHPO_4$) 10; limestone ($CaCO_3$) 19; sodium bicarbonate ($NaHCO_3$) 1; MgP 2; mineral and vitamin mixture 1
[2]digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively
[3]net energy of lactation
[4]digestible amino acids in the intestine
[5]values in the parenthesis: requirements for the given amino acids according to Rulquin at al. (2001b)

Analytical procedures

During the experiment feed intake and refusals were monitored daily, an aliquot of them was taken for subsequent analyses. In both feed and feed refusals the dry matter (DM) was determined by drying at 103°C for 4 h, crude protein, crude fibre and fat were estimated according to AOAC (1984) and neutral detergent fibre by using α-amylase was estimated according to Van Soest et al. (1991).

Cows were milked twice daily at 7:15 and 17:15 h. During the experimental period milk yield was monitored and milk samples were taken at each milking, preserved with 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. Milk yield was corrected for energy content according to Sjaunja et al. (1991).

On the last day of each experimental period, blood samples were taken into heparinised tubes from the jugular vein (at 7:45 h) for determination of the AA profile and plasma metabolites. The samples were immediately centrifuged for 15 min at 1 500 g. Plasma 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 the AA profile the blood plasma was deproteinised with sulphosalicylic acid and centrifuged for 10 min at 3 000 g. The supernatant was stored at –80°C until the AA profile was determined on the AAA 400 Automatic Aminoanalyser (Ingos, Prague, Czech Republic).

Statistical analysis

Data obtained in the experiment were analysed using the GLM procedure of the Statgraphics 7.0 package (Manugistics Inc. and Statistical Graphics Corporation, Rockville, Maryland, USA) according to the following model:

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

Table 2. Effect of duodenally infused leucine on nutrient intake in lactating dairy cows

Nutrient	Control[1]		Leucine[2]		P[7]
	mean	SEM	mean	SEM	
Dry matter (kg/day)	15.90	1.00	16.00	1.18	NS
Crude protein (kg/day)	1.98	0.15	2.05	0.18	NS
Fat (kg/day)	0.36	0.02	0.36	0.03	NS
Crude fibre (kg/day)	2.46	0.11	2.44	0.13	NS
NDF (kg/day)[3]	5.37	0.30	5.34	0.33	NS
PDIN (kg/day)[4]	1.29	0.10	1.34	0.12	NS
PDIE (kg/day)[4]	1.40	0.10	1.43	0.11	NS
NEL (MJ/day)[5]	108.30	7.14	109.80	8.39	NS
LysDI (% PDIE)[6]	7.85	0.03	7.87	0.03	NS
MetDI (% PDIE)[6]	2.71	0.02	2.73	0.03	NS
LeuDI (% PDIE)[6]	8.14	0.02	9.40	0.03	***
HisDI (% PDIE)[6]	2.68	0.02	2.69	0.03	NS

[1]control group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day) and histidine (10.7 g/day)

[2]experimental group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day), histidine (10.7 g/day) and leucine (19.3 g/day)

[3]neutral detergent fibre with α-amylase

[4]digestible protein in the intestine when rumen fermentable N supply or energy supply are limiting, respectively

[5]net energy of lactation

[6]digestible amino acids in the intestine

[7]NS – not significant; †$P < 0.10$ (tendency); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

where:
μ = general mean
T_i = treatment effect (i = 2)
C_j = cow effect (j = 4)
P_k = period effect (k = 4)
ε_{ijk} = error term

For all statistical evaluations period means were used.

RESULTS

Due to health problems (leg injury) cow No. 3 had to be removed from the fourth period from the evaluation.

Intake of nutrients and milk yield and composition

The intake of DM and other nutrients is presented in Table 2. No significant differences between the treatments were determined ($P > 0.05$). The content of LeuDI (% PDIE) was significantly higher in Leucine compared to Control ($P < 0.001$).

Milk yield and content and yield of milk components are given in Table 3. No significant effect of Leu infusion on milk yield and composition was observed ($P > 0.05$), but the concentration of protein and casein in milk tended to be higher in Leucine than in Control ($P < 0.10$). The yield of milk components was not affected by the treatment ($P > 0.05$).

Blood parameters and plasma AA

The blood parameters are documented in Table 4. Duodenal infusion of Leu did not have any effect on plasma metabolites ($P > 0.05$).

Changes in plasma AA concentrations are shown in Table 5. Duodenal infusion of Leu resulted in an increased level of isoleucine (Ile) in the treatment Leucine compared to Control ($P < 0.01$).

Table 3. Effect of duodenally infused leucine on yield, daily yield of milk components and milk composition

Component	Control[1]		Leucine[2]		P^4
	mean	SEM	mean	SEM	
Milk yield (kg/day)	18.3	2.2	18.8	2.2	NS
ECM (kg/day)[3]	22.2	2.7	22.7	2.7	NS
Composition of milk					
Fat (kg/day)	56.0	1.6	54.9	1.2	NS
Protein (kg/day)	37.4	0.8	38.3	0.8	†
Casein (kg/day)	30.4	0.7	31.3	0.5	†
Lactose (kg/day)	47.0	0.6	46.4	0.4	NS
Urea (mg/100 ml)	187.3	21.3	197.7	39.4	NS
Yield of milk components					
Fat (g/day)	1 025	125	1 035	127	NS
Protein (g/day)	679	73	715	77	NS
Casein (g/day)	552	61	585	65	NS
Lactose (g/day)	864	111	878	108	NS

[1]control group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day) and histidine (10.7 g/day)
[2]experimental group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day), histidine (10.7 g/day) and leucine (19.3 g/day)
[3]energy corrected milk calculated according to Sjaunja et al. (1991)
[4]NS – not significant; †$P < 0.10$ (tendency); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 4. Effect of duodenally infused leucine on blood parameters of lactating dairy cows

Component	Control[1]		Leucine[2]		P^7
	mean	SEM	mean	SEM	
Total protein (g/l)	76.81	0.286	76.84	0.256	NS
Albumin (g/l)	33.26	0.273	33.10	0.244	NS
Urea (mmol/l)	4.66	0.057	4.56	0.051	NS
Glucose (mmol/l)	3.26	0.054	3.26	0.048	NS
AST (U/l)[3]	93.56	1.229	94.50	1.099	NS
GMT (U/l)[4]	26.25	0.728	25.95	0.651	NS
NEFA (mmol/l)[5]	0.68	0.058	0.55	0.052	NS
BHB (mmol/l)[6]	0.74	0.037	0.696	0.033	NS

[1]control group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day) and histidine (10.7 g/day)
[2]experimental group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day), histidine (10.7 g/day) and leucine (19.3 g/day)
[3]aspartate aminotransferase
[4]γ-glutamyltransferase
[5]nonesterified fatty acids
[6]β-hydroxybutyrate
[7]NS – not significant; †$P < 0.10$ (tendency); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Furthermore, concentrations of Leu, cysteine (Cys) and citruline (Cit) tended to be higher and the concentration of tyrosine (Tyr) tended to be lower in Leucine in comparison with Control ($P < 0.10$).

DISCUSSION

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 ($P > 0.05$). Similar responses to Leu supplement have been reported in other studies in which Leu was supplemented either in the ruminally protected form (Křížová et al., 2008) or in the form of abomasal (Huhtanen et al., 2002; Korhonen et al., 2002) or duodenal infusions (Rulquin and Pisulewski, 2006).

In our study no effect of Leu infusion on milk yield and content and yield of milk components was observed ($P > 0.05$) except for the protein and casein concentration that tended to be higher in Leucine than in Control ($P < 0.10$). Our findings are in agreement with minor effects of additional Leu on milk yield and composition reported in other studies (e.g. Kröber et al., 2001; Huhtanen et al., 2002; Korhonen et al., 2002; Křížová et al., 2008). On the other hand, in a dose response study (duodenal infusions of 0, 40, 80 and 120 g/day Leu) Rulquin and Pisulewski (2006) found that milk yield was not affected by the treatments, whereas the content and yield of protein and casein varied quadratically ($P < 0.05$), with a maximum reached by 40 g/day Leu. In contrast, contents and yields of fat and lactose decreased linearly ($P < 0.05$) over the entire range of treatments.

Blood parameters and plasma AA

Blood parameters determined in this experiment were not affected by the treatment ($P > 0.05$). This is in accordance with Křížová et al. (2008). Similarly, Huhtanen et al. (2002) and Rulquin and Pisulewski (2006) reported that the infusion of Leu had no effect on plasma glucose or nonesterified fatty acids.

Table 5. Effect of duodenally infused leucine on plasma concentrations of free amino acids (in mg/l of plasma)

Amino acids (mg/l)	Control[1]		Leucine[2]		P^7
	mean	SEM	mean	SEM	
Arginine	11.0	0.50	10.9	0.82	NS
Histidine	9.1	0.54	9.7	0.63	NS
Ileucine	11.0	1.39	8.3	1.04	**
Leucine	5.9	0.86	7.6	1.26	†
Lysine	10.0	0.75	10.0	0.75	NS
Methionine	9.0	1.37	8.8	1.18	NS
Phealanine	7.9	2.78	6.9	2.78	NS
Threonine	8.9	1.01	8.5	0.92	NS
Valine	16.9	1.70	13.8	1.66	NS
Alanine	16.2	1.45	16.0	1.44	NS
Asparagine	6.1	0.15	6.5	0.59	NS
Aspartic acid	1.7	0.16	1.8	0.33	NS
Citrulline	8.7	0.67	9.7	0.90	†
Cysteine	10.9	1.62	12.8	1.05	†
Glutamine	47.2	5.97	51.2	6.28	NS
Glutamic acid	8.7	1.11	10.0	1.86	NS
Glycine	28.2	4.42	30.3	3.97	NS
Ornithine	6.4	0.44	6.2	0.44	NS
Proline	6.6	0.43	7.2	0.46	NS
Serine	10.6	1.28	11.0	1.33	NS
Tyrosine	5.2	0.47	4.1	0.49	†
EAA[3]	89.7	3.71	84.5	4.15	NS
NEAA[4]	156.6	15.87	166.9	12.02	NS
BCAA[5]	33.8	3.47	29.7	3.33	NS
TAA[6]	246.2	19.20	251.4	12.46	NS

[1]control group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day) and histidine (10.7 g/day)
[2]experimental group supplemented with duodenally infused methionine (12.6 g/day), lysine (20.7 g/day), histidine (10.7 g/day) and leucine (19.3 g/day)
[3]essential amino acids
[4]non-essential amino acids
[5]branched chain amino acids
[6]total amino acids
[7]NS – not significant; †$P < 0.10$ (tendency); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

In the present study, we found that the plasma concentration of Leu tended to be higher ($P < 0.10$) after Leu infusion. This fact conforms with the findings of other studies which also described an elevated plasma concentration of Leu when it was supplied in a rumen protected form (e.g. Křížová et al., 2008) or directly infused into the abomasum (e.g. Huhtanen et al., 2002) or duodenum (Rulquin and Pisulewski, 2006). Similarly, Kröber et al. (2001) confirmed that the variation in supply was clearly reflected in different plasma levels of the respective AA after feeding the experimental diets for 18 days on average. The response in terms of blood plasma concentrations of other AA to Leu supplementation is scarce and inconsistent. Our study showed that duodenal infusion of Leu significantly decreased the concentration of isoleucine (Ile) ($P < 0.01$). Further, there was a tendency to a lower concentration of Tyr and to higher concentrations of Cys and Cit after Leu infusion ($P < 0.10$). This is in disagreement with Kröber et al. (2001), who did not find any significant effects of Leu supply on plasma levels of Lys, Ile, threonine (Thr), valine (Val), His, and phenylalanine (Phe) as the other essential AA except arginine, and the same was ascertained for non-essential AA with the exception of asparagine and Tyr. Huhtanen et al. (2002) found that the only effect of Leu infusion on plasma concentrations of other AA was a tendency of increased Phe levels and decreased Val levels. According to Harper et al. (1984) Leu can decrease plasma concentrations of Met and aromatic AA such as Phe. Although non-significant ($P > 0.1$), decreased concentrations of Val, Met and Phe were also noted in our experiment. This discrepancy in response in plasma AA concentrations to Leu infusions is probably caused by the basal diet that seems to be the main factor in determining AA supply, as documented by various responses to Leu supplementation on grass silage or maize silage based diets (Schwab et al., 1992a,b; Vanhatalo et al., 1999; Varvikko et al., 1999; Rulquin and Pisulewski, 2006; Křížová et al., 2008). Further, according to Kröber et al. (2001) alterations in blood plasma levels of other AA would reflect interactions with the supplemented AA which, in the case of antagonism, could indicate the necessity of supplementing not only the primarily limiting AA but also others.

CONCLUSION

Duodenal infusion of Leu had only a small positive effect on milk protein and casein content in milk ($P < 0.1$). The absence of response to Leu infusion confirms the conclusions of previous studies that with the typical mixed diets the margin between the first-, second- and even third-limiting amino acid (AA) is very small, thus the responses in milk protein synthesis even to the first-limiting AA are generally relatively small.

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