

Response of Dairy Cows Fed Grass Silage Diets to Abomasal Infusions of Histidine Alone or in Combinations with Methionine and Lysine

A. VANHATALO, P. HUHTANEN, V. TOIVONEN,
and T. VARVIKKO

Animal Production Research, Agricultural Research Centre of Finland,
FIN-31600 Jokioinen, Finland

ABSTRACT

The response of dairy cows fed grass silage-based diets to the abomasal infusion of water (control) or 6.5 g of His alone or in combination with either 6.0 g of Met or 19.0 g of Lys or both was studied in an incomplete 4×5 Latin square experiment with 14-d periods. Each cow received a basal diet of 8 kg/d of cereal concentrate [12.1% crude protein (CP)] and free access to grass silage (14.1% CP) ensiled with an acid-based additive. Postruminal infusions increased arterial plasma concentrations of the amino acids (AA) infused, but compared with control, only the infusion of His (18 vs. 57 $\mu\text{mol/L}$) was associated with significant increases in milk and milk protein yields. Infusions of His did not affect dry matter intake of grass silage, rumen fermentation, or diet digestibility. Milk protein content was unchanged by treatments, but His infusions decreased lactose and fat contents. The combinations of AA did not produce any further responses compared with His alone. However, milk protein percentage was slightly higher, and milk fat percentage tended to be higher when Met rather than Lys was infused with His. We concluded that His is the first-limiting AA when grass silage-based diets are supplemented with cereal concentrates, while neither Met nor Lys are the second-limiting AA with grass silage feeding.

(Key words: dairy cows, histidine, infusions, grass silage)

Abbreviation key: AV = arteriovenous, BCAA = branched chain AA, EAA = essential AA, ECM = energy corrected milk, HI = histidine infusion, HL = histidine and lysine, HM = histidine and methionine, HML = histidine, methionine, and lysine, NEAA = nonessential AA, TAA = total AA.

INTRODUCTION

There is increasing interest in optimizing the delivery of AA to the duodenum to accurately meet the AA re-

quirements of the ruminant animal. Such delivery is of interest not only to increase the efficiency of conversion of dietary N into milk protein, thereby reducing feed costs and improving the composition of milk products, but also to address environmental concerns in dairy cow feeding to suppress N excretion in manure. Optimization of AA supply can be done, e.g., by increasing diet CP content with appropriate protein supplements, reducing rumen degradability of feed CP provided that rumen-degradable CP is sufficient in the diet, increasing production of microbial protein in the rumen, or using rumen-protected AA. In principle, the last mentioned mean is the most effective approach to optimize AA supply because AA specifically needed can be supplemented without increasing the content of AA not needed in the diet; i.e., the animal does not need to excrete surplus N from supplemental AA. Most of the commercial products of rumen-protected AA are based on Met or Lys or both because they have often been implied as first-limiting AA in corn silage but also in grass silage-based diets (27, 31).

In our previous experiments (35), infusing linearly increasing amounts of Met or Lys into the abomasum of cows fed grass silage-based diets had no significant effects on milk or milk protein yield. Instead, milk fat content and yield increased significantly in response to Met, and milk urea content increased in response to Lys. These results suggested that neither Met nor Lys is the first-limiting AA on grass silage-based diets. However, these AA were not infused in the presence of each other. Therefore, possible co-limitation of these AA is open for discussion. The collected data were, however, further used to calculate output to input ratios of essential AA (EAA). These theoretical calculations suggested His to be the most probable candidate for the first-limiting AA, and Met and Lys were in the group of AA potentially becoming limiting after His. The objective of the present experiment was to investigate the lactational and metabolic responses of cows to the abomasal infusion of His alone or in combination with either Met or Lys or both.

Received November 9, 1998.
Accepted April 26, 1999.

MATERIALS AND METHODS

Cows and Feeding

Four Finnish Ayrshire cows (90 to 120 DIM) at third or fourth lactation were used in the experiment. The mean live weight of cows was 509 ± 72 kg at the beginning of the experiment, and 542 ± 64 kg at the end of the experiment. The cows were fitted with rumen cannulas, and infusion catheters were introduced into their abomasums as described earlier (35). The cows were kept in individual stanchions and milked twice daily, at 0700 and 1700 h.

The cows were given free access to grass silage so thatorts were not more than 10% of the daily portion. Grass silage was made of primary growth of timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), and red clover (*Trifolium pratense*). It was cut and ensiled with an acid based additive as described previously (35). The concentrate mixture given to the animals twice daily at a rate of 8 kg/d as fed consisted of barley (41%), oats (41%), molassed sugar beet pulp (15%), commercial mineral mixture (2.7%), and salt (0.3%). The mineral mixture contained 16% Ca, 6.4% P, 9.0% Na, 8.0% Mg, 150,000 IU of vitamin A/kg, 100,000 IU of vitamin D/kg, and 500 mg of vitamin E/kg. The mineral mixture also contained (per kilogram) 500 mg of Cu, 10 mg of Se, 3000 mg of Zn, 8 mg of Mo, 1000 mg of Mn, and 100 mg of I. Animals were fed at 0600 and 1800 h. Water was freely available.

Experimental Design and Treatments

The experimental design used was a 4×5 incomplete Latin Square with 14-d periods, of which the latter 7 d formed a collection period. The five treatments allocated to the four cows were continuous abomasal infusions of AA dissolved in 6 L of water/d as follows: 1) only water was infused (control); 2) 6.5 g/d of His (**HI**); 3) 6.5 g/d of His and 6.0 g/d of Met (**HM**); 4) 6.5 g/d of His and 19.0 g/d of Lys (**HL**), and 5) 6.5 g/d of His, 6.0 g/d of Met, and 19.0 g/d of Lys (**HML**). The experimental solutions were infused with a peristaltic pump as described previously (35).

Sampling, Recordings, and Chemical Analyses

Feed intake and milk yield of cows were recorded daily throughout the experiment. However, data from collection period only was used in statistical analyses. Feed, milk, blood, and rumen fluid sampling have been described earlier (35). Briefly, blood samples from mammary and tail veins and ruminal fluid samples were taken on the last day of each experimental period before the morning feeding and two blood samples and three

ruminal fluid samples were taken at 3-h intervals thereafter.

Both blood and ruminal fluid samples were reduced to period means for each cow before analysis. To determine the total digestibility of experimental diets, a 5-d fecal grab sampling with acid-insoluble ash as an internal marker, was carried out (34).

Feeds and feces for chemical composition and ruminal fluid for VFA were analyzed as previously described (35). Silage in vitro cellulase digestibility was analyzed according to Friedel (13). Plasma glucose and NEFA were measured colorimetrically with commercial GOD-Perid and NEFA-C reagent kits, respectively. Plasma AA were analyzed with Biochrom 20 AA analyzer. Details of the procedures as well as analyses on milk for content of protein, fat, lactose, and urea are described in our previous paper (35).

Calculations and Statistical Analyses

Mammary extraction of blood metabolites and AA were calculated as arteriovenous (**AV**) difference divided by arterial concentration. Mammary plasma flow was estimated in reference to Phe and Tyr output in milk protein by an application of the Fick principle as described by Cant et al. (5) with the exception that the free milk Phe and Tyr values were neglected. Uptake of blood metabolites and AA by the mammary gland were then calculated as AV difference \times mammary plasma flow.

Data were analyzed by the analysis of variance for Latin square: cow (df 3), period (df 4), treatment (df 4), and the residual effects (df 8) were the sources of variation. The general linear models procedure of SAS (28) was used. Treatment differences were further partitioned into a single degree of freedom comparisons by orthogonal contrasts: 1) control versus HI + HM + HL + HML; 2) HI versus HM + HL + HML; 3) HM+HL versus HML, and 4) HM versus HL. Results were expressed as least squares means and are so presented throughout the text.

RESULTS

Feed Intake, Rumen Fermentation, and Diet Digestibility

The chemical composition of feeds given to the animals is presented in Table 1. Grass silage was restrictively fermented and of reasonably good fermentation quality. However, in vitro digestibility and N content of the silage were rather low.

Data on feed intake and rumen fermentation are given in Table 2. Grass silage DMI was not affected by abomasal His infusions ($P > 0.05$), but total DMI was

TABLE 1. Chemical composition of feedstuffs.

	Grass silage	Concentrate ¹
pH	4.25	
DM, g/kg of feed	209	898
	(g/kg of DM)	
Ash	75	65
Total N	22.6	19.3
NDF	605	292
Water-soluble carbohydrates	23.2	
Lactic acid	37.5	
Acetic acid	22.4	
Butyric acid	2.9	
Ammonia N, g/kg of N	63	
Soluble N, g/kg of N	506	
In vitro cellulase digestibility, g/kg of OM	661	

¹Concentrate was a mix of barley (41%), oats (41%), sugar beet pulp (15%), and minerals (3%).

slightly higher ($P = 0.05$) with His infusions as compared with the control. Rumen fermentation parameters were not affected by His infusions. The lower ($P = 0.008$) proportion of acetic acid and higher ($P = 0.02$) proportion of valeric acid in VFA with the His alone treatment versus His given in combination with other AA needs to be explained. However, these differences, although statistically significant, were extremely small, less than 1 percentage unit, and thus are proba-

bly without any biological significance. Digestibility coefficients of the diets (on average 70.8% for OM, 68.9% for N, and 60.6% for NDF) were not significantly affected by the treatments.

Milk Yield and Composition

Infusions of His increased milk yield ($P = 0.01$), but energy corrected milk (ECM) yield was not affected ($P > 0.05$) (Table 3). Neither was protein content of milk, but lactose and fat contents decreased ($P \leq 0.03$). However, milk protein content was higher ($P = 0.02$), and fat content tended to be higher ($P = 0.08$) when His was combined with Met rather than Lys. The His infusions did not affect lactose and fat yields but increased milk protein yield ($P = 0.007$), and, consequently, raised the protein to fat ratio of milk ($P = 0.007$). Infusions of His had no influence on milk urea content. However, milk urea tended ($P = 0.07$) to be lower when all three AA were infused together as compared with the combinations of two AA.

Plasma Metabolites and AA

Arterial concentrations, calculated AV differences, extractions, and uptakes of glucose and NEFA by the

TABLE 2. Least squares means for feed intake and rumen fermentation parameters.

	AA Infused postruminally ¹					SEM	Contrast (P) ²			
	Control	HI	HM	HL	HML		1	2	3	4
DMI, kg/d										
Silage	8.9	9.1	9.1	9.0	9.2	0.08	†	NS ³	†	NS
Concentrate	7.2	7.2	7.2	7.2	7.2					
Total	16.1	16.3	16.3	16.2	16.4	0.08	*	NS	†	NS
Rumen fermentation										
pH	6.28	6.35	6.47	6.40	6.39	0.060	NS	NS	NS	NS
Ammonia N, mmol/L	5.68	5.50	5.26	5.06	5.37	0.423	NS	NS	NS	NS
Total VFA, mmol/L	115	112	109	111	109	2.3	NS	NS	NS	NS
Molar proportion of VFA, mmol/mol										
Acetic	700	694	699	702	705	1.9	NS	**	†	NS
Propionic	144	147	144	143	142	1.8	NS	†	NS	NS
Butyric	113	117	116	113	111	1.9	NS	NS	NS	NS
Isobutyric	9.2	9.2	9.1	9.1	8.9	0.08	NS	NS	NS	NS
Valeric	11.6	11.9	11.1	11.4	11.5	0.15	NS	*	NS	NS
Isovaleric	13.8	13.5	13.7	13.5	13.4	0.40	NS	NS	NS	NS
Caproic	7.5	7.5	7.0	7.6	7.9	0.33	NS	NS	NS	NS

¹Control = No infusion of AA postruminally; HI = 6.5 g of His/d infused postruminally; HM = 6.5 g of His/d and 6 g of Met/d infused postruminally; HL = 6.5 g of His/d, and 19 g of Lys/d infused postruminally; and HML = 6.5 g of His/d, 6.0 g of Met/d, and 19 g of Lys/d infused postruminally.

²Contrasts: 1 = Control versus all other infusions, 2 = HI versus HM, HL and HML, 3 = HM and HL versus HML, and 4 = HM versus HL.

³ $P > 0.10$.

† $P \leq 0.10$.

* $P \leq 0.05$.

** $P \leq 0.01$.

TABLE 3. Least squares means for milk yield and milk components.

	AA Infused postruminally ¹					SEM	Contrast (<i>P</i>) ²			
	Control	HI	HM	HL	HML		1	2	3	4
Milk yield, kg/d	22.9	23.6	23.7	24.2	23.7	0.25	**	NS ³	NS	NS
ECM ⁴ , kg/d	23.3	23.4	23.6	23.4	23.8	0.21	NS	NS	NS	NS
Milk composition, g/kg										
Protein	30.4	30.6	31.0	29.8	31.1	0.27	NS	NS	†	*
Lactose	47.7	46.9	46.9	47.3	47.3	0.18	*	NS	NS	†
Fat	43.4	41.1	42.1	40.3	42.5	0.61	*	NS	NS	†
Yield, g/d										
Protein	695	721	728	717	729	6.9	**	NS	NS	NS
Lactose	1094	1107	1110	1143	1115	11.8	†	NS	NS	†
Fat	988	968	986	961	994	12.3	NS	NS	NS	NS
Protein:fat	0.71	0.75	0.76	0.75	0.74	0.010	**	NS	NS	NS
Urea, mg/L	179	177	190	178	160	8.93	NS	NS	†	NS

¹Control = No infusion of AA postruminally; HI = 6.5 g of His/d infused postruminally; HM = 6.5 g of His/d and 6 g of Met/d infused postruminally; HL = 6.5 g of His/d and 19 g of Lys/d infused postruminally; and HML = 6.5 g of His/d, 6.0 g of Met/d, and 19 g of Lys/d infused postruminally.

²Contrasts: 1 = Control versus all other infusions, 2 = HI versus HM, HL, and HML, 3 = HM and HL versus HML, and 4 = HM versus HL.

³*P* > 0.10.

⁴ECM, Energy-corrected milk; calculated according to Sjaunja et al. (32).

†*P* ≤ 0.10.

**P* ≤ 0.05.

***P* ≤ 0.01.

mammary gland are given in Table 4. Infusions of His had no influence on these arterial metabolites.

Arterial plasma AA and urea concentrations, AV differences, and extractions by the mammary gland are given in Tables 5, 6 and 7, respectively. Plasma urea concentrations were not significantly affected by the treatments. Infusions of His did not affect plasma branched-chain AA (**BCAA**), EAA, nonessential AA

(**NEAA**), or total AA (**TAA**) concentrations as compared with control, but increased His (*P* < 0.001) and decreased Leu, Phe, Thr, Gly, Ser, and Tyr (*P* ≤ 0.07 at least). Concentrations of BCAA, EAA, and TAA in agreement with those of many single AA, e.g., Lys, Met, Phe, Trp, and Tau, were lower (*P* ≤ 0.07) when His was infused alone rather than in combination with other AA. The same was true (*P* ≤ 0.08) for the plasma concen-

TABLE 4. Least squares means for plasma metabolites.

	AA Infused postruminally ¹					SEM	Contrast (<i>P</i>) ²			
	Control	HI	HM	HL	HML		1	2	3	4
Glucose, mmol/L										
Arterial	3.24	3.15	3.24	3.16	3.15	0.065	NS ³	NS	NS	NS
AV ⁴ Difference	0.77	0.77	0.79	0.80	0.84	0.065	NS	NS	NS	NS
Extraction, %	23.9	24.8	24.9	25.7	27.4	2.14	NS	NS	NS	NS
Uptake, g/kg of milk	72.2	76.9	61.3	68.9	69.7	7.42	NS	NS	NS	NS
NEFA, μmol/L										
Arterial	96.2	84.8	96.5	105.8	105.4	8.11	NS	†	NS	NS
AV Difference	-23.8	-23.3	-8.6	-10.5	-19.2	5.91	NS	NS	NS	NS
Extraction, %	-27.9	-31.6	-12.0	-12.9	-24.3	7.13	NS	NS	NS	NS
Uptake, g/kg of milk	-3.40	-3.30	-0.93	-1.64	-2.49	0.806	NS	NS	NS	NS

¹Control = No infusion of AA postruminally; HI = 6.5 g/d of His infused postruminally; HM = 6.5 g of His/d and 6 g of Met/d infused postruminally; HL = 6.5 g of His/d and 19 g of Lys/d infused postruminally; and HML = 6.5 g of His/d, 6.0 g of Met/d, and 19 g of Lys/d infused postruminally.

²Contrasts: 1 = Control versus all other infusions, 2 = HI versus HM, HL, and HML, 3 = HM and HL versus HML, and 4 = HM versus HL.

³*P* > 0.10.

⁴Arteriovenous.

†*P* ≤ 0.10.

trations of AA involved in urea cycle in the liver (Arg, Cit, Orn). Plasma His, Thr, and NEAA due to Asp, Glu, and Gly concentrations were higher ($P < 0.01$) on HM and HL treatments than on HML treatment. Plasma Met concentration was higher ($P < 0.001$) and Lys concentration lower ($P = 0.004$) on HM than on HL treatment.

Infusions of His did not affect AV for any single AA (Table 6), except for increases in Thr ($P = 0.08$) and Ser ($P = 0.009$). Arteriovenous differences of Ile, Leu, Lys, Phe, Thr, Ser, and Tau were larger ($P \leq 0.07$) on treatments when His was infused in combination with Met and Lys rather than alone. The AV difference of NEAA and Asp ($P \leq 0.04$) were higher on HM and HL treatments than on HML treatment, and that of Asn was higher ($P = 0.06$) on HM than on HL treatment.

The mammary gland extraction rate of His was reduced ($P < 0.001$), and those of Leu, Phe, Thr, Asn, Ser, and Tyr increased ($P \leq 0.03$) in response to His infusions (Table 7). Extraction rates of His, Thr, Ser, and Tau were lower ($P \leq 0.03$) when His was infused alone rather than together with other AA. Extractions of Asp and NEAA were higher ($P \leq 0.07$) on HM and HL than on HML treatment. Extractions of Lys, Asn, and BCAA were higher ($P \leq 0.03$), but that of Met was lower ($P = 0.08$) on HM than on HL treatment.

Calculated mammary plasma flow rates for the control, HI, HM, HL, and HML treatments in terms of liters per day (11,915, 13,218, 10,408, 12,137, 11,348; SEM 975.6) or liters per kilogram of milk (528, 560, 445, 509, 478, SEM 41.5) were not affected by the abomasal AA infusions. However, both of these estimates

TABLE 5. Least squares means for arterial plasma AA and urea concentration.

AA ³	AA Infused postruminally ($\mu\text{mol/L}$) ¹					SEM	Contrast (P) ²			
	Control	HI	HM	HL	HML		1	2	3	4
Arg	65	66	73	82	76	4.0	†	*	NS ⁴	NS
His	18	53	57	47	38	4.5	***	NS	*	NS
Ile	124	112	133	153	147	7.1	NS	**	NS	†
Leu	102	78	92	94	91	5.6	†	†	NS	NS
Lys	82	77	90	120	115	5.2	**	***	NS	***
Met	21	17	33	18	30	1.4	*	***	*	***
Phe	49	37	43	42	43	2.2	*	†	NS	NS
Thr	104	91	98	96	85	4.0	*	NS	*	NS
Trp	25	24	32	29	27	2.4	NS	†	NS	NS
Val	201	168	186	205	189	9.2	NS	*	NS	NS
Ala	236	218	245	239	239	8.6	NS	*	NS	NS
Asn	52	47	55	55	51	2.8	NS	†	NS	NS
Asp	11	10	11	12	10	0.6	NS	NS	*	NS
Cit	69	66	74	71	74	3.2	NS	†	NS	NS
Cys	21	26	22	24	21	4.6	NS	NS	NS	NS
Gln	155	147	157	148	139	8.1	NS	NS	NS	NS
Glu	151	162	181	175	153	8.0	NS	NS	*	NS
Gly	333	308	313	313	267	12.0	*	NS	**	NS
Orn	41	40	45	46	46	2.0	NS	*	NS	NS
Pro	82	76	80	84	77	4.1	NS	NS	NS	NS
Ser	110	95	97	103	88	4.8	*	NS	†	NS
Tau	37	32	39	36	40	1.3	NS	***	NS	NS
Tyr	48	35	42	43	40	2.6	*	†	NS	NS
BCAA	427	358	410	452	427	20.4	NS	*	NS	NS
EAA	790	722	837	886	840	37.0	NS	**	NS	NS
NEAA	1198	1124	1204	1197	1087	29.1	NS	NS	**	NS
TAA	1988	1846	2041	2084	1927	59.7	NS	*	†	NS
Urea	3288	3273	3446	3272	2867	261.7	NS	NS	NS	NS

¹Control = No infusion of AA postruminally; HI = 6.5 g of His/d infused postruminally; HM = 6.5 g of His/d and 6 g of Met/d infused postruminally; HL = 6.5 g of His/d and 19 g of Lys/d infused postruminally; and HML = 6.5 g of His/d, 6.0 g of Met/d, and 19 g of Lys/d infused postruminally.

²Contrasts: 1 = Control versus all other infusions, 2 = HI versus HM, HL, and HML, 3 = HM and HL versus HML, and 4 = HM versus HL.

³BCAA = Branched-chain AA (Ile, Leu, and Val), EAA = essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val), NEAA = nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr), and TAA = EAA + NEAA.

⁴ $P > 0.10$.

† $P \leq 0.10$.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

TABLE 6. Least squares means for mammary arteriovenous difference of plasma AA.

AA ³	AA Infused postruminally ($\mu\text{mol/L}$) ¹					SEM	Contrast (<i>P</i>) ²			
	Control	HI	HM	HL	HML		1	2	3	4
Arg	30.5	37.9	39.1	40.2	33.0	4.61	NS ⁴	NS	NS	NS
His	11.2	10.1	13.4	16.1	11.7	1.76	NS	NS	NS	NS
Ile	38.2	31.1	43.9	38.9	36.6	3.49	NS	†	NS	NS
Leu	49.6	45.7	56.9	53.8	54.3	3.48	NS	*	NS	NS
Lys	44.4	42.3	49.2	55.9	55.3	4.31	NS	†	NS	NS
Met	10.2	10.3	12.0	11.7	12.8	0.98	NS	NS	NS	NS
Phe	19.5	17.3	22.3	19.0	20.5	1.37	NS	†	NS	NS
Thr	24.4	24.8	32.9	30.4	28.8	2.17	†	*	NS	NS
Trp	1.3	3.0	3.3	3.4	4.5	1.45	NS	NS	NS	NS
Val	47.7	41.6	55.0	50.9	49.4	5.38	NS	NS	NS	NS
Ala	20.4	20.0	26.8	27.1	15.3	7.18	NS	NS	NS	NS
Asn	12.2	12.9	18.8	14.1	14.8	1.52	NS	NS	NS	†
Asp	4.1	3.9	4.9	5.4	3.6	0.43	NS	NS	*	NS
Cit	4.0	-0.6	4.6	3.8	2.1	1.67	NS	†	NS	NS
Cys	1.3	1.0	1.4	5.1	1.0	0.48	NS	NS	NS	NS
Gln	30.6	30.0	36.9	35.2	22.3	5.59	NS	NS	†	NS
Glu	9.5	4.0	18.9	22.2	10.1	7.42	NS	NS	NS	NS
Gly	9.5	4.0	18.8	22.2	10.1	7.42	NS	NS	NS	NS
Orn	19.0	17.5	20.1	19.2	19.1	1.11	NS	NS	NS	NS
Pro	14.1	4.9	13.9	8.4	4.6	3.82	NS	NS	NS	NS
Ser	6.1	10.7	24.0	18.5	15.2	2.84	**	*	NS	NS
Tau	2.3	-1.8	3.0	4.3	2.2	1.79	NS	*	NS	NS
Tyr	16.9	16.1	21.6	19.5	19.8	2.10	NS	NS	NS	NS
BCAA	135.5	118.4	155.7	143.4	140.1	11.86	NS	†	NS	NS
EAA	277.1	264.1	327.8	320.0	306.7	26.19	NS	NS	NS	NS
NEAA	176.8	167.8	237.9	229.4	166.9	21.43	NS	NS	*	NS
TAA	453.9	431.9	565.7	549.3	473.6	41.53	NS	†	NS	NS

¹Control = No infusion of AA postruminally; HI = 6.5 g of His/d infused postruminally; HM = 6.5 g of His/d and 6 g of Met/d infused postruminally; HL = 6.5 g of His/d and 19 g of Lys/d infused postruminally; and HML = 6.5 g of His/d, 6.0 g of Met/d, and 19 g of Lys/d infused postruminally.

²Contrasts: 1 = Control versus all other infusions, 2 = HI versus HM, HL, and HML, 3 = HM and HL versus HML, and 4 = HM versus HL.

³BCAA = Branched-chain AA (Ile, Leu, and Val), EAA = essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val), NEAA = nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr), and TAA = EAA + NEAA.

⁴ $P > 0.10$.

† $P \leq 0.10$.

* $P \leq 0.05$.

** $P \leq 0.01$.

tended ($P \leq 0.13$) to decrease when His was infused in combinations with Met or Lys or both rather than alone. Uptakes (grams per kilogram of milk) of EAA, NEAA, or TAA by the mammary gland were generally not affected by the AA infusions (Table 8). However, uptake of Ser was higher ($P = 0.01$) on His containing treatments as compared with control. Uptake of Arg was higher ($P = 0.04$) and those of Cit and Tau lower ($P \leq 0.06$) when His was infused alone rather than together with other AA. Uptake of NEAA and Asp tended to be ($P \leq 0.11$) higher on HM and HL treatments than on HML. The uptake of Lys was higher ($P = 0.06$) on HL than on HM treatment.

DISCUSSION

Characteristics of the Basal Diet

As in our previous experiments (35), AA infusions had generally no effect on rumen fermentation or diet

digestibility, indicating that infusions had no effect on nutrient supply from the basal diet. Assuming metabolizable energy concentration of the experimental diet (11.0 MJ/kg of DM) on the basis of digestibility of OM measured, cows consumed metabolizable energy slightly in excess of requirement. However, as the experimental diets were not protein supplemented to avoid the masking influence of AA supplementation, the calculated supply of AA in terms of AAT (AA absorbed from the small intestine; 21) was slightly insufficient to meet the requirements. With grass silage diets protein supplements, e.g., rapeseed meal and postruminally infused casein, have consistently increased milk production with high yielding cows (18, 33) but also with low producing cows even when their AAT needs were met (16).

Rumen fermentation patterns of diets based on re-strictively fermented grass silage are characterized

with a small proportion of propionate and high proportions of lipogenic VFA (16), which in turn may be a reason for limited supply of glucose from grass silage diets (23). In the present study, the average value of 144 mmol/mol for the molar proportion of propionate was even lower than typically found for these type of diets (16). The mean ammonia N concentration in the rumen liquor was 5.4 mmol/L, well exceeding the minimum amount for bacterial needs (30). The amount of rumen-degradable N available was thus obviously adequate despite the low CP content of the diet (13.2%). That microbial protein production did not respond to

urea supplementation in similar dietary conditions (1) further supports this finding.

Plasma Metabolites

Amino acid supply. Arterial concentration and blood flow are the major determinants of AA supply to the mammary gland (2). Enhanced arterial plasma concentrations of His, Met, and Lys indicate that abomasally infused AA in this experiment increased the supply of potentially absorbable His, Met, and Lys. However, increased yields of milk and milk protein

TABLE 7. Least squares means for extraction (AV¹ Difference/arterial concentration) of plasma AA by the mammary gland.

AA ⁴	AA Infused postruminally (%) ²					SEM	Contrast (P) ³			
	Control	HI	HM	HL	HML		1	2	3	4
Arg	47.5	58.3	53.8	47.8	43.1	5.14	NS ⁵	NS	NS	NS
His	62.2	18.8	24.5	33.4	34.0	4.04	***	*	NS	NS
Ile	30.8	27.9	33.2	25.0	24.8	2.21	NS	NS	NS	*
Leu	48.1	59.1	61.9	57.2	59.2	1.80	***	NS	NS	NS
Lys	53.7	55.0	55.4	46.2	47.7	2.54	NS	NS	NS	*
Met	49.1	59.7	35.7	63.6	42.8	2.78	NS	**	†	***
Phe	39.4	47.8	50.5	45.2	47.7	2.86	*	NS	NS	NS
Thr	23.7	27.3	33.1	31.2	34.0	1.42	***	**	NS	NS
Trp	5.7	13.5	10.4	12.3	16.1	4.50	NS	NS	NS	NS
Val	23.8	24.6	29.5	24.4	26.0	2.04	NS	NS	NS	NS
Ala	8.6	8.6	11.0	12.1	6.3	3.00	NS	NS	NS	NS
Asn	23.3	27.1	34.2	25.1	28.8	1.82	*	NS	NS	**
Asp	37.6	38.4	43.6	43.8	35.5	2.35	NS	NS	*	NS
Cit	5.4	-1.1	6.1	5.5	2.5	2.20	NS	*	NS	NS
Cys ⁶	6.1	3.9	6.2	8.3	3.7	1.97	NS	NS	NS	NS
Gln	21.1	19.5	23.8	24.0	17.6	3.62	NS	NS	NS	NS
Glu	40.8	40.5	41.3	43.3	38.1	4.15	NS	NS	NS	NS
Gly	2.8	0.9	6.0	6.9	3.9	2.40	NS	NS	NS	NS
Orn	46.9	44.2	45.4	41.6	41.4	2.39	NS	NS	NS	NS
Pro	17.6	6.4	16.8	9.4	5.3	4.59	NS	NS	NS	NS
Ser	5.8	12.2	25.3	18.5	18.2	2.77	***	*	NS	NS
Tau	5.8	-6.3	7.2	11.2	4.9	4.87	NS	*	NS	NS
Tyr	35.3	46.4	51.0	44.9	49.5	3.87	*	NS	NS	NS
BCAA	31.7	32.9	37.9	31.4	32.6	1.91	NS	NS	NS	*
EAA	49.8	51.1	58.9	54.4	51.5	3.07	NS	NS	NS	NS
NEAA	14.9	14.6	19.8	18.9	15.4	1.55	NS	†	†	NS
TAA	22.8	23.1	27.7	26.0	24.5	1.57	NS	NS	NS	NS

¹Arteriovenous.

²Control = No infusion of AA postruminally; HI = 6.5 g of His/d infused postruminally; HM = 6.5 g of His/d and 6 g of Met/d infused postruminally; HL = 6.5 g of His/d and 19 g of Lys/d infused postruminally; and HML = 6.5 g of His/d, 6.0 g of Met/d, and 19 g of Lys/d infused postruminally.

³Contrasts: 1 = Control versus all other infusions, 2 = HI versus HM, HL, and HML, 3 = HM and HL versus HML, and 4 = HM versus HL.

⁴BCAA = Branched-chain AA (Ile, Leu, and Val), EAA = essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val), NEAA = nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr), and TAA = EAA + NEAA.

⁵P > 0.10.

⁶N = 18, because arterial concentration and AV difference in two cases were 0; thus it was not possible to include these values in the analysis; SEM in these cases was 0.0249.

†P ≤ 0.10.

*P ≤ 0.05.

**P ≤ 0.01.

***P ≤ 0.001.

TABLE 8. Least squares means for mammary uptakes of AA from plasma.

AA ³	AA Infused postruminally (g/kg of milk) ¹						Contrast (P) ²			
	Control	HI	HM	HL	HML	SEM	1	2	3	4
Arg	2.75	3.76	3.00	3.22	2.65	0.278	NS ⁴	*	NS	NS
His	0.86	0.87	0.92	1.14	0.85	0.095	NS	NS	NS	NS
Ile	2.56	2.30	2.47	2.44	2.21	0.157	NS	NS	NS	NS
Leu	3.34	3.33	3.23	3.39	3.30	0.211	NS	NS	NS	NS
Lys	3.32	3.45	3.13	3.84	3.76	0.232	NS	NS	NS	†
Met	0.78	0.85	0.78	0.82	0.88	0.042	NS	NS	NS	NS
Phe	1.62	1.60	1.57	1.50	1.57	0.076	NS	NS	NS	NS
Thr	1.48	1.64	1.71	1.72	1.60	0.120	NS	NS	NS	NS
Trp	0.13	0.44	0.32	0.25	0.53	0.171	NS	NS	NS	NS
Val	2.85	2.72	2.76	2.77	2.69	0.161	NS	NS	NS	NS
Ala	0.91	0.91	0.97	1.12	0.69	0.257	NS	NS	NS	NS
Asn	0.94	1.08	1.23	1.01	1.02	0.125	NS	NS	NS	NS
Asp	0.28	0.29	0.29	0.36	0.22	0.041	NS	NS	†	NS
Cit	0.35	-0.06	0.33	0.31	0.19	0.133	NS	†	NS	NS
Cys	0.08	0.07	0.07	0.12	0.05	0.033	NS	NS	NS	NS
Gln	2.28	2.40	2.31	2.11	1.61	0.453	NS	NS	NS	NS
Glu	6.00	6.75	5.90	7.39	5.29	1.039	NS	NS	NS	NS
Gly	0.32	0.16	0.59	0.76	0.36	0.243	NS	NS	NS	NS
Orn	1.27	1.28	1.14	1.18	1.16	0.078	NS	NS	NS	NS
Pro	0.83	0.32	0.69	0.37	0.27	0.236	NS	NS	NS	NS
Ser	0.34	0.67	1.16	0.98	0.79	0.147	**	NS	NS	NS
Tau	0.18	-0.15	0.19	0.28	0.15	0.096	NS	**	NS	NS
Tyr	1.55	1.60	1.68	1.62	1.69	0.085	NS	NS	NS	NS
BCAA	8.75	8.35	8.46	8.60	8.20	0.459	NS	NS	NS	NS
EAA	19.7	20.9	19.9	21.1	20.0	1.13	NS	NS	NS	NS
NEAA	15.3	15.3	16.5	17.6	13.5	1.64	NS	NS	NS	NS
TAA	35.0	36.3	36.5	38.7	33.5	2.53	NS	NS	NS	NS

¹Control = No infusion of AA postruminally; HI = 6.5 g of His/d infused postruminally; HM = 6.5 g of His/d and 6 g of Met/d infused postruminally; HL = 6.5 g of His/d and 19 g of Lys/d infused postruminally; and HML = 6.5 g of His/d, 6.0 g of Met/d, and 19 g of Lys/d infused postruminally.

²Contrasts: 1 = Control versus all other infusions, 2 = HI versus HM, HL, and HML, 3 = HM and HL versus HML, and 4 = HM versus HL.

³BCAA = Branched-chain AA (Ile, Leu, and Val), EAA = essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val), NEAA = nonessential AA (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr), and TAA = EAA + NEAA.

⁴ $P > 0.10$.

† $P \leq 0.10$.

* $P \leq 0.05$.

** $P \leq 0.01$.

(Table 3) were associated only with increased supply of His. The low concentration of His in arterial plasma when cows were fed the control diet (Table 5) coupled with the highest extraction rate (62%) of all AA by the mammary gland (Table 7) support His as the most critical AA on this particular diet. Note that plasma His concentration with control diet in the present study was more than four times lower than that on corn-based control diet (18 vs. 76 $\mu\text{mol/L}$) in the infusion study of Fisher (11), suggesting that His is deficient on a grass silage-based diet, but not on a corn-based diet. Extremely high plasma His concentration (135 $\mu\text{mol/L}$) and reduced intake caused by the highest level of intravenous His infusion (50 g/d) in Fisher's study may have indicated an adverse effect of excess AA (29). In our study, infusing only of 6.5 g/d of His on a grass silage diet was an amount high enough to

elevate arterial plasma His concentration from 18 up to 57 $\mu\text{mol/L}$. The latter estimate represents plasma His concentration often found on corn-based diets (e.g., 11, 25). However, the low His concentration in arterial plasma as found on the control diet in the present study appears to be typical for grass silage diets, be it extensively (7, 10, 23) or restrictively fermented silage (23, 36). These plasma His concentrations are almost as low as those found in lactating goats with induced His deficiency (2). On the other hand, in some experiments plasma His concentrations up to 40 $\mu\text{mol/L}$ on grass silage feeding have been found (22, 35). As compared with our previous infusion studies with Met and Lys (35), it appears that arterial plasma His concentration is more sensitive than Met or Lys to the changes in the supply.

Decreased arterial concentrations of Leu, Phe, Thr, Gly, Ser, and Tyr (Table 5) may be interpreted as an indication of the increased uptake of AA used for milk protein synthesis on His containing treatments. Especially decreased concentration of Gly reflects the improved protein status of the cows (3), as is also found in response to abomasal casein infusions (23). Oxidation of Leu, Phe, Thr, and Tyr is limited (4) and, therefore, these AA were most probably used as precursors for protein synthesis. Of the AA mentioned above, Gly and Ser are also known to be glucogenic (3), although not to the extent found for highly glucogenic AA such as Gln, Asp, Ala, and Glu (4). In the present study, plasma concentrations of Gln and Glu were confounded, obviously because of degradation during the storage of the samples. Mammary AV difference, extraction or uptake of EAA, NEAA, TAA, or BCAA were not affected by His infusions. Increased uptake of Ser by the mammary gland with AA infusions has been found earlier, and it has been linked with possible metabolism between Ser and lactate or the ability of Ser to be synthesized from glucose within the mammary gland (22). Based on the decreased concentration in arterial plasma and the high extraction rate on His containing treatments (Table 7), Leu was probably the second-limiting AA in the present study.

Variation in milk yield is usually accompanied by a similar variation in mammary blood flow, although this close relationship does not always hold (26). It has been suggested that AA may influence the rate of blood flow in ruminants (15). In the present study, despite enhancing milk yield, infusion of His did not affect mammary plasma flow. Infusion of Met seemed, however, to be associated with decreased mammary plasma flow, as also found previously (15). This association may be related to increased arterial concentration of Tau resulting from abomasal Met infusions. Taurine is an intermediate of Met metabolism, and it has been found to affect blood flow by suppressing blood vessels (37).

NEFA and glucose. Of the energy-yielding metabolites examined, neither arterial NEFA nor glucose were affected by the treatments. Arterial NEFA concentration, on average 98 $\mu\text{mol/L}$, was typical for grass silage-fed cows in mid-lactation being in positive energy balance. The average glucose concentration of 3.19 mmol/L was rather low, but it was in the range found for cows fed on restrictively fermented grass silage diets (23). In the present study, the low proportion of propionate in the rumen VFA and the significantly decreased milk lactose content on His treatments suggest that an insufficient supply of glucose limited milk production on His supplemented diets. In other studies with diets based on restrictively fer-

mented grass silage increasing the supply of glucose precursors has increased plasma glucose and milk lactose contents as reviewed by Huhtanen (16). In these diets, some of the AA are catabolized to produce glucose for lactose synthesis instead of being used in milk protein synthesis as enhanced milk protein yield with increased supply of glucose precursors suggest (16, 17). Increasing the molar proportion of propionate in ruminal VFA by changing the amount of concentrate in grass silage diets has been difficult (16). For instance, increasing the proportion of barley up to 75% of diet DM led to a decrease in the proportion of acetate and an increase in the proportion of butyrate, but the proportion of propionate remained unchanged (20). Therefore, in AA infusion studies, it seems essential to ensure adequate glucose supply of cows in order to specifically identify production responses to AA under study irrespective of the glucose effect. The present results clearly warrant further investigation on the possible interaction between His and glucose.

Production Responses to AA Infusions

Effect of His. The significant increases in yields of milk and milk protein (on average 0.9 kg and 29 g, respectively) caused by infusions of 6.5 g of His into the abomasum of cows confirms our hypothesis (35) that His is the first-limiting AA on grass silage-based diets. This finding is further confirmed in our later studies [(19) M. Korhonen, A. Vanhatalo, T. Varvikko, and P. Huhtanen, 1996, unpublished]. Thus, with milk yield and milk protein yield as primary response criteria, our results suggest that the general claim that Met and Lys are the first-limiting AA for production in ruminants (27, 31) does not apply for grass silage-based diets. Infusions of His did not affect milk protein content, and the significant decrease ($P < 0.03$) in milk fat content was because of the dilution of fat into the greater amount of milk produced with His infusions, resulting in a significant increase in the protein to fat ratio of milk. Because milk urea content was not affected by His infusions, the increase in the protein yield was obviously a result of a stimulated casein synthesis. Increases in milk protein yield achieved by postruminal infusions of AA have repeatedly been associated with increased casein yield, whereas whey and NPN fractions have not been affected (31). Increases in milk protein yield with Met and Lys on corn-based diets have often resulted from increases in milk protein content (25, 31) rather than from increases in milk yield, as was found in response to His on grass silage-based diet in the present study.

Published results from infusion studies, when His as a sole AA has been infused postruminally are scarce.

Fisher (11) observed that increasing amounts of intravenous infusion of His increased DMI of cows at low amounts of His (20 g/d), but reduced it at high His levels (50 g/d), as compared with the saline control. In contrast to our studies, milk protein content decreased at both levels of His infusion. Lack of positive response to His was obviously related to sufficient His in the basal diet, which consisted of corn and corn silage, as will be discussed later. Despite the lack of actual production data, other references in the literature speculate on the role of His as a candidate for the first-limiting AA. Virtanen (36) found that His and Trp in milk protein were more weakly labeled than were other EAA after cows were given a single dose of ^{15}N -labeled ammonium sulfate. This outcome was true for cows fed purified diets and grass silage diets supplemented with oats. Based on these results, he suggested that the synthesis of these AA, especially of His, may form a bottleneck in protein synthesis. Later, Fraser et al. (12) and Chen et al. (9) suggested His in microbial protein to be the only limiting AA for milk production. Fraser et al. (12) have also reported significant reductions in milk and milk protein yields due to removal of Lys and His, but not Met, from abomasal infusion of EAA mixture in cows that were maintained by total intragastric infusion of nutrients. Chen et al. (9) and Cant et al. (6) have similarly shown that removal of His from abomasally infused mixtures of AA caused reduced yields of milk protein. Detailed information on the diets, amounts of AA infused, or on other production parameters measured was not, however, given in the above reports. Choung and Chamberlain (10) reported an increase of 26% in protein yield of cows fed grass silage diet in response to intravenous infusion of AA mixture including His, Lys, Met, and Trp. Removal of Met from the mixture did not affect protein yield. From comparison of AA concentrations in microbial and whole digesta and feeds used in the experiment relative to that in milk protein, they concluded that His is the first-limiting AA in the mixture of the remaining three AA infused abomasally in their experiment. Similarly, by comparing bacterial AA composition in relation to that of milk protein, Oldham (26) suggested that His could be the first-limiting AA in ruminants. Our theoretical calculation based on actual input to output ratios of EAA from experimental data (35), and the present experimental data on His, justifies and confirms these earlier observations and suggestions.

Amino acid infusion studies have mostly been focused merely on the idea of identifying the first-limiting AA in terms of production response to infusion of the particular AA under interest. Less attention has been paid to the diet effect itself, i.e., whether the

variation in the delivery of AA from the basal diet has any influence on the limiting AA. Our results—His differing from the general concept that Met and Lys are the first limiting AA—suggest that AA that first limits milk production may vary according to diets. Thus, the role of basal feeding is an important determinant of the first-limiting AA in milk production. Because the AA flow to the lower tract originates mainly from microbial and dietary protein, the supply of AA to the host animal is dependent on the AA composition and proportional amounts of these protein fractions in the total protein entering to the lower tract. Thus, the variation in the first-limiting AA assessed for different diets, e.g., for grass- and corn-based diets, may be explained by varying proportions of microbial protein in the digesta entering the duodenum. Microbial protein is a more important AA source in grass silage than in corn-based feeding. This difference emphasizes the role of His as a first-limiting AA on grass silage feeding, since His is probably the first-limiting AA in microbial protein (9, 12). On the other hand, on corn-based diets RUP may be a more important AA source. Corn rich in His but deficient in Lys, is often supplemented with soybean meal, which in turn is deficient in Met. Therefore, diets based on corn may be adequate in His but deficient in Met and Lys.

Effects of Met and Lys. No role was noted for Met or Lys in the increases of milk or milk protein yields in this experiment. Our earlier results (35) demonstrated that neither Met nor Lys are the first-limiting AA in grass silage diet. However, Met and Lys are often considered as co-limiting AA. The present results do not support, however, that they are either separately or together the second-limiting AA on grass silage diets. Instead, Met seemed to increase milk fat content (+1.8 g/kg) as found and discussed previously (35), although a slight Met-associated increase in milk protein content (+1.2 g/kg) contrasted with our earlier findings. Also, Chamberlain et al. (7) reported that milk production responses to Met and Lys in a grass silage diet have often been limited to inconsistent responses in milk fat content. For instance, despite a significant increase in milk yield in response to infusion of protein or of complete AA mixture on grass silage feeding, an infusion of both Met and Lys in a similar diet tended to increase milk fat content without affecting milk yield (14). It should be noted, however, that the increases in milk fat and protein contents in the present experiment were associated with Met containing infusions only when there was lack of corresponding response to HL treatment. This association suggests that Met rather than Lys is responsible for fat synthesis.

CONCLUSIONS

Infused His significantly increased yields of milk and protein, which indicates that His is the first-limiting AA in milk production of cows fed grass silage diets supplemented with cereal concentrates. Additional His decreases milk fat and lactose contents. Lowered milk lactose content with His suggests that glucose is the next factor limiting milk production on grass silage diets. Therefore, the deficiency in glucose supply of grass silage fed cows needs to be taken into consideration in further AA research, and the possible interaction between His and glucose should be studied. Lack of milk production responses to Met or Lys or both in combinations with His suggests that neither of these two AA is the second-limiting AA on grass silage-based diets. Instead, the role of Leu in this respect deserves further research. Further investigation is also needed to quantify lactational and metabolic responses as a function of graded concentration of additional His in the diet. Our results—His differing from the general concept that Met and Lys are the first-limiting AA—illustrates that AA limiting milk production varies according to dietary situations. Thus, the role of basal feeding is an important determinant of the first-limiting AA in milk production.

ACKNOWLEDGMENTS

The skilled technical assistance and care of the experimental animals by Aino Matilainen and her staff is gratefully acknowledged. This work was supported financially by Rehuraisio Ltd., 21201 Raisio, Finland.

REFERENCES

- Ahvenjärvi, S., A. Vanhatalo, P. Huhtanen, and T. Varvikko. Effects of supplementation of a grass silage and barley diet with urea, rapeseed meal and heat-moisture-treated rapeseed cake on omasal digesta flow and milk production in lactating dairy cows. *Acta Agric. Scand.* In press.
- Bequette, B. J., and F.R.C. Backwell. 1997. Amino acid supply and metabolism by the ruminant mammary gland. *Proc. Nutr. Soc.* 56:593–605.
- Bergman, E. N., and R. N. Heitmann. 1978. Metabolism of amino acids by the gut, liver, kidneys, and peripheral tissues. *Fed. Proc.* 37:1228–1232.
- Black, A. L., R. S. Anan, M. L. Bruss, M. L. and C. A. Brown. 1990. Partitioning of amino acids in lactating cows: oxidation to carbon dioxide. *J. Nutr.* 120:700–710.
- Cant, J. P., E. J. DePeters, and R. L. Baldwin. 1993. Mammary amino acid utilization in dairy cows fed fat and its relationships to milk protein depression. *J. Dairy Sci.* 76:762–774.
- Cant, J. P., D. R. Trout, F. Qiao, and B. W. McBride. 1997. Milk composition responses to unilateral arterial infusion of amino acid mixtures. *J. Dairy Sci.* 80 (Suppl. 1):153. (Abstr.)
- Chamberlain, D. G., J.-J. Choung, and S. Robertson. 1992. Protein nutrition of dairy cows receiving grass silage diets: effects of feeding a protein supplement of unbalanced amino acid composition. *J. Sci. Food Agric.* 60:425–430.
- Chamberlain, D. G., P. A. Martin, and S. Robertson. 1989. Optimizing compound feed use in dairy cows with high intakes of silage. Pages 175–193 in *Recent Advances in Animal Nutrition*. W. Haresign and D.J.A. Cole, eds. Butterworths, London.
- Chen, X. B., D. J. Kyle, and E. R. Ørskov. 1994. Limiting amino acids in microbial protein for productive purposes in lactating cows. Page 120 in *Proc. 45th Annu. Mtg Europ. Assoc. Anim. Prod.* Edinburgh, UK.
- Choung, J.-J., and D. G. Chamberlain. 1995. The effects of intravenous supplements of amino acids on milk production of dairy cows consuming grass silage and a supplement containing feather meal. *J. Sci. Food Agric.* 68:265–270.
- Fisher, L. J. 1972. Response of lactating cows to the intravenous infusion of amino acids. *Can. J. Anim. Sci.* 52:377–384.
- Fraser, D. L., E. R. Ørskov, F. G. Whitelaw, and M. F. Franklin. 1991. Limiting amino acids in dairy cows given casein as the sole source of protein. *Livest. Prod. Sci.* 28:235–252.
- Friedel, K. 1990. Die Schätzung des energetischen Futterwertes von Grossfutter mit Hilfe einer cellulosemethode. *Wiss. Z. Univ. Rostock, N-Reihe* 39 (8):78–86.
- Girdler, C. P., P. C. Thomas, and D. G. Chamberlain. 1988. Effect of intraabomasal infusions of amino acids or of a mixed animal protein source on milk production in the dairy cow. *Proc. Nutr. Soc.* 47:82A. (Abstr.)
- Guinard, J., and H. Rulquin. 1995. Effects of graded amounts of duodenal infusions of methionine on the mammary uptake of major milk precursors in dairy cows. *J. Dairy Sci.* 78:2196–2207.
- Huhtanen, P. 1998. Supply of nutrients and productive responses in dairy cows given diets based on restrictively fermented silage. *Agric. Food Sci. Finl.* 7:219–250.
- Huhtanen, P., R. Blauwiekel, and I. Saastamoinen. 1998. Effects of intraruminal infusions of propionate and butyrate with two different protein supplements on milk production and blood metabolites in dairy cows receiving grass silage-based diet. *J. Sci. Food Agric.* 77:213–222.
- Huhtanen, P., H. Miettinen, and V. Toivonen. 1997. Effects of silage fermentation and post-ruminal casein supplementation in lactating dairy cows: 1. Diet digestion and milk production. *J. Sci. Food Agric.* 74:450–458.
- Huhtanen, P., A. Vanhatalo, A., and T. Varvikko. 1997. Effects of abomasal infusions of histidine, leucine and glucose on milk production in cows given grass silage-based diet. *J. Dairy Sci.* 80 (Suppl. 1):247. (Abstr.)
- Jaakkola, S., and P. Huhtanen. 1993. The effects of the forage preservation method and the proportion of concentrate on nitrogen digestion and rumen fermentation in cattle. *Grass Forage Sci.* 48:145–154.
- Madsen, J., T. Hvelplund, M. Weisbjerg, J. Bertilsson, I. Olsson, R. Spörndly, O. M. Harstadt, H. Volden, M. Tuori, T. Varvikko, P. Huhtanen, and B. L. Olafson. 1995. The AAT/PBV protein evaluation systems for ruminants. A revision. *Nor. J. Agric. Sci.* (Suppl.) 19:1–37.
- Metcalfe, J. A., D. E. Beever, J. D. Sutton, D. Wray-Cahen, R. T. Evans, and D. J. Humphries. 1994. The effect of supplementary protein on in vivo metabolism of the mammary gland in lactating dairy cows. *J. Dairy Sci.* 77:1816–1827.
- Miettinen, H., and P. Huhtanen. 1997. Effects of silage fermentation and post-ruminal casein supplementation in lactating dairy cows: 2. Energy metabolites and plasma amino acids. *J. Sci. Food Agric.* 74:459–468.
- Oldham, J. D. 1987. Efficiencies of amino acid utilization. Pages 171–186 in *Feed Evaluation and Protein Requirement Systems for Ruminants*. R. Jarrige and G. Alderman, eds. European Economic Union, Brussels.
- Pisulewski, P. M., H. Rulquin, J. L. Peuraud, and R. Verite. 1996. Lactational and systemic responses of dairy cows to post-ruminal infusions of increasing amounts of methionine. *J. Dairy Sci.* 79:1781–1791.
- Prosser, C. G., S. R. Davis, V. C. Farr, and P. Lacasse, P. 1996. Regulation of blood flow in the mammary microvasculature. *J. Dairy Sci.* 79:1184–1197.
- Rulquin, H., and R. Verite. 1993. Amino acid nutrition of dairy cows: productive effects and animal requirements. Pages 55–77

- in* Recent Advances in Animal Nutrition—1993. P. C. Garnsworthy and D.J.A. Cole ed. Nottingham University Press, Nottingham, UK.
- 28 SAS. 1987. SAS/STAT Guide for Personal Computers, Version 6 edition. SAS Institute Inc., Cary, NC.
- 29 Satter, L. D., R. L. Lang, J. W. Van Loo, M. E. Carlson, and R. W. Kepler. 1975. Adverse effect of excess methionine or methionine hydroxy analog on feed consumption in cattle. *J. Dairy Sci.* 58:521–525.
- 30 Satter, L. D., and R. E. Roffler. 1975. Nitrogen requirement and utilization in dairy cattle. *J. Dairy Sci.* 58:1219–1237.
- 31 Schwab, C. G. 1996. Rumen-protected amino acids for dairy cattle: progress towards determining lysine and methionine requirements. *Anim. Feed Sci. Technol.* 59:87–101.
- 32 Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1990. A nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 *in* 27th Session Int. Comm. Recording and Productivity of Milk Animals. Paris, France.
- 33 Tuori, M. 1992. Rapeseed as a supplementary protein for dairy cows on grass silage-based diet, with the emphasis on the Nordic AAT-PBV feed protein evaluation system. *Agric. Sci. Finl.* 1:367–439.
- 34 Vanhatalo, A., T. Varvikko, and I. Aronen. 1992. The effect of type of additive on rumen fermentation and digestion of grass silage in cattle. *Agric. Sci. Finl.* 1:163–175.
- 35 Varvikko, T., A. Vanhatalo, T. Jalava, and P. Huhtanen. 1999. Lactation and metabolic responses to graded abomasal doses of methionine or lysine in cows fed grass silage diets. *J. Dairy Sci.* 82:2659–2773.
- 36 Virtanen, A. I. 1966. Milk production of cows on protein-free feed. *Science* 153:1603–1614.
- 37 Zelikovic, I. and R. W. Chesney. 1989. Taurine in biology and nutrition. Pages 199–227 *in* Absorption and utilization of amino acids. Volume I. M. Friedman, ed. CRC Press, Boca Raton, FL.