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Responses of cows to abomasal infusion of lysine and methionine at two levels of dietary protein*

S.J. Mabjeesh[1,4], E. Smoler[1], S. M. Abramson[1], I. Bruckental[2], S. Zamwel[1], K. Nanjappan[3] and A. Arieli[1]

[1]Department of Animal Sciences, The Faculty of Agricultural, Food and Environmental Quality Sciences
P.O. Box 12, Rehovot 76100, Israel
[2]Physiology and Nutrition Department,
Institute of Animal Sciences, Agriculture Research Organization, The Volcani Center
Bet Dagan 50250, Israel
[3]Department of Physiology, Veterinary College Research Institute
Namakkal, 637002, India

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ABSTRACT

The response in dairy cows fed high-concentrate diets to abomasal infusion of lysine (Lys) and methionine (Met) at two levels of dietary CP was examined. Four multiparous Israeli Holstein cows (day in milk=180±30, means ± SE) were utilized in a 4×4 Latin square design experiment that included a 2×2 factorial arrangement with 18-d periods. Cows were surgically prepared with abomasal cannulae and catheters implanted in the costoabdominal artery. Two diets were composed to contain high and low crude protein (CP) content (152 vs 132 g/kg dry matter). Abomasal infusion of either water or Lys (38 g/d) plus Met (14 g/d) was performed with each diet. On the last day of the experimental period the metabolism of amino acids (AA) across the mammary gland was monitored. Dry matter intakes and milk and protein yields were not affected by either dietary CP level or postruminal infusion of Lys plus Met and averaged 15.9, 21.4, and 0.694 kg/d, respectively. Milk fat content and yield were not affected by dietary CP concentration, but did increase with abomasal infusion of Lys plus Met (33.3 vs 37.2 g/kg; $P<0.04$, and 0.703 vs 0.762 kg/d; $P<0.05$, respectively). Arterial plasma concentration of Lys and Met increased by 2.4- and 3.5-fold, respectively, when these AA were infused abomasally.

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[4] Corresponding author

Net mammary gland uptake of both AA increased during the abomasal infusion in the low CP diets but not in the high CP diets. The ratio of uptake across the udder of these AA to their corresponding output value in milk, suggested that there was no shortage of supplying Lys and Met from dietary intake. It was also apparent that increased uptake of Met across the udder was accompanied with higher fat secretion in milk.

KEY WORDS: dairy cows, milk production, amino acid, lysine, methionine

INTRODUCTION

Milk and milk protein yields can be influenced by dietary means, including increasing the crude protein (CP) consumed by the cow. Such a response was achieved by increasing dietary CP up to 140 g/kg dry matter (DM) relative to diets deficient in CP (90 g/kg DM); however, further increases in dietary CP content often showed inconsistent responses (Clark and Davis, 1980). Increasing the supply of CP to the small intestine by adding undegradable protein (UDP) supplements increased milk and milk protein yields of cows maintained on protein-rich diets (Forster et al., 1983; McGuffey et al., 1990; Cunningham et al., 1996). Such an increase is unlikely to depend only on the supplementary CP in the diet; the quality and composition of the basic diets are also important. Predicting the quantity and profile of amino acids (AA) reaching the small intestine of dairy cows has been, and still is, equivocal in the current prediction systems of dairy cows (NRC, 1989). Nevertheless, efforts have been made to predict such AA profiles, in order to improve the balance and availability of AA such that requirements for milk protein secretion will be fulfilled (O'Connor et al., 1993). Consequently, limiting essential AA (EAA) are defined for milk protein synthesis under particular nutritional states.

Lys and Met are often considered to be co-limiting AA for milk protein synthesis, particularly when maize-based diets are fed to lactating dairy cows (Schwab et al., 1992a,b). Lys appears to be the first limiting AA when maize-based rations are supplemented with protein sources of maize origin, and Met may be first limiting when all, or most, of the UDP intake is supplemented by legume or animal by-products, or both (Schwab et al., 1976; Rulquin, 1987). Most of the reported responses in milk and milk protein yields to infused Lys and Met have been in cows given diets prepared to be severely limiting in one or both AA (King et al., 1991; Guinard and Rulquin, 1994, 1995; Nichols et al., 1998). Indeed, other workers (Overton et al., 1998; Robinson et al., 1998) failed to observe any productive responses to supplementing diets with these AA where the ration had been better formulated to meet requirements of high-yielding dairy cows. This inconsistency in the productive response of dairy cows given extra EAA directly into the duodenum stresses the importance of the composted basic diets consumed by the animals.

Besides the increasing interest in higher milk protein content, environmental concepts of N disposal in modern dairy farming emphasize the need for more precise information on supplementary protein and EAA in diets of dairy cows in accordance to requirements. Dairy cow rations in Israeli farming systems contain at least 650 to 700 g/kg DM of a concentrate mixture with 300 to 350 g/kg DM of a roughage source which often consists of maize silage, legume or grass hay. Protected Lys and Met, in different commercial forms, are offered to farmers as a promising supplement to improve milk and milk protein production in high-yielding dairy cows. However, there is little information available on the addition of Lys and Met to rations that are high in concentrates and contain more than one source of CP in the diet. Therefore, the objective of this study was to investigate whether Lys and Met are limiting AA for milk and milk protein production in diets typically prepared on commercial farms in Israel. In this study, we combined continuous abomasal infusion of an AA mixture with diets that contained two levels of CP (high and low) relative to production requirements.

MATERIAL AND METHODS

Diets

Two diets with similar ingredients were prepared with two different levels of CP: one sufficient to meet cow requirements (NRC, 1989), referred here as high-CP, and one lower relative to CP allowances (low-CP; Table 1). Maize silage and wheat straw, comprising 330 g/kg DM, served as the main sources of forage for both diets. All feedstuffs were incubated in the rumen using a standard *in situ* method described below. The *in situ* effective degradability values were used in linear programming software (Gavish, Giva't Brenner 60948, Israel) to compose the two diets. Complementary ingredients were calculated using a least-cost linear program (Gavish) to supply (per kg DM) 7.03 MJ of net energy for lactation, neutral detergent fibre (NDF) 330 g/kg, acid detergent fibre (ADF) 214 g, and CP 132 g in the low-CP diet, 255 g/kg CP of this being UDP, CP 152 g in the high-CP diet, with 290 g/kg of it UDP.

Cows and experimental design

Four Israeli-Holstein cows (live body weight = 560±37 kg, day in milk = 180±30, means ± SE) were housed in a metabolic barn, each in individual pens. The cows were fitted with abomasal cannulae and arterial catheters. Cows were milked at 08.00 and 16.00 each day and milk yields were recorded.

TABLE 1

Compositions of formulated diets given to dairy cows in this trial, g/kg dry matter

Item	Diets	
	low crude protein	high crude protein
Components		
whole cottonseed	231	213
cracked maize grain	223	206
barley grain	114	106
maize gluten meal	15	57
rapeseed meal	30	28
sunflower meal	15	14
vitamins and minerals†	44	47
maize silage	253	253
wheat straw	75	75
Chemical composition and nutritive value		
dry matter	647	647
organic matter	902	900
RDOM	503	491
crude protein	132	152
UDP	255	290
neutral detergent fibre	336	330
acid detergent fibre	222	206
net energy for lactation, MJ/kg	7.03	7.03

UDP, rumen undegradable crude protein; RDOM, rumen degradable organic matter, both determined *in situ*

† containing, per kg: vit. A 1.2 g, vit. D 10 mg, vit. E 2.01 g, Mn 11 mg, Zn 12 mg, Fe 4 mg, I_2 240 mg, Co 40 mg, Se 100 mg, Cu 800 mg, $(NH_3)_2SO_4$ 1.4 mg, $MgSO_4$ 1 mg, Ca 180 mg, P 90 mg, NaCl 90 mg

Two weeks prior to the experiment, cows were surgically implanted with an arterial catheter (i.d. 0.35 mm, heparin-coated Teflon, T-10 X HN 6.0-35-90-M-10S-PIG, COOK Inc., Bloomington, IN 47402-0489, USA), under local anaesthesia, into the dorsal aorta, *via* a costoabdominal artery following the method described by Haibel et al. (1989). Local anaesthesia was maintained by infiltrating a transverse line of lidocaine hydrochloride (20 g/l, Xylocaine; Teva Medical Ltd, Ashdod 77100, Israel) into the subcutaneous tissue near the proximal border of each surgical site. A Teflon guide-wire (code 10 X TCMT-35-125-3-BH; COOK) technique was used to help insert the catheter in to the artery. A second temporary polyvinyl chloride catheter was inserted, 1 d before blood sampling, into a subcutaneous abdominal vein. Patency of catheters was maintained by daily flushing with sterile heparinized (300 i.u./ml) 154 mM NaCl.

The cows were allowed to recover from the operations and were then used in a 4×4 Latin square experimental design with 2×2 factorial arrangement. Each ex-

perimental period consisted of 14 d of adaptation and 4 d of sampling and collection. Continuous abomasal infusion of either 5 l of H_2O alone or with Lys plus Met (LM, 38 and 14 g/d) was utilized to form four dietary treatments: high CP-H_2O, high CP-LM, low CP-H_2O, and low CP-LM. The infusions were through a plastic tube, attached to the abomasal cannula, with a peristaltic pump (minipuls 2, GILSON, Villieres le Bel 95400, France).

Experimental protocol

Diets were prepared each morning, machine mixed, and were offered at 11.00 as a total mixed ration. Diets were adjusted to the refusals of the previous day to allow about ~5% refusals. Each morning, refusals were removed from the feeders at 08.00, collected and weighed. During the last 4 d of each experimental period, diets and refusals were sampled daily and stored at -20°C for analysis. Milk samples were taken at each milking from d 15 to 17 of the experimental period and analyzed for fat, protein and lactose contents by infrared procedures (Milkoscan 605; Foss Electric, DK-3400 Hillerød, Denmark) at the Israeli Cattle Breeders Association Milk Recording Laboratory (Industrial Park, Caesarea 38900, Israel).

On d 15 to 17 of each experimental period, eight faecal grab samples were taken at 9-h intervals. The indigestible NDF (INDF) fraction was used to calculated the apparent total-tract digestibilities of nutrients. On the last day (d 18) of each period, cows were machine-milked at 08.00, followed by hand stripping to remove any residual milk, at 09.00, blood samples were collected. Blood samples were drawn concurrently from both the arterial and venous catheters at 60 min intervals into heparinized (20 i.u./ml) syringes over an 8-h period. Cows were allowed to stand for 15 min prior to blood sampling to prevent any analytical bias that might exist as a result of blood flow fluctuations and to ensure steady-state conditions (Zeiler, 1961). Catheters were flushed between samplings with sterile heparinized (50 i.u./ml) physiological saline (154 mM NaCl). Blood samples were immediately placed on ice and plasma was separated by centrifugation at 3000 g for 10 min. Plasma was stored at -20°C until analysis. At the end of sampling period, half udders of cows were milked separately as described earlier. Milk from the relevant half was sampled and stored appropriately for further analysis.

In situ measurements

For *in situ* incubations of feeds and faeces, polyester bags were suspended in the rumen in four replicates for each incubation time. Two dairy cows in mid-lactation with semi-permanent cannulae in the rumen were used for the *in situ* incubation. The cows were maintained on a standard diet with a

roughage:concentrate ratio 35:65 and containing (per kg DM) CP 160 g CP, NDF 340, and 7.11 MJ net energy for lactation.

Dry-milled samples (5 g) were weighed into 12×6 cm polyester bags with a 45 μm mean pore size. Bags were introduced serially into the rumen and incubated for 96, 48, 36, 24, 12, 9, 6, or 3 h. Solubility at 0 h was evaluated by immersing the bags for two consecutive 30-min periods in warm water (39°C). The rumen-incubated polyester bags were removed together, immediately rinsed with cold tap water and washed in a washing machine with cold water for 45 min without spinning.

Chemical analyses and calculations

Feed DM was determined by drying at 105°C for 24 h. Diets, refusals, silage and faeces were dried at 55°C for 48 h. All dried samples were ground to pass through a 2-mm mesh sieve and pooled on a DM basis. The concentrations of organic matter (OM), NDF and CP (6.25×N) were measured for all dried pooled samples. Total CP (6.38×N) in milk on the last day of the experimental period, blood-sampling day, was measured in fresh samples.

The concentration of INDF in the diets (corrected for refusals) and faeces was used to calculate the apparent digestibilities of the dietary components - DM, OM, NDF and CP. For the INDF determination, 5 g samples of dry and pooled faeces, diets, and refusals were weighed into 12×6 cm polyester bags. Each sample was incubated in duplicate in the rumen of two ruminally cannulated dairy cows maintained on a standard diet as described earlier. Bags were removed from the rumen after 168 h and machine-washed with cold water. Dry matter content of residuals was determined as described earlier. Residuals from the same sample were pooled and ground to pass a 1-mm sieve. The NDF fraction was measured according to the method of Van Soest et al. (1991) and was assumed to represent INDF.

Effective ruminal degradabilities of the different feed fractions were calculated according to Ørskov and McDonald (1979) using a fractional passage rate of 6.5%/h. Plasma urea N concentration was determined according to Coulomb and Faverau (1963) on pooled 8 h samples.

Whole milk samples (0.5 ml) and a similar volume of an L-norleucine standard were added and then hydrolyzed in 4 M HCl (3 ml) at 110°C for 18 h for AA analysis.

For plasma free AA analysis, 100 ml of sulphosalicylic acid (50 g/100 g) was added to 900 ml plasma and total protein was precipitated by centrifuging at 15100 *g* for 5 min at 4°C (model 12-24 centrifuge; Mikro, D-78532 Hettich, Germany). AA were separated on a reverse-phase column (Superpher 60 RP 8, 4 μm, LiChro Cartridge 250-4; Merck, D-64271 Darmstadt, Germany) by HPLC after derivatizing with 9-fluorenylmethyloxycarbonyl chloride. AA concentration was measured on pooled 8 h samples.

AA net fractional extraction by the mammary gland was calculated as the arteriovenous (AV) concentration difference, and expressed as percentage of the arterial concentration. Plasma flow was calculated according to the method described by Cant et al. (1993) using the Fick principle and Phe and Tyr as internal markers. Whole milk Phe and Tyr concentrations, corrected for 4% of milk protein AA derived from non-mammary synthesized protein appearing in the milk (Whitney et al., 1976), were used in the model instead of casein-bound and milk-free Phe and Tyr. Net uptake of individual AA by the gland was calculated as the AV concentration difference × mammary plasma flow. Amino acid balance across the mammary gland was calculated as uptake by the mammary gland divided by AA secreted in milk, corrected for milk protein AA derived from non-mammary synthesized protein (Whitney et al., 1976). Plasma flow and balance calculations were performed over the 8 h sampling period for the udder half in which the venous catheter was implanted.

Statistical analyses

Data were analyzed using the General Linear Model procedure of SAS (1985). The 4×4 Latin square design included a 2×2 factorial arrangement of treatments. A single degree of freedom and orthogonal comparisons were used to determine the effects of dietary CP levels (high vs low), abomasal infusion (H_2O vs LM) and their interaction. Means were considered significantly different at $P<0.05$. Results are presented as Lsmeans ± SEM.

RESULTS

Intakes, digestibilities, milk yield and composition

Nutrient intakes and digestibilities are listed in Table 2. Intakes of DM and OM were similar among treatments, averaging 15.9 and 14.4 kg/d, respectively. However, CP intake differed between low-CP and high-CP treatments with CP content in the ration being 2.01 and 2.50 kg/d, respectively. Apparent total-tract digestibility of CP was similar for all treatments, averaging 684 g/kg. However, DM and OM digestibilities were significantly higher in the high-CP diets and were not affected by LM infusion. Digestibility of high-CP diets averaged 700 and 726 g/kg for DM and OM, 630 and 675 g/kg for low-CP diets, respectively. This enhancement in digestibility coefficients of nutrients in ruminant diets is a result of improving the ruminal utilizing efficiency of N (Oldham, 1984; Argyle and Baldwin, 1989).

Milk production was similar among treatments, averaging 21.4 kg/d (Table 3). Likewise, milk protein and lactose contents and yields were similar across dietary

TABLE 2

Daily intake and apparent total-tract digestibility of feed for dairy cows given diets with high or low crude protein (152 and 132 g/kg) coupled with abomasal infusion of 5 l/d containing H_2O or lysine 38 g plus methionine 14 g

Item	Diets				SE	Effect of level, P< †
	low crude protein		high crude protein			
	H_2O	LM	H_2O	LM		crude protein
Intake, kg						
dry matter	15.9	15.2	16.4	16.2	0.69	NS
organic matter	14.6	13.6	14.7	14.5	0.62	NS
crude protein	2.10	2.01	2.49	2.46	0.09	0.01
Digestibility coefficient						
dry matter	0.62	0.64	0.70	0.70	0.03	0.05
organic matter	0.68	0.67	0.73	0.73	0.01	0.05
crude protein	0.71	0.69	0.67	0.67	0.01	NS

LM, Lys plus Met infusion

† NS, not significant; there was no significant effect of LM infusion or interactive effect

TABLE 3

Daily milk production and composition of dairy cows given diets with high or low crude protein (152 and 132 g/kg) coupled with abomasal infusion of 5 l/d containing H_2O or lysine 38 g plus methionine 14 g

Item	Diets				SE	Effect of level, P< †
	low crude protein		high crude protein			
	H_2O	LM	H_2O	LM		LM
Milk, kg/d	21.2	20.9	22.2	21.2	1.02	NS
Milk protein						
g/kg	32.8	33.4	31.9	34.5	0.7	NS
kg/d	0.688	0.687	0.698	0.703	0.04	NS
Milk fat						
g/kg	34.6	38.9	31.9	35.5	0.15	0.04
kg/d	0.727	0.789	0.679	0.734	0.03	0.05
Milk lactose						
g/kg	42.2	42.6	43.0	41.8	0.11	NS
kg/d	0.895	0.894	0.989	0.886	0.06	NS

LM, Lys plus Met infusion

† NS, not significant; there was no significant effect of dietary crude protein level or interactive effect

treatments and averaged 33.2 and 42.4 g/kg, and 0.694 and 0.916 kg/d, respectively. However, fat content was higher (P<0.04) with LM infusion in both high- and low-CP diets, averaging 37.2 and 33.3 g/kg for LM and H_2O infusion, respectively. Consequently, milk fat yield was higher with LM vs H_2O infusion (0.762 vs 0.702 kg/d).

Plasma urea N and arterial AA concentrations, and metabolism across the mammary gland

Plasma urea N concentration was significantly higher in the high-CP vs low-CP diets averaging 16.8 and 12.2 mM, respectively (Table 4). Arterial plasma AA concentrations was similar between treatments and was not affected by either abomasal infusion or dietary CP concentration (Table 4). Met concentration increased by 3.5- and 2.4-fold in the low-CP and high-CP diets with LM infusion, respectively. Lys concentration decreased as a result of higher CP concentration in the diet and increased during LM infusion, being 2.19- and 1.25-fold higher in the low-CP and high-CP treatments, respectively, which resulted in an interactive effect on plasma concentration.

TABLE 4

Concentration of amino acids (μM) and urea N (mM) in the arterial plasma of dairy cows given diets with high or low crude protein (152 and 132 g/kg) coupled with abomasal infusion of 5 l/d containing H_2O or lysine 38 g plus methionine 14 g

Amino acid	Diets				SE	Effect of level, P < †		
	low crude protein		high crude protein					
	H_2O	LM	H_2O	LM		CP	LM	CP×LM
Thr	80.8	75.1	101.3	77.7	7.20	NS	NS	NS
Arg	88.3	98.1	94.3	106.5	7.30	NS	NS	NS
Ala	299.8	297.1	244.2	218.9	67.2	NS	NS	NS
Tyr	58.7	70.2	68.4	60.0	6.56	NS	NS	NS
Pro	77.0	101.0	96.4	90.0	12.8	NS	NS	NS
Met	19.5	67.3	25.1	53.6	5.06	NS	0.001	NS
Val	185.2	221.4	200.7	187.5	24.6	NS	NS	NS
Phe	47.4	63.3	44.2	41.5	9.51	NS	NS	NS
Ile	85.3	95.3	90.2	74.5	13.5	NS	NS	NS
Leu	156.3	183.7	218.7	205.7	22.6	NS	NS	NS
His	38.7	52.9	44.6	35.1	11.8	NS	NS	NS
Lys	86.3	188.9	95.7	120.3	11.7	0.01	0.002	0.02
Plasma urea N	11.7	12.6	17.3	16.3	0.65	0.01	NS	NS

LM, Lys plus Met infusion; CP, protein content in diet

† NS, not significant

Net fractional extractions of plasma AA by the udder are shown in Table 5. Net fractional extraction of non-EAA was similar for all treatments and ranged from 15.1% for Pro to 35.3% for Val. Branched-chain AA exhibited higher fractional extraction rates and averaged 56, 35 and 36% for Leu, Ile and Val, respectively. Arg extraction decreased by 25% with LM infusion. Net fractional extraction of Met and Lys was influenced by LM infusion and that of Lys by dietary CP content, being higher in the low-CP vs the high-CP diets. Abomasal LM infusion considerably decreased the net fractional extraction of plasma Met by ~100% and Lys by 78%.

TABLE 5

Net fractional extraction of amino acids by the half udder of dairy cows given diets with high or low crude protein (152 and 132 g/kg) coupled with abomasal infusion of 5 l/d containing H_2O or lysine 38 g plus methionine 14 g (values are expressed as 100× arteriovenous difference / arterial concentration)

Amino acid	Diets				SE	Effect of level, P < †	
	low crude protein		high crude protein				
	H_2O	LM	H_2O	LM		CP	LM
Thr	36.8	35.2	38.8	35.4	2.58	NS	NS
Arg	57.4	46.3	51.2	40.4	2.93	NS	0.01
Ala	33.7	33.5	40.6	33.5	2.74	NS	NS
Tyr	23.4	18.8	22.0	26.8	2.90	NS	NS
Pro	13.6	13.5	12.9	20.2	3.86	NS	NS
Met	55.1	33.3	51.0	19.5	4.09	NS	0.001
Val	36.7	39.2	32.3	32.4	4.10	NS	NS
Phe	48.8	59.3	58.9	60.9	3.83	NS	NS
Ile	36.0	34.0	34.1	34.1	1.06	NS	NS
Leu	55.3	54.0	54.8	57.7	2.87	NS	NS
His	36.4	54.2	42.5	46.3	9.37	NS	NS
Lys	57.4	34.4	51.2	26.8	3.46	0.002	0.001

LM, Lys plus Met infusion; CP, protein content in diet, there was no interactive effect
† NS, not significant

Net uptakes of all AA were similar among treatments with the exception of Met and Lys (Table 6). Met and Lys had higher uptakes in the low-CP vs high-CP diets (6.8 vs 4.3 mmol/h and 24.8 vs 15.1 mmol/h, respectively). Met uptake was doubled during LM infusion in the low-CP diets whereas it decreased by 19.5% in the high-CP diets. Lys net uptake across the mammary gland was not affected by LM infusion in both CP levels. Plasma flow calculated from the Fick principle was similar across treatments and averaged 358 l/ h and 402 l/ l milk (Table 6).

The AA balance ratio of EAA in general was equal or exceeded unity for all dietary treatments and was not affected by either LM infusion or CP consumption (Table 7). However, Lys balance across the gland was influenced by diet CP con-

TABLE 6

Net uptake (mmol/h) of amino acids and plasma flow (l/h) across the half udder of dairy cows given diets with high or low crude protein (152 and 132 g/kg) coupled with abomasal infusion of 5 l/d containing H_2O or lysine 38 g plus methionine 14 g

Amino acid	Diets				SE	Effect of level, P < †		
	low crude protein		high crude protein					
	H_2O	LM	H_2O	LM		CP	LM	CP×LM
Thr	12.8	11.3	13.9	10.1	1.61	NS	NS	NS
Arg	22.1	19.8	17.6	15.8	2.72	NS	NS	NS
Ala	40.7	44.2	37.8	27.1	10.7	NS	NS	NS
Tyr	5.8	5.4	5.5	5.9	0.61	NS	NS	NS
Pro	4.3	6.6	4.5	6.7	1.81	NS	NS	NS
Met	4.5	9.0	4.8	3.8	0.68	0.01	0.04	0.01
Val	28.5	43.3	24.3	22.3	10.8	NS	NS	NS
Phe	9.4	9.7	9.6	9.3	0.61	NS	NS	NS
Ile	13.1	14.6	11.7	9.4	2.91	NS	NS	NS
Leu	37.4	45.0	44.4	43.2	8.60	NS	NS	NS
His	6.1	10.9	6.5	6.1	2.33	NS	NS	NS
Lys	21.6	27.9	18.3	11.8	2.94	0.02	NS	NS
Plasma flow								
l/h	368	384	352	329	37.6	NS	NS	NS
l/l milk	424	434	381	370	40.1	NS	NS	NS

LM, Lys plus Met infusion; CP, protein content in diet
† NS, not significant

tent, being higher for the low-CP than the high-CP diet. Lysine balance across the gland was affected from the CP level and the LM×CP level interaction. The LM infusion caused an apparent increase of 96 and 23% in the balance ratio of Met and Lys, respectively, in the low-CP diets and a decrease of 19 and 37%, respectively, in the high-CP diet resulting in a significant interaction effect of LM infusion by dietary CP for Met ratio. The balance ratio for Met did differ from unity within all treatments except for that in the LM-low-CP diet where it was significantly higher than unity. However, Lys balance ratio was equal to unity in both the high- and low-CP diets with H_2O treatment and significantly higher than unity in the LM low-CP and lower than unity in the high-CP diets with the LM infusion (Table 7).

DISCUSSION

This study was conducted to examine the effect of abomasal infusion of Lys plus Met on the production of milk and milk components in rations prepared to be

TABLE 7

Balance ratio of amino acid uptake to milk output in udder of dairy cows given diets with high or low crude protein (152 and 132 g/kg) coupled with abomasal infusion of 5 l/d containing H_2O or lysine 38 g plus methionine 14 g

Amino acid	Diets				SE	Effect of level, P < †		
	low crude protein		high crude protein					
	H_2O	LM	H_2O	LM		CP	LM	CP×LM
Thr	1.05	0.91	1.17	0.83	0.15	NS	NS	NS
Arg	2.58‡	2.20‡	2.01‡	1.87‡	0.30	NS	NS	NS
Ala	2.38‡	2.39‡	2.17‡	1.60‡	0.59	NS	NS	NS
Tyr	1.21	1.10	1.14	1.27	0.13	NS	NS	NS
Pro	0.20‡	0.28‡	0.22‡	0.29‡	0.08	NS	NS	NS
Met	1.05	2.06‡	1.08	0.87	0.18	0.02	NS	0.02
Val	2.24‡	3.10‡	1.91‡	1.81‡	0.69	NS	NS	NS
Phe	1.14	1.24	1.16	1.15	0.10	NS	NS	NS
Ile	1.70	1.77	1.47	1.27	0.32	NS	NS	NS
Leu	2.08‡	2.31‡	2.36‡	2.44‡	0.42	NS	NS	NS
His	1.67	2.62	1.60	1.55	0.49	NS	NS	NS
Lys	1.24	1.52‡	1.03	0.65‡	0.18	0.03	NS	NS

LM, Lys plus Met infusion; CP, protein content in diet

† NS, not significant

‡ $P < 0.05$ when $\mu \neq 1$ within treatment

high in concentrates. Two levels of dietary CP were chosen, with a relatively low level of UDP (NRC, 1989), in order to magnify the effect of LM supplementation in diets.

Milk and milk protein production were not affected by abomasal LM infusion despite the fact that plasma Lys and Met concentrations were drastically increased. Production responses to added AA, especially ruminally protected Met plus Lys, have been reported when added to diets of dairy cow in early, mid and late lactation (Xu et al., 1998). This increase was related to milk casein content in all stages of lactation. Milk protein content increased when ruminally protected Lys plus Met were added to dairy cow diets (57 days in milk) based on maize-distillers grains (Nichols et al., 1998). Others reported that Met, unlike Lys, limits milk and milk protein production when added to dairy cows diets (Armentano et al., 1997; Robinson et al., 1998). Those responses were achieved when rations contained 500 g/kg (on a DM basis) roughage and a concentrate mixture based on soyabean proteins, or were composed to be deficient in metabolic allowances of one or both of these EAA. In the current study, however, diets contained a higher content of concentrates.

The availability of precursors, such as AA, from blood plasma may provide a better explanation for the unresponsiveness of cows fed the high-CP diet and re-

ceiving the LM infusion. Plasma AA concentrations were similar for high-CP and low-CP diets except for Lys, which was lower in the high-CP diets. The higher CP content in the high-CP diet was achieved by increasing the content of maize gluten meal, which is highly undegradable in the rumen (NRC, 1989). Maize gluten meal is relatively poor in Lys (NRC, 1996), which would explain the lower plasma Lys concentration. In the low-CP diets, the increase in the plasma concentration of Met and Lys during LM infusion was much greater than that in the high-CP diets, resulting in an interaction effect of dietary CP concentration by LM infusion. This suggests that in the high-CP diets, as a result of greater amounts of absorbed CP, larger plasma pools of AA occurred. Larger plasma fluxes of Phe, Met and Lys were observed when Phe or Lys plus Met were infused intravenously into dairy goats at different stages of lactation (Bequette et al., 1999; Mabjeesh et al., 2000). Despite the apparent evidence of more EAA absorbed in the high-CP diets and during LM infusion, milk production was similar among treatments, raising the question of the metabolic fate of these AA at the mammary gland and whole body levels.

Plasma flow measured in this study might be over estimated because certain amount of Phe and Tyr (up to 11% for Phe and 25% for Tyr; Bequette et al., 1999) might be taken up across the mammary gland as plasma-circulating peptides. In order to compare uptake of EAA across the mammary gland, however, it was assumed that the experimental accuracy was similar for all treatment. The plasma flow and the ratio of plasma flow per milk volume measured in the present study compare well with other reported values measured by dye dilution method (Metcalf et al., 1991) or the Fick principle with Phe+Tyr (Mabjeesh et al., 1999). Overall, the uptake and balance ratio of EAA across the mammary gland were adequate for milk protein secretion in both the high-CP and low-CP diets. Net fractional extraction of Arg was decreased during the LM infusion in both the low-CP and high-CP diets, which suggests a transporter competition at the level of the mammary gland. Both Lys and Arg share the same specific transport system (Y+) in bovine mammary gland and have been found to be strong competitors of each other's uptake into bovine mammary tissue (Baumrucker, 1984). Net fractional extraction of Met decreased upon LM infusion in the low-CP diet and net uptake was almost doubled, resulting in a higher balance ratio without altering protein secretion, but increasing fat production and content in milk. Met could supply S for the *de novo* mammary synthesis of Cys *via* a trans-sulphuration pathway. This contribution, however, might be small in the mammary gland (Lee et al., 1997). Met is essential for *de novo* synthesis of short-chain (C_4 to C_{14}) fatty acids in the mammary gland (Varvikko et al., 1999). Hence higher milk fat secretion in the present study might occur at the level of mammary tissue *via* this metabolic route in the low-CP diet when LM was infused. However, in the high-CP diet the net uptake of Met at the mammary gland was decreased and still milk fat secretion was elevated. It has been thought that shortage of Met may limit the formation of apoprotein associated with lipoproteins,

resulting in reducing the synthesis of lipoproteins and subsequent transport of triglycerides from the liver toward peripheral tissues, particularly the mammary gland (Pullen et al., 1989). In the current study the fatty acid profile secreted in milk was not measured, hence the precise pathway in which Met elevated milk fat secretion could not be clarified in the high- and low-CP diets. However, it may be speculated that milk fat secretion was increased as a result of increased supply to both the mammary gland (e.g., in the low-CP LM diet) and liver (e.g., high-CP LM diet). Similar results were observed when plasma Met concentration was increased during abomasal infusion of LM or LM plus His in dairy cows fed grass-silage-based diets (Vanhatalo et al., 1999; Varvikko et al., 1999). Lys uptake and balance ratios were higher in the low-CP compared to high-CP diets regardless of the LM infusion without any alteration in protein secretion. The balance ratios across the gland in the high-CP diets during LM infusion decreased for Met and Lys, however the ratio was similar to unity for Met and less than unity for Lys. This result for Lys might be explained by several reasons. In the current study, uptake was calculated from free plasma AA concentrations alone and the peptide-bound AA that might contribute to the mammary tissue metabolism was not considered. Up to 16% of Lys secretion in milk was contributed from plasma peptides in lactating goats at different stages of lactation (Bequette et al., 1996; Mabjeesh et al., 2000). Interestingly, this fraction was not affected by the availability of free Lys in plasma. Hence, if this fraction is considered in the current study then Lys supply to milk protein synthesis would not limit milk production. A second point that might affect the balance ratio measurements is the fact that plasma concentration and not whole blood values were used. During LM infusion, erythrocytes might serve as a carrier for AA, supplying Lys to the mammary gland. Indeed, Hanigan et al. (1991) showed, in dairy cows, that the uptake of AA (including Met and Lys) by the mammary gland differs between whole blood and plasma pools. Erythrocyte and plasma exchanges were also found to occur and plasma uptake therefore may inadequately represents whole blood uptake. A third point that should be borne in mind that these balance measurements across the mammary gland were done under the assumption that AA are taken up by the gland and contribute to a biosynthetic pathway (anabolism). The oxidative pathway was activated in the mammary gland of goats when surplus EAA (e.g., Leu, Lys), relative to requirements, were supplied intravenously (Bequette et al., 1996; Mabjeesh et al., 2000). Up to 31% of the Lys taken up by the gland during LM infusion was oxidized (Mabjeesh et al., 2000). This mechanism may be a disposal route for excess AA taken up by the gland. If this pathway were to be taken as general for other EAA, the balance ratio would fall beyond unity and these measurements would have to be reconsidered with respect to classifying limiting EAA for protein synthesis. The exact metabolic fate of the extra Lys and Met taken up by the gland remains to be clarified; nonetheless, it is apparent that on excess of these EAA might be disposed of *via* the oxidative path-

way, at the level of either the mammary or liver tissue (Mabjeesh et al., 1999). Indeed, plasma urea N concentrations were higher for high-CP diets suggesting that there was increased deamination and oxidation of dietary AA.

CONCLUSIONS

In this study we show that diets containing high concentrations of grains are adequate to supply AA to the mammary gland of dairy cows and fulfill metabolic requirements. Abomasal infusion of Lys plus Met caused a dramatic increase in the blood plasma concentration of these AA without any effect on milk protein secretion. It was suggested that an increased supply of Met might cause higher fat secretion by the mammary gland. It was concluded that excess ingested AA are catabolized in the high-CP diets and upon LM infusion.

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STRESZCZENIE

Reakcja krów otrzymujących dawki o różnej zawartości białka na infuzję lizyny i metioniny do trawieńca

Doświadczenie przeprowadzono na 4 krowach - wieloródkach, holsztynach izraelskich (w 180±30 dniu laktacji), w układzie kwadratu łacińskiego 4 x 4, zawierającym układ 2 x 2 czynnikowy z 18-to dniowymi okresami. Krowy miały założone kaniule do trawieńca oraz katetery do tętnicy żebrowo-brzusznej. Skarmiano dwie dawki o wysokiej lub niskiej zawartości białka ogólnego (152 vs 132 g/kg s.m.). Przy podawaniu każdej z dawek infundowano do trawieńca wodę lub lizynę (Lys) 38 g/dzień z metioniną (Met) 14 g/dzień. W ostatnim dniu okresu doświadczalnego oznaczano metabolizm aminokwasów (aa) w gruczole mlekowym.

Pobranie s.m. paszy oraz wydajność mleka i białka w mleku nie zależały ani od zawartości białka w dawce ani od infuzji Lys+Met i wynosiły średnio 15,9; 21,4 i 0,694 kg/dzień, odpowiednio. Podobnie, zawartość i wydajność tłuszczu mleka nie zależały od ilości białka w dawce, lecz wzrastały po infuzji Lys+Met (odpowiednio, 33,3 vs 37,2 g/kg; $P<0,04$, oraz 0,703 vs 0,762 kg/dzień; $P<0,05$). Stężenie Lys i Met w plazmie krwi tętniczej wzrosło 2,4- i 3,5-cio krotnie, odpowiednio po infuzji tych aa do trawieńca. Pobranie netto obydwóch aa przez gruczoł mlekowy zwiększało się podczas ich infuzji do trawieńca, gdy krowy otrzymywały dawkę o niskiej zawartości białka, czego nie stwierdzono przy podawaniu dawki wysokobiałkowej. Stosunek pobrania tych aa przez gruczoł mlekowy do odpowiadających im wartościom wydalanym w mleku sugeruje, że pobierane dawki nie były niedoborowe pod względem zawartości Lys i Met. Wydaje się także, że zwiększeniu pobrania Met przez gruczoł mlekowy towarzyszyło zwiększenie produkcji tłuszczu mleka.