

Responses to Graded Postruminal Doses of Histidine in Dairy Cows Fed Grass Silage Diets

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ABSTRACT

Five Finnish Ayrshire cows were used in a 4×4 Latin square experiment designed to study the effects of graded doses of postruminal His infusion on milk production, arterial concentrations, and mammary uptakes of plasma amino acids (AA) as well as utilization of added His. Grass silage (16.9% CP) was given ad libitum with 8 kg of cereal-based concentrate per day (11.3% CP). Treatments were abomasal or duodenal infusions of 250 g of glucose/d in combination with 0, 2, 4, or 6 g of His/d. Infusions did not affect dry matter intake (mean 18 kg/d). Infusion of His increased milk yield linearly from 27.0 to 28.8 kg/d, protein yield from 861 to 919 g/d and lactose yield from 1345 to 1457 g/d. Milk fat yield and content changed in a cubic manner (1240, 1167, 1296, and 1177 g/d and 4.60, 4.16, 4.60, and 4.09). Infusion of His had no influence on milk protein or lactose concentrations. Arterial Lys and His concentrations increased linearly, but other AA concentrations were unaffected as well as calculated arteriovenous differences and mammary AA uptakes. The extraction of His decreased linearly with an increasing amount of His. The utilization of added His (28%) was not affected by the level of infusion, and mammary AA uptake seemed to be regulated by an inverse relationship between arteriovenous difference of essential AA and calculated mammary plasma flow. This experiment confirmed that His is the first-limiting AA on grass silage-cereal based diets.

(Key words: dairy cows, histidine, amino acids, grass silage)

Abbreviation key: AV = arteriovenous, BCAA = branched chain AA, EAA = essential AA, MBF = mammary blood flow, NEAA = nonessential AA, TAA = total amino acids

INTRODUCTION

Because of economic and environmental considerations, the utilization of feed protein and milk protein yields have become important issues in dairy production. Different milk and milk protein yield responses with varying protein feeds (Rulquin and Verite, 1993) indicate that the milk protein yield response depends on the AA composition of the supplementary protein. Furthermore, it has been found that the sufficient supply of limiting AA may enhance feed nitrogen utilization by increasing the amount of AA used for milk protein synthesis (Guinard and Rulquin, 1994). However, this is true only in cases where the limiting AA is known and the supply of other nutrients is sufficient.

Recent AA infusion studies (Huhtanen et al., 1997; Vanhatalo et al., 1999) suggest that His is the first-limiting AA on grass silage, cereal-based diets. In these experiments, the addition of 6.5 g of His/d increased both milk and milk protein yields, but the conversion efficiencies of added His into milk protein with an addition rate of 6.5 g/d were low (10%). Low utilization of added His in milk protein synthesis suggests that either the dose of His was too high or the utilization of His is also dependent on factors other than its arterial supply.

Because of the decreased milk lactose content in the first His infusion experiment (Vanhatalo et al., 1999), it was suggested that the insufficient glucose supply from the basal diet may have compromised protein yield responses. This assumption was verified in the next experiment (Huhtanen et al., 1997), in which His infused in combination with glucose increased milk protein yield more than either His or glucose infused as a sole nutrient. In the present experiment, glucose was infused for all treatments to ensure adequate glucose supply of cows fed restrictively fermented grass silage and cereal grains.

The objectives of this study were to examine the effect of graded doses of postruminally infused His on milk and milk protein yields, utilization of added His, and plasma AA and mammary metabolism that mediate the responses in milk yield.

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MATERIALS AND METHODS

Experimental Design and Treatments

The experimental design was a 4×4 Latin square with one extra animal and 14-d periods, of which the last 5 d formed a collection period. Treatments consisted of increasing amounts of His of 0, 2, 4, and 6 g/d, all combined with 250 g of glucose/d. Both glucose and His were dissolved in 6 L of tap water/d and the solution was infused continuously via a peristaltic pump (Watson Marlow, Falmouth, Cornwall, UK) into the abomasum or the duodenum.

Cows and Feeding

Experimental animals were five multiparous Finnish Ayrshire cows (mean BW 552 kg) in early lactation (on average, 35 DIM). Two of the cows received the same treatment sequence throughout the experiment. This arrangement was used because we had five cows available, of which two had minor health problems before the beginning of the study. However, one other cow had to be removed from the experiment just before the last period because of mastitis, with the consequence that one observation was lost. All cows were fitted with rumen cannulas and infusion lines were installed into the abomasum similar to earlier experiments (Huhtanen et al., 1997; Vanhatalo et al., 1999; Varvikko et al., 1999). Two of the cows also had cannulas in the proximal duodenum, and with these cows infusion tubes were installed into the duodenum via this cannula. Cows were housed in individual stalls and milked twice daily, at 0700 and 1700 h.

The wilted grass silage was made from primary growth of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward, which was ensiled in a bunker silo with a formic acid-based additive (5.4 L/tonne of grass with 800 g of formic acid and 20 g of orthophosphoric acid per kg of additive). Silage was offered ad libitum so thatorts were about 10% of the daily portion. The concentrate, which consisted of barley (41%), oats (41%), sugar beet pulp (13.2%), and minerals (4.8%), was fed at a rate of 8 kg/d (as-fed basis) in two equal meals at 0600 and 1600 h. The mineral mixture was 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 trace mineral mixture was (per kilogram): 500 mg of Cu, 10 mg of Se, 3000 mg of Zn, 8 mg of Mo, 10 mg of Co, 1000 mg of Mn, and 100 mg of I. Cows had free access to water and salt block.

Sampling, Recordings, and Chemical Analyses

Feed, feces, and ruminal contents were sampled to allow calculations of quantity and quality of the nutri-

ent supply from the basal diet. Feed intake and milk production were recorded daily throughout the experiment. Feed samples were collected for analysis during each collection period. For statistical analysis, intake and milk production data from the last 5 d of each period were used. Sampling of milk, rumen fluid, and blood have been described earlier (Varvikko et al., 1999). Briefly, blood samples from mammary and tail veins and rumen samples were taken just before morning feeding and 3 and 6 h after morning feeding and rumen samples 9 h after morning feeding on the last day of each collection period. Glucose, NEFA, and urea were analyzed from plasma and BHBA from whole blood samples. For AA analyses, both arterial and venous plasma were pooled over the sampling times to provide one sample from each plasma source per cow per day. Fecal grab samples of about 200 g were taken during the last 5 d of each period twice daily at 0700 and 1600 h to determine the total digestibility of the diet. Acid-insoluble ash was used as an internal marker similar to an earlier experiment (Vanhatalo et al., 1999). Details for analysis of chemical composition of feeds and feces, rumen fermentation parameters, and other analyses have been described previously (Vanhatalo et al., 1999; Varvikko et al., 1999).

After the actual infusion experiment, the cows were kept on the basal diet for 14 d, and omasal digesta samples were taken on the last day as described by Ahvenjärvi et al. (2000) to determine the supply of AA from the basal diet. The total digesta flow into the omasum was calculated by the triple-marker method described by France and Siddons (1986) with CoEDTA, Yb, and Cr as markers for liquid phase, large, and small particles, respectively. Details for marker input and analyses and handling of digesta samples were similar to those previously described by Ahvenjärvi et al. (2000). Briefly, Yb and CoEDTA were continuously infused into the rumen and Cr was given twice daily into the rumen as Cr-mordanted straw. Large particles were first separated from digesta samples by filtering through cheesecloth, and then supernatant was divided into small particles and liquid phase by centrifugation. After centrifugation, samples were freeze-dried. Marker concentrations were determined from each phase and the portion of each phase in total digesta was estimated with reconstitution. Based on the portion of each phase in true digesta the representative samples for AA analysis were formed. Single AA were analyzed after acid hydrolysis according to the Official Community Methods of Analysis for the Determination of AA in Feedingstuffs (European Commission, 1998), with an AA analyzer (Biochrom 20; Pharmacia Biotech, Cambridge, UK) equipped with a 90- \times 4.6-mm PEEK sodium prewash, and a 250- \times 4.6-mm Bio 20 PEEK

sodium high-performance columns. The running program was that recommended by the manufacturer with minor modifications in running times and temperatures. To determine Met and Cys concentrations, we oxidized samples by performic acid before acid hydrolysis.

Calculations and Statistical Analyses

Calculations of energy balance were done as described by Varvikko et al. (1999). Estimations of mammary plasma flow, extractions, arteriovenous (AV) differences, and mammary uptakes of AA have also been described earlier (Vanhatalo et al., 1999; Varvikko et al., 1999). Briefly, mammary plasma flow was estimated in reference to Phe and Tyr output in milk protein using an application of the Fick principle, with the exception that the free milk Phe and Tyr values were neglected. Extraction was calculated as AV difference divided by arterial concentration. Mammary uptake values were calculated as AV difference \times mammary plasma flow. The utilization of incremental amounts of His for milk protein synthesis was estimated by regressing infused His on His output in milk. Utilization of AA absorbed from the small intestine for milk protein production was calculated based on measured omasal canal total AA (TAA) flow to omasum, milk protein output, and estimated AA requirements for maintenance according to the protein evaluation system used in Nordic countries (Madsen et al., 1995). In this system, the maintenance requirement for AA absorbed in the small intestine is 3.25 g/kg of BW^{0.75} per day, and the digestibility of undegraded feed and microbial proteins are 82 and 85%, respectively. Because, on grass silage and cereal-based diets, AA of microbial origin form the major part of duodenal AA supply, the intestinal digestibility of AA was estimated to be 84%.

Data were analyzed by the analysis of variance for Latin square design using the general linear models procedure of SAS (1987). Because data collected included the observations of an extra cow, they were analyzed both with and without these values. Because no differences between these analyses existed in the statistical interpretation of the data, data from the extra cow were included in the analyses presented here. Animal (df 4), treatment (df 3), period (df 3), and the residual effects (df 8) were used as sources of variation in the model. The sums of squares from the analyses were further divided into single degree of freedom comparisons to provide linear, quadratic, and cubic effects of level of His infusion. A mean value derived from four rumen samples and from three whole blood and plasma samples was calculated for each cow and was used to analyze data for rumen ammonia, VFA, plasma glucose,

Table 1. Chemical composition of feedstuffs.

	Grass silage	Concentrate ¹
pH	4.16	
DM, %	19.3	87.9
	% of DM	
Ash	7.7	7.1
Total N	2.7	1.8
NDF	53.3	29.2
Water-soluble carbohydrates	3.9	
Lactic acid	4.4	
Acetic acid	2.4	
Butyric acid	0	
Ammonia N, % of N	3.3	
Soluble N, % of N	68.8	
In vitro cellulase digestibility, % of OM	77.1	

¹Concentrate was a mixture of barley (41%), oats (41%), sugar beet pulp (13.8%), and minerals (4.2%).

NEFA, and whole blood BHBA. Results are expressed as least squares means.

RESULTS

Rumen Fermentation, Digestibility, and Energy Utilization

The silage was good quality, restrictively fermented grass silage, as indicated by low concentrations of fermentation acids and ammonia and the absence of butyrate (Table 1). The mean CP and NDF contents (% of DM) of the diet were 14.8 and 44.1, respectively. According to the Finnish feeding standards (Tuori et al., 1996), the intake of energy (MJ/d) and absorbed AA were approximately 96 and 93% of the requirements during the experiment.

The infusion of His had no influence ($P > 0.05$) on calculated energy balance (average -4 MJ/d) or energy utilization [milk energy/(ME intake $-$ ME maintenance), average 64%]. Infusion of His induced a cubic change in rumen caproate concentration (Table 2), but did not significantly ($P > 0.05$) affect other rumen fermentation parameters (Table 2). Infusion of His increased linearly digestibility of CP ($P = 0.02$) and tended to increase linearly digestibility of NDF ($P = 0.09$) and of OM ($P = 0.08$) (Table 2).

Feed Intake, Milk Yield, and Composition

Feed intake and milk yield parameters are shown in Table 3. Infusion of His had no effect on grass silage, concentrate, or total DMI. The infusion of His linearly increased milk, milk protein, and milk lactose yields ($P = 0.02$). Milk fat yield changed in a cubic manner due to similar changes in milk fat content ($P = 0.04$). Consequently, the opposite pattern was seen in the milk pro-

Table 2. Least squares means for rumen fermentation parameters and diet digestibility.

	His infused postruminally					Contrast		
	0 g/d	2 g/d	4 g/d	6 g/d	SEM	Linear	Quadratic	Cubic
						P		
pH	6.06	5.96	5.97	5.97	0.034	NS ¹	NS	NS
NH ₃ -N, mmol/L	5.33	5.75	5.06	5.53	0.5	NS	NS	NS
Total VFA, mmol/L	121.7	130.2	126.6	124.2	2.9	NS	NS	NS
Proportion, mmol/100 mmol								
Acetate	64.2	65.1	64.4	64.8	0.4	NS	NS	NS
Propionate	18.1	17.8	17.3	19.0	0.4	NS	†	NS
Butyrate	12.9	12.8	13.5	11.8	0.4	NS	†	NS
Isobutyrate	0.94	0.85	0.90	0.93	0.03	NS	†	NS
Valerate	1.47	1.36	1.47	1.40	0.05	NS	NS	NS
Isovalerate	1.45	1.51	1.46	1.37	0.1	NS	NS	NS
Caproate	0.85	0.64	0.97	0.73	0.06	NS	NS	**
Digestibility, %								
OM	73.8	74.9	75.4	75.6	1.0	†	NS	NS
NDF	64.4	66.5	66.8	67.3	1.0	†	NS	NS
CP	69.0	69.6	70.3	72.1	1.0	*	NS	NS

¹*P* > 0.10.†*P* ≤ 0.10.**P* ≤ 0.05.***P* ≤ 0.01.

tein:fat ratio (*P* = 0.01). Milk protein, lactose, and urea concentrations were not affected by graded amounts of infused His (*P* > 0.05).

Plasma Metabolites and AA

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

by mammary gland are given in Table 4. The infusion of His did not affect (*P* > 0.05) these metabolites.

Arterial concentrations of AA and urea and AV differences, extractions, and uptakes of AA are presented in Tables 5, 6, 7, and 8, respectively. Infusion of His had no influence (*P* > 0.05) on arterial concentrations of branched chain AA (BCAA), nonessential AA (NEAA), or TAA, but it linearly increased the concentration of

Table 3. Least squares means for feed intake, milk yield, and milk composition.

	His infused postruminally					Contrast		
	0 g/d	2 g/d	4 g/d	6 g/d	SEM	Linear	Quadratic	Cubic
						P		
DMI, kg/d								
Silage	10.8	11.2	11.3	11.4	0.4	NS ¹	NS	NS
Concentrate	7.0	7.0	6.6	6.5				
Total	17.8	18.2	17.9	17.9	0.4	NS	NS	NS
Yield, kg/d								
Milk	27.0	28.1	28.1	28.8	0.4	*	NS	NS
ECM ²	29.0	28.5	30.3	29.3	0.6	NS	NS	NS
Protein, g/d	861	877	907	919	14	**	NS	NS
Lactose, g/d	1345	1402	1401	1457	23	**	NS	NS
Fat, g/d	1240	1167	1296	1177	36	NS	NS	*
Milk composition, %								
Protein	3.19	3.13	3.22	3.20	0.04	NS	NS	NS
Lactose	4.98	5.01	4.97	5.05	0.03	NS	NS	NS
Fat	4.60	4.16	4.60	4.09	0.1	*	NS	**
Protein:fat	0.69	0.76	0.72	0.79	0.01	**	NS	**
Urea, mg/dl	15.5	15.7	17.7	16.7	0.1	†	NS	NS

¹*P* > 0.10.†*P* ≤ 0.10.**P* ≤ 0.05.***P* ≤ 0.01.²ECM, Energy-corrected milk; calculated according to Sjaunja et al. (1990).

Table 4. Least squares means for mammary plasma flow, arterial plasma concentrations, extractions, arteriovenous (AV) differences, and mammary uptake of energy metabolites.

	His infused postruminally				SEM	Contrast		
	0 g/d	2 g/d	4 g/d	6 g/d		Linear	Quadratic	Cubic
						<i>P</i>		
Mammary plasma flow								
L/d	14934	13778	15114	11832	2024.8	NS ¹	NS	NS
L/kg of milk	569	487	533	415	73.5	NS	NS	NS
Glucose, mmol/L								
Arterial concentration	3.18	3.22	3.18	3.21	0.05	NS	NS	NS
AV difference	0.74	0.80	0.74	0.82	0.03	NS	NS	NS
Extraction, %	23.3	24.8	23.2	25.4	0	NS	NS	NS
Uptake, g/kg of milk	71	72	71	59	8	NS	NS	NS
NEFA, μ mol/L								
Arterial concentration	115.3	105.4	122.6	121.8	9.3	NS	NS	NS
AV difference	-20.3	-23.0	-29.7	-24.4	11	NS	NS	NS
Extraction, %	-25	-33	-40	-26	0	NS	NS	NS
Uptake, g/kg of milk	-3.6	-3.9	-4.4	-3.0	1.7	NS	NS	NS
BHBA, mmol/L								
Arterial concentration	0.65	0.65	0.70	0.59	0.04	NS	NS	NS
AV difference	0.18	0.17	0.18	0.17	0.02	NS	NS	NS
Extraction, %	29	29	28	30	0	NS	NS	NS
Uptake, g/kg of milk	10.4	8.6	9.6	7.0	1.5	NS	NS	NS

¹*P* > 0.10.

Cys (*P* = 0.03). The concentration of essential AA (EAA) also increased linearly (*P* = 0.05) because of linear increases in His (*P* < 0.01) and Lys (*P* = 0.03) concentrations. Elevated concentration of EAA also increased the EAA:TAA ratio (*P* < 0.01). Plasma urea concentration increased for cows infused with 2 g of His per day, decreased for cows infused with 4 g/d, and increased for cows infused with 6 g/d, producing a cubic effect (*P* = 0.06). Concentrations of Cit (*P* = 0.04) and Tau (*P* = 0.05) varied similarly, and that of Thr (*P* = 0.08) and Pro (*P* = 0.10) tended to vary similarly.

The AV differences, extractions, and mammary uptakes of AA were not significantly altered (*P* > 0.05) by His infusion with the exceptions that uptake of Orn decreased (*P* = 0.03), and extractions of His decreased over the first two infusions and then increased, as indicated by the significant (*P* = 0.04) cubic effect. Furthermore, AV difference of taurine (*P* = 0.09) and extraction of Orn (*P* = 0.08) tended to vary similarly to His extraction, uptake of Arg tended to decrease (*P* = 0.07), and extraction of Leu tended to increase (*P* = 0.10) linearly.

Calculated mammary plasma flow rates in terms of liters per day and of liters per kilogram of milk (Table 4) were not significantly affected (*P* > 0.05) by graded amounts of His infusion, but flow decreased numerically when doses of His increased. There was a high inverse correlation ($r^2 = 0.83$) between calculated mammary plasma flow and AV difference of EAA as shown in Figure 1.

AA Supply

The measured omasal AA flows (g/d) and omasal digesta AA composition (g/100 g of AA) are shown in Table 9. The amount of TAA flowing into the omasum was 1999 g/d and EAA flow was 924 g/d. In response to feed intake, the TAA flow was 107 g/kg of DMI (SD = 7.0).

DISCUSSION

Feed Intake, Digestibility, and Energy Metabolism

The slight, although not statistically significant, increase in silage DMI (Table 3) agrees with results of earlier His infusion experiments (Huhtanen et al., 1997; Vanhatalo et al., 1999), in which His was infused together with glucose or as a sole nutrient. However, in the present study the total DMI remained unchanged because concentrate DMI appeared to decrease with increasing amounts of infused His. This changed the forage-to-concentrate ratio of the diet. Huhtanen (1998) found that increasing the concentrate in the diet could decrease total digestibility of the diet based on high quality grass silage. Therefore, small but statistically significant differences in diet digestibility in the present study may be attributed to changes in forage-to-concentrate ratio.

The cows were estimated to be in a slightly negative energy balance as typically found for cows in early lactation. This is supported by higher apparent efficiency of metabolizable energy utilization in the present study

(64%) compared with 51% in a study of Varvikko et al. (1999), where the basal diet was similar with energy balance about zero or positive.

Milk Yield and Composition

Increases in milk, protein, and lactose yields and unchanged protein content of milk (Table 3) are in agreement with a previous study carried out with animals fed a similar diet supplemented with infusions of 6.5 g/d of His and of 250 g/d of glucose (Huhtanen et al., 1997). These responses were smaller, but statistically significant, when His or glucose were infused as a sole nutrient (Huhtanen et al., 1997; Vanhatalo et al., 1999). However, no milk or milk protein yield responses to Met or Lys infused as a sole nutrient or in combination with His were found in our previous studies with similar basal diets (Vanhatalo et al., 1999; Varvikko et al.,

1999). These earlier results and milk and milk protein yield responses in the present study are in accordance with our earlier conclusion that His is the first-limiting AA on the grass silage, cereal-based diet.

In ruminants, propionate is the main precursor of glucose, and glucose is the main precursor of lactose, which is known to regulate the volume of secreted milk (Mather and Keenan, 1983). The rumen fermentation pattern in cows fed restrictively fermented grass silage-based diets has been characterized by a small molar proportion of propionate in total VFA (Huhtanen, 1998; Vanhatalo et al., 1999). Therefore, to provide a sufficient mammary glucose supply, glucose formed in the liver may also originate from other sources, such as AA. This can lead to a situation in which liver and mammary AA metabolism are competitive. In the present study added glucose may reduce the utilization of AA for energy metabolism, and thus more AA in blood may be

Table 5. Least squares means for arterial plasma AA and urea.

AA ¹	His infused postruminally				SEM	Contrast		
	0 g/d	2 g/d	4 g/d	6 g/d		Linear	Quadratic	Cubic
	(μmol/L)					P		
Arg	62	67	68	72	4	NS ²	NS	NS
His	23	28	51	64	4	***	NS	NS
Ile	100	111	111	113	6	NS	NS	NS
Leu	89	94	91	97	5	NS	NS	NS
Lys	72	82	86	91	5	*	NS	NS
Met	21	23	21	23	1	NS	NS	NS
Phe	45	47	43	48	2	NS	NS	NS
Thr	105	137	116	133	9	NS	NS	†
Trp	36	36	35	36	2	NS	NS	NS
Val	189	199	188	200	8	NS	NS	NS
Ala	249	278	256	279	12	NS	NS	NS
Asn	60	72	66	74	5	NS	NS	NS
Asp	6.9	7.2	6.9	7.5	0.3	NS	NS	NS
Cit	68	77	67	73	3	NS	NS	*
Cys	21	22	23	26	1	*	NS	NS
Gln	294	332	301	303	14	NS	NS	NS
Glu	60	61	57	62	2	NS	NS	NS
Gly	332	400	340	388	26	NS	NS	NS
Orn	36	38	39	40	2	NS	NS	NS
Pro	72	87	77	86	5	NS	NS	†
Ser	121	139	116	131	9	NS	NS	NS
Tau	38	41	31	37	3	NS	NS	*
Tyr	52	58	55	56	3	NS	NS	NS
EAA	735	823	809	876	40	*	NS	NS
NEAA	1268	1456	1295	1411	68	NS	NS	NS
BCAA	378	403	389	410	19	NS	NS	NS
TAA	2003	2278	2104	2287	104	NS	NS	NS
EAA:TAA	0.37	0.36	0.38	0.38	0.01	**	NS	NS
Urea	2705	3217	2696	3015	170	NS	NS	†

¹BCAA = Branched-chain AA (Val, Ile, and Leu). 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). TAA = EAA + NEAA.

²P > 0.10.

†P ≤ 0.10.

*P ≤ 0.05.

**P ≤ 0.01.

***P ≤ 0.001.

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

AA ¹	His infused posturally				SEM	Contrast		
	0 g/d	2 g/d	4 g/d	6 g/d		Linear	Quadratic	Cubic
	($\mu\text{mol/L}$)					<i>P</i>		
Arg	34.3	34.2	30.4	40.4	2	NS ²	NS	NS
His	12.3	12.8	11.6	17.2	1.7	NS	NS	NS
Ile	37.6	40.7	40.1	48.1	5.4	NS	NS	NS
Leu	49.8	54.4	52.3	62.5	5.3	NS	NS	NS
Lys	44.6	47.1	46.5	56.2	4.3	NS	NS	NS
Met	11.0	12.4	12.2	14.4	1.2	NS	NS	NS
Phe	19.1	20.5	18.9	24.3	2.3	NS	NS	NS
Thr	29.4	30.7	26.0	37.1	6	NS	NS	NS
Trp	4.8	4.0	2.9	5.7	2	NS	NS	NS
Val	50.1	51.2	46.6	63.8	9.4	NS	NS	NS
Ala	42.2	37.4	26.6	63.3	15.5	NS	NS	NS
Asn	16.5	17.5	16.4	25.3	4	NS	NS	NS
Asp	2.7	3.0	2.6	3.3	0.3	NS	NS	NS
Cit	7.1	3.5	-0.0	8.2	3.5	NS	NS	NS
Cys	1.8	1.1	-0.1	3.2	1.2	NS	NS	NS
Gln	62.8	75.0	63.6	80.5	15.4	NS	NS	NS
Glu	44.5	44.7	41.4	49.3	2.3	NS	NS	NS
Gly	18.8	22.0	-2.6	38.0	19.8	NS	NS	NS
Orn	19.4	17.7	16.3	20.7	1.8	NS	NS	NS
Pro	11.5	12.0	6.5	16.4	4.3	NS	NS	NS
Ser	12.7	12.7	13.2	28.2	8.2	NS	NS	NS
Tau	3.1	2.1	0.3	6.0	1.6	NS	†	NS
Tyr	18.9	19.1	19.7	25.1	2.9	NS	NS	NS
EAA	293	308	287	370	40	NS	NS	NS
NEAA	232	245	187	333	71	NS	NS	NS
BCAA	138	146	139	174	20	NS	NS	NS
TAA	525	553	475	702	109	NS	NS	NS

¹BCAA = Branched-chain AA (Val, Ile, and Leu). 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). TAA = EAA + NEAA.

²*P* > 0.10.

†*P* ≤ 0.10.

partitioned into the mammary gland and used for protein synthesis. This is supported by increased utilization of absorbed AA (from 72 to 76%) into milk protein synthesis with increasing amounts of His. Higher utilization of AA may have increased the amount of synthesized α -lactalbumin, and this together with added glucose, may have increased milk lactose synthesis, because α -lactalbumin is a key component in lactose synthesis in the mammary gland (Rogers et al., 1979). It is likely that, due to infused glucose, glucose supply was not a limiting factor in the present study as it was in our previous study (Vanhatalo et al., 1999). In support of this, rumen propionate (Table 2) was higher than in our previous studies with His infusion (Huhtanen et al., 1997; Vanhatalo et al., 1999), and plasma concentrations of major glucogenic AA (Ala, Gln, Gly) did not change (Table 5) as they did in our previous study (Vanhatalo et al., 1999). Secondly, milk lactose concentration did not decrease with increasing milk yield (Table 3). Thirdly, the estimated mammary uptake of glucose, expressed as grams per kilogram of

milk, was in good accordance with the theoretical value proposed by MacRae et al. (1988).

Interestingly, His infusion in the present and in our earlier study (Huhtanen et al., 1997) increased milk protein yields by increasing the volume of milk excretion rather than by increasing milk protein content. According to Oldham (1994), glucose and energy metabolism may have implications for AA metabolism, e.g., sufficient supply of glucogenic nutrients other than AA from the basal diet may increase availability of limiting AA, which could either increase milk yield, protein content of milk, or both. This may explain the present results since glucose was added, and therefore it is not likely that glucose was limiting. This may not be the case with our previous His infusion studies (Huhtanen et al., 1997, Vanhatalo et al., 1999), where His was infused alone. Possibly, different responses may be associated with the relationships between AA partitioning into liver and mammary gland, or the mammary gland's tendency to maintain a constant milk protein:lactose ratio. The actual mechanisms in the mammary gland tissue that de-

termine partitioning of AA to protein synthesis and to other purposes remains to be established.

Lowered milk fat content and yield and higher protein to fat ratio because of His infusion are in agreement with our earlier results (Huhtanen et al., 1997; Vanhatalo et al., 1999) and were most likely caused by a dilution effect. However, the cause of the increase in fat content at His infusion level of 4 g/d remains unclear. Generally, the effects of added AA or dietary protein supplements on milk fat content or yield have been variable (Chamberlain et al., 1989). In our previous studies (Varvikko et al., 1999), infusing increasing amounts of Met linearly increased milk fat content and yield, and increasing amounts of Lys tended to have a slight quadratic effect on milk fat content and yield. In the present study, not only fat content and yield but also other parameters, such as rumen caproate (Table 2), arterial concentrations of Thr, citrulline, Pro, taurine, and urea (Table 5), and extraction of His (Table 7) changed in a cubic man-

ner. In addition, numerical, although not statistically significant, cubic changes were also seen in AV differences of EAA and NEAA (Table 6). These changes may be related to possible imbalances of nutrient supply at gut or mammary level. This is supported by numerical cubic changes in mammary plasma flow rates (Table 4), because controlling the mammary blood flow (MBF) is one mechanism to regulate the uptake of nutrients by the mammary gland to maintain the supply of one nutrient commensurate with others. Slight changes in milk urea concentrations (Table 3) as well as changes in arterial plasma urea concentrations (Table 5) may also be related to small variations in the dietary CP concentration or in the utilization of AA as an energy source.

His Utilization

Utilization of added His for milk protein synthesis was calculated by regression based on the addition rate

Table 7. Least squares means for mammary extraction rates¹ of plasma AA.

AA ²	His infused postruminally				SEM	Contrast		
	0 g/d	2 g/d	4 g/d	6 g/d		Linear	Quadratic	Cubic
	(%)					<i>P</i>		
Arg	55.4	52.3	46.2	55.9	3.4	NS ³	NS	NS
His	55.7	52.4	22.6	27.6	5	***	NS	**
Ile	37.6	36.7	35.5	42.1	3.7	NS	NS	NS
Leu	55.7	58.6	58.7	64.2	3	†	NS	NS
Lys	61.2	58.0	55.0	61.5	3.1	NS	NS	NS
Met	55.3	54.2	57.2	61.9	3.9	NS	NS	NS
Phe	42.1	43.8	44.8	51.2	4.1	NS	NS	NS
Thr	27.7	21.8	22.4	28.2	4.7	NS	NS	NS
Trp	14.6	10.9	7.6	15.3	5.7	NS	NS	NS
Val	26.8	25.8	24.9	31.4	4.3	NS	NS	NS
Ala	16.7	14.2	9.5	22.2	5.3	NS	NS	NS
Asn	25.9	24.4	23.6	34.6	5.2	NS	NS	NS
Asp	40.4	41.2	38.2	44.6	3.3	NS	NS	NS
Cit	9.4	5.1	1.4	11.0	5.2	NS	NS	NS
Cys	6.8	5.4	-0.6	12.2	5.3	NS	NS	NS
Gln	22.3	22.3	19.6	27.5	4.8	NS	NS	NS
Glu	74.7	73.5	73.9	79.3	2.3	NS	NS	NS
Gly	5.5	4.7	-1.0	9.7	5.6	NS	NS	NS
Orn	53.0	47.0	43.4	50.7	3	NS	†	NS
Pro	14.5	14.2	7.2	18.9	5.4	NS	NS	NS
Ser	9.8	9.3	11.3	22.3	6.3	NS	NS	NS
Tau	-0.9	-8.2	4.2	6.5	5.8	NS	NS	NS
Tyr	35.8	33.0	36.0	46.3	4.8	NS	NS	NS
EAA	17.9	16.5	13.3	23.5	5	NS	NS	NS
NEAA	39.1	37.4	35.6	41.8	3.8	NS	NS	NS
BCAA	36.4	36.4	35.7	41.9	3.8	NS	NS	NS
TAA	25.7	24.1	21.9	30.5	4.6	NS	NS	NS

¹Arteriovenous difference/arterial concentration.

²BCAA = Branched-chain AA (Val, Ile, and Leu). 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). TAA = EAA + NEAA.

³*P* > 0.10.

†*P* ≤ 0.10.

***P* ≤ 0.01.

****P* ≤ 0.001.

Table 8. Least squares means for mammary AA uptake.

AA ¹	His infused postruminally					Contrast		
	0 g/d	2 g/d	4 g/d	6 g/d	SEM	Linear	Quadratic	Cubic
	(g/kg of Milk)					<i>P</i>		
Arg	3.14	2.87	2.71	2.77	0.14	†	NS ²	NS
His	0.98	0.96	0.89	1.04	0.06	NS	NS	NS
Ile	2.48	2.53	2.61	2.48	0.08	NS	NS	NS
Leu	3.40	3.44	3.46	3.21	0.16	NS	NS	NS
Lys	3.45	3.32	3.45	3.24	0.19	NS	NS	NS
Met	0.85	0.90	0.91	0.84	0.03	NS	NS	NS
Phe	1.61	1.63	1.60	1.57	0.04	NS	NS	NS
Thr	1.68	1.72	1.53	1.73	0.17	NS	NS	NS
Trp	0.53	0.42	0.34	0.50	0.21	NS	NS	NS
Val	2.88	2.82	2.70	2.90	0.22	NS	NS	NS
Ala	1.56	1.44	1.07	2.23	0.54	NS	NS	NS
Asn	1.15	1.24	1.20	1.47	0.15	NS	NS	NS
Asp	0.20	0.19	0.18	0.18	0.03	NS	NS	NS
Cit	0.49	0.23	-0.02	0.54	0.29	NS	NS	NS
Cys	0.07	0.06	-0.02	0.15	0.07	NS	NS	NS
Gln	4.55	5.05	4.63	4.67	0.71	NS	NS	NS
Glu	4.69	4.11	4.07	3.75	0.47	NS	NS	NS
Gly	0.38	0.67	-0.13	1.07	0.73	NS	NS	NS
Orn	1.34	1.13	1.14	1.06	0.07	*	NS	NS
Pro	0.54	0.63	0.33	0.70	0.2	NS	NS	NS
Ser	0.48	0.61	0.60	1.10	0.36	NS	NS	NS
Tau	0.19	0.12	0.03	0.29	0.08	NS	†	NS
Tyr	1.74	1.65	1.78	1.79	0.04	NS	NS	NS
EAA	21.0	20.6	20.2	20.3	0.54	NS	NS	NS
NEAA	17.4	17.1	14.9	19.0	2.5	NS	NS	NS
TAA	38.4	37.7	35.1	39.3	2.7	NS	NS	NS

¹BCAA = Branched-chain AA (Val, Ile, and Leu). 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). TAA = EAA + NEAA.

²*P* > 0.10.

†*P* ≤ 0.10.

**P* ≤ 0.05.

and increased milk protein yield. The amount of excreted His in milk was based on average milk protein His content (Tuori et al., 1996). The mean conversion efficiency of added His was 28%, and it was not dependent on the amount of His infused. The efficiency of utilization of added His was similar to that with cows in our earlier experiment (24%), in which an equal amount of glucose and 6.5 g/d of His were infused (Huh-tanen et al., 1997). The efficiency of His utilization was, however, much higher than the respective efficiency values (10 and 11%) in our previous experiments for cows with similar infusion level of His and without infused glucose (Huhtanen et al., 1997, Vanhatalo et al., 1999) or values (10 and 12%) for cows with graded doses of Met and Lys, respectively (King et al., 1990; Pisulewski et al., 1996). In most infusion studies, the efficiency of utilization of added AA has varied from 5 to 30% (King et al., 1990; Guinard and Rulquin, 1994, 1995; Pisulewski et al., 1996; Seymour et al., 1990).

In the light of linear production responses to increased His supply in the present study, and compared with the efficiency of utilization of absorbed AA in grow-

ing ruminants (MacRae et al., 1995), the conversion efficiency of His in the present study was low. Oldham (1994) has speculated that protein, glucose, and energy supply from the basal diet, and protein-to-energy ratio of the diet may be decisive in partitioning dietary AA into protein synthesis, energy formation, or gluconeogenesis. This associative effect between the nitrogen and energy supplies has been demonstrated by Rulquin et al. (1982) in an experiment in which casein was infused into the duodenum of dairy cows fed at two energy intake levels (85 and 100% of requirements). They found that the conversion efficiency of casein into milk was almost doubled (24 and 42%) at a higher energy intake. Similarly, according to Choung and Chamberlain (1995b), the conversion efficiency of casein was lower for a diet with unbalanced AA composition versus a diet with higher quality protein. In the experiment of Schwab et al. (1992), utilization of Lys was much higher in early lactation (60%) than in later lactation (15%). Better utilization of added His in the present study than utilization of added Met or Lys in the studies of King et al. (1990) and Pisulewski et al. (1996) or in our

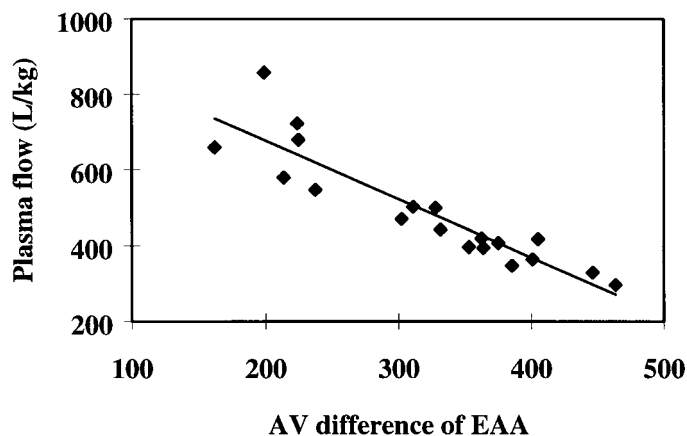


Figure 1. Relationship between arteriovenous (AV) difference of essential AA (EAA) and mammary plasma flow ($y = -1.54x + 984.7$; $r^2 = 0.82$).

earlier studies, in which His and glucose were infused separately (Huhtanen et al., 1997; Vanhatalo et al., 1999), may be due to the slightly deficient supply of energy and protein from the basal diet, and the metabolic interactions between glucose and AA. The sufficient supply of glucose, as discussed earlier, and increased arterial His concentration in the present study, as well as low oxidation of His in the body tissues (Black

et al., 1990) suggest that other factors limit plasma AA utilization.

Kirchgessner and Kreutzer (1987) found that milk AA composition is constant and independent of AA supply. Thus, it is likely that the AA needed per kilogram of milk produced is constant. This assumption leads to a suggestion that the metabolism in the mammary gland controls uptake of AA directed into protein synthesis. Constant His uptake-to-output ratio (mean, 1.06) and a tendency for decreased blood flow in the present study support this. Therefore, low utilization of His may be associated with partition efficiency of plasma His flux to mammary gland and the partition efficiency may be driven by AA metabolism inside the mammary gland rather than by supply level.

The actual mechanism affecting the partition of plasma AA fluxes is unclear. One possible mechanism may be the transport system of AA. Histidine is transported into mammary gland by a sodium dependent transport system (Baumrucker, 1985), thus transportation is not driven by a concentration gradient of His. This was observed in the present study and also in a study by Guinard and Rulquin (1995). Uptake of His was not related to its arterial concentration. It is possible that low utilization may partly be due to restricted capacity or selectivity of transport systems, which leads to a low partition of His into the mammary gland. The

Table 9. Least squares means for measured AA flows into the omasum and AA profile of omasal digesta.

AA ¹	Flow				Profile	
	Mean	SD	Min	Max	Mean	SD
	(g/d)				(g/100 g AA)	
Arg	102	10	91	115	5.1	0.1
His	49	4	44	54	2.4	0
Ile	116	10	103	126	5.8	0.1
Leu	165	13	151	183	8.2	0.1
Lys	127	12	111	139	6.3	0.2
Met	55	5	51	61	2.7	0.1
Phe	111	8	101	120	5.6	0.1
Thr	107	9	96	117	5.3	0
Val	94	11	84	108	4.7	0.2
Ala	137	13	124	154	6.8	0.1
Asp	219	16	200	238	11.0	0.1
Cys	28	1	27	29	1.4	0.1
Glu	299	21	281	329	15.0	0.4
Gly	111	10	101	125	5.6	0.1
Orn	2.5	0	2.3	2.8	0.1	0
Pro	81	6	73	85	4.0	0.2
Ser	94	8	86	106	4.7	0.1
Tau	2.6	0.2	2.4	2.8	0.1	0
Tyr	101	12	87	114	5.0	0.5
EAA	924	79	833	1024	46.2	0.5
NEAA	1075	80	985	1179	53.8	0.5
BCAA	375	33	338	417	18.7	0.3
TAA	1999	158	1818	2203	100	0

¹BCAA = Branched-chain AA (Val, Ile, and Leu). EAA = Essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val). NEAA = Nonessential AA (Ala, Asp, Glu, Gly, Pro, Ser, Tyr). TAA = EAA + NEAA.

efficiency of utilization of Lys higher than that of His or Met may also be related to partition efficiency, since Lys is transported into the mammary gland by more than one transport system, which are mainly driven by concentration gradient. Thus, increased plasma supply of Lys may increase mammary Lys uptake as observed by Guinard and Rulquin (1995) and by Varvikko et al. (1999).

AA Metabolism

Linear increases in His concentration (Table 5) show clearly that arterial His concentration is responsive to increased postruminal supply when added as a free AA. The highest and the lowest concentrations of His in this study were similar to those in our earlier His infusion experiments (Huhtanen et al., 1997; Vanhatalo et al., 1999), where His was added at rate of 6.5 g/d.

Arterial His concentration on corn silage diets has varied in a range of 35 to 76 $\mu\text{mol/L}$ (Cant et al., 1993; Fisher, 1972; Pisulewski et al., 1996; Schwab et al., 1992; Seymour et al., 1990), and has thus been much higher than respective concentrations ($<25 \mu\text{mol/L}$) on grass silage diets (Choung and Chamberlain, 1992, 1995a; Miettinen and Huhtanen, 1997; Vanhatalo et al., 1999). This emphasizes differences in responses to AA infusions between grass and corn silage diets. These differences suggest that the basal diet and the nutritional status of the cow have important effects on responses, as discussed earlier (Vanhatalo et al., 1999). In the present experiment, low arterial His concentration (23 $\mu\text{mol/L}$) and its high extraction rate (56%) on control diet, and high production response (1.1 kg/d) even at the lowest level of His infusion, confirm the decisive role of His as the first-limiting AA on grass silage-grain diets.

In the present study, infusion of His did not decrease the concentrations of BCAA (Table 5) as it did in our earlier study (Huhtanen et al., 1997). Further, graded amounts of His increased linearly plasma Lys and His concentrations in this study. These elevations may also be partly responsible for slightly increased plasma total concentration of TAA. Due to an early stage of lactation and slightly negative energy and protein balance, it is also possible that body tissues were catabolized. Another explanation could be increased liver AA being released into the blood circulation, or changes in the liver AA metabolism when greater amounts of His were infused.

The infusion of His did not decrease arterial Gly concentration as it did in our earlier His infusion experiment (Vanhatalo et al., 1999). In the experiment of Huhtanen et al. (1997), combinations of glucose and His, or glucose with His and Leu, increased plasma Gly

concentration compared with infusing with His or His and Leu without glucose. This may refer to positive glucose status of the animals because Gly is a glucogenic AA (Bergman and Heitman, 1978). Because of added glucose in the present study, there was no need to use AA for gluconeogenesis in the liver and thus the concentrations of glucogenic AA remained unchanged.

Lack of significant responses to His in AV difference (Table 6), extraction (except for His) (Table 7), and mammary uptake of AA (Table 8) are in agreement with those in our earlier His infusion experiments (Huhtanen et al., 1997; Vanhatalo et al., 1999). However, increased milk protein synthesis in mammary gland associated with a slight increase ($P = 0.12$) in AV difference of His is in accordance with the finding that mammary His uptake is positively correlated to His output in milk protein. Direct uptake-to-output ratio, lowered His extraction rate, decreased mammary plasma flow, and a low correlation ($r^2 = 0.38$) between arterial concentration and AV difference of His in this experiment, all suggest that the arterial concentration alone does not regulate the mammary uptake of His. This is in accordance with the suggestion of Bequette and Backwell (1997), that based on low extraction rates, mammary metabolism determines the uptake of AA. Also, Guinard and Rulquin (1994) found that AV difference of His was not related to its arterial supply. In this respect, Met has been found to be similar to His (Guinard and Rulquin, 1995; Varvikko et al., 1999), while Lys uptake seems to be dependent on arterial supply (Guinard and Rulquin, 1994).

Nutrient concentrations in blood, MBF, and nutrient transport systems are the three major determinants that regulate mammary gland nutrient uptake (Mephram, 1982; Baumrucker, 1985). Judged from an inverse relationship ($r^2 = 0.82$) between AV difference of EAA and mammary plasma flow (Figure 1), it seems that the so-called 'pull' hypothesis regulates a portion of uptake of AA by the mammary gland. This is supported by the work of Cant and McBride (1996). They suggested, based on modeling of uptake of nutrients by the mammary gland, that MBF is a function of metabolic activity of mammary gland. Lowered mammary plasma flow with increasing His supply in the present study is in agreement with the results of Bequette and Backwell (1997) who observed that infusion of His into the abomasum decreased MBF in goats with induced His deficiency. Guinard and Rulquin (1995) found that MBF decreased with increasing Met infusion. Interestingly, in those experiments mentioned above, MBF was reduced when the supply of AA used primarily for milk protein synthesis in mammary gland (His and Met) increased. However, the uptake of individual EAA per kilogram of milk (Table 8) did not change. This suggests

that uptake of these AA is regulated for protein synthesis requirements and, that changing the MBF is one mechanism controlling the uptake processes. One reason to maintain the uptake of some AA commensurate with the uptakes of other AA may be that by avoiding excess nutrient uptake and, consequently, their catabolism in the mammary tissue, mammary gland can reduce extra energy costs.

Histidine Supply

Omasal AA flows and digesta AA composition on basal diet was measured because such data from grass silage-based diets are scarce. The CP flow into the omasum, 127 g of CP/kg of DMI in the present study, was comparable to values measured in our earlier study carried out using similar methods and with cows fed a similar diet (Ahvenjärvi et al., 2000). The total AA flow was 1999 g/d, of which 46% was in the form of EAA.

Because of different intake levels, feeds, and digesta flow measurement methods, the actual AA flow values can differ between studies. Thus, to increase knowledge about differences in AA supply between diets, it is more useful to compare digesta AA profiles (g/100 g of AA) than individual AA flows. From the individual AA, Met and Lys are widely discussed in the literature, since they are often ranked as the first- and second-limiting for AA-milk protein synthesis. Based on the results of earlier experiments (Huhtanen et al., 1997; Vanhatalo et al., 1999), His and Leu are also possible candidates for limiting AA on grass silage-cereal based diets. The digesta contents (g/100 g of AA) for His, Lys, and Leu (2.4, 6.3, and 8.2) in the present study were similar to studies of corn-based feeding reported by Schwab et al. (1992) (2.3, 6.2, and 9.7) or by Pisulewski et al. (1996) (2.3, 7.1, and 8.9). However, Met content in our study (2.7) was almost twice as high as in the two other experiments (1.8 and 1.6) mentioned above. Teller et al. (1992) measured duodenal AA flows in cows fed direct cut or wilted grass silage supplemented with concentrates consisting mainly of corn and oil plant seed meals. The contents of His, Lys, Leu, and Met for direct-cut silage feeding were 2.7, 7.8, 4.1, and 1.8 g/100 g of AA. The respective values for wilted silage feeding were 2.5, 7.4, 4.3, and 2.2 g/100 g of AA.

The limiting AA have been ranked by comparison of digesta and milk AA composition. Because of stable milk AA composition (Kirchgessner and Kreutzer, 1987), the literature values for milk AA composition can be used for this purpose. According to Tuori et al. (1996) Met and His contents in milk protein are equal (2.7 g/100 g of AA). These AA are not synthesized from other AA and are quantitatively transferred into mammary gland as needed for protein synthesis (Annison,

1982). Thus, when the dietary supply of Met and His is limiting, supplementing the diet with these AA should increase milk or milk protein yield. Digesta His content was smaller than Met content in the present study, but on corn silage-based diets, His content is usually much higher than Met content (Pisulewski et al., 1996; Schwab et al. 1992). With the assumption of similar requirements for milk protein synthesis, it can be concluded that differences in AA supply between basal diets likely are the main causes of varying responses to these AA among infusion studies. The variation in digesta AA composition between different diets also suggests that the composition of basal diet has a marked influence on AA composition of protein absorbed from the intestine, and thus, the first-limiting AA may be dependent on basal diet.

CONCLUSIONS

Linear increases in milk and milk protein yields with increased His in the present experiment confirm the conclusions of earlier studies that His is the first-limiting AA on grass silage, cereal-based diets. Milk protein yield responses were associated entirely with increased milk yield. Despite the linear increase in protein yield and arterial concentration of His, the efficiency of utilization of incremental His was low. This suggests that the mammary gland has an ability to control extraction of AA from blood. Mammary plasma flow seemed to be involved in this control because there was an inverse relationship between AV difference of EAA and calculated mammary plasma flow. Basal diet has a marked influence on digesta AA composition and thus AA supply from the intestine.

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