Responses in Milk Constituents to Intravascular Administration of Two Mixtures of Amino Acids to Dairy Cows

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ABSTRACT

Four Holstein-Friesian cows were used to investigate the effects of intravascular infusions of AA mixtures on milk constituents. Cows were in wk 11 to 28 of lactation and were fed a basal concentrate (142 g of CP/kg of DM) and grass silage (149 g of CP/kg of DM) in a 60:40 ratio (percentage of DM). Cows were fed hourly, and feed intake was fixed at 95% of ad libitum intake for each experimental period. Each cow received a 4-d jugular saline infusion, followed by a 5-d jugular infusion of a mixture of AA. Two mixtures of AA were used in a crossover design. The first mixture contained both the essential AA and nonessential AA found in milk protein (total AA); this mixture was infused at 400 g of AA/d. The other mixture represented the essential AA fraction only and was infused at 208 g/d. Infusion of total AA increased milk protein concentration from 32.4 to 35.0 g/kg, and essential AA increased milk protein concentration from 32.5 to 36.9 g/kg; milk protein yield increased by 87 g/d (total AA) and 143 g/d (essential AA). Intravascular administration of AA specifically stimulated milk protein concentration, and the efficiency with which the AA were used was higher than had been previously reported when AA supply was increased either by dietary supplementation or by abomasal infusion.

(**Key words**: amino acids, lactation, milk, protein)

Abbreviation key: **EAA** = essential AA, **NEAA** = nonessential AA, **TAA** = total AA.

INTRODUCTION

Supplemental dietary protein for lactating dairy cows has resulted in variable and generally modest increases in milk protein output (8, 12, 13). Rarely has extra dietary protein increased milk protein concentration. Responses of cows to abomasal infusion of protein or AA have also been variable (7). For example, abomasal infusions of mixtures containing free component AA of casein (6) or only the essential AA (**EAA**) of casein (18) have induced most of the milk protein response observed with casein; similar infusions of soy protein isolate, even when supplemented with free AA, failed to increase milk protein output to the extent that casein did (4, 5). The variability of these data may relate to different stages of lactation (17), but other factors, such as the basal diet, supplementary protein source, and even the incremental levels of AA infused, may be important determinants, particularly in the partitioning of the infused AA into protein stores in milk or the body (19). Intravenous infusions of AA mixtures (10, 14, 15) have increased milk protein output, suggesting that, under some conditions, the regulatory mechanisms for milk protein biosynthesis might be limited by the substrate; however, responses have been variable here also. Thus, jugular infusion of Met (13 g/d) increased milk protein yield, primarily as increased milk protein concentration (10). However, at higher rates of Met infusion (26 g/d), even though protein concentration appeared to increase further, protein yield did not increase because of an associated decrease in milk production. In the same study (10), infusion of His decreased milk protein concentration, but infusion of Lys had no effect on milk protein. In a separate study (9) in the same laboratory, Lys and Met in combination failed to alter milk protein content or yield. Similarly, jugular infusion of Met or of a mixture of 10 EAA into lactating goats failed to produce any marked improvement in milk output or protein concentration (3). The EAA mixture appeared to decrease blood urea concentration more than did Met

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infusion in this study, perhaps indicating increased efficiency of utilization of the infused AA for net protein synthesis within the whole body.

In view of the variable responses in both the yield and concentration of milk protein as a response to increased protein or mixed AA supplements, particularly when supplied enterally, we decided to examine whether responses in milk protein yield and concentration could be improved by infusion of AA directly into the peripheral circulation via the jugular vein, thereby eliminating or reducing the modifying effect of the splanchnic tissues. Two mixtures of AA were compared: a total AA (**TAA**) mixture, consisting of the EAA and the nonessential AA (**NEAA**) in the same composition as milk protein, and EAA only.

MATERIALS AND METHODS

Four multiparous Holstein-Friesian dairy cows (mean BW, 557 ± 25.5 kg) in midlactation (wk 11 to 28) were fed hourly a basal ration consisting of a 60:40 mixture of concentrates (142 g of CP/kg of DM) and grass silage (149 g of CP/kg of DM; Table 1) at a rate fixed at 95% of ad libitum intake prior to each infusion period. The DMI averaged 16.2 and 16.7 (±1.62) kg/d for saline and treatment periods, respectively; CP intakes were 2.25 and 2.34 (±0.313) kg/d, representing 87% of the NRC (16) recommendation, and the metabolizable protein (1) intakes during the saline period were 1.37 kg/d, equivalent to 104% of the requirement. Estimated intake of metabolizable energy for these cows was 48 Mcal/d (201 MJ/d), which represented 104% of the calculated NRC (16) requirement or 106% of the estimated requirement of the Agriculture and Food Research Council (1). Saline was infused via a previously implanted jugular catheter for 4 d (covariate period), followed by one of two mixtures of AA for 5 d. The differing AA treatments were administered in a simple crossover design following the saline covariate periods; a 2-wk rest was allowed between treatments. The AA (Forum Chemicals Ltd., Redhill, Surrey, England) were dissolved in pyrogen-free saline, and the pH of the infusate was adjusted to 7.4 with sodium hydroxide prior to filtering through a 0.22-μm filter for sterilization. The TAA mixture was equivalent to the proportions of EAA and NEAA found in milk protein (Table 2), although Tyr was quantitatively replaced by Phe because of solubility problems; TAA was infused at 400 g of AA/d. The second mixture, EAA, consisted only of the EAA contained in the TAA mixture and was infused at 207.8 g of AA/d. Solutions of both mixtures and the covariate saline infusions were infused into the jugular vein at 2 ml/min. Milk samples were taken at each twice daily milking (0630 and 1630 h), and the respective concentrations of fat, true protein, and lactose were determined by infrared analysis of milk using an infrared milk analyzer (model 131; Foss Electric, York, England) at the end of each period. Means of data for the last 3 d of each infusion period were used for statistical comparison. Urea concentrations in defatted milk were determined as previously described (13). Additionally, a 100-ml aliquot of milk was frozen on the last day of infusion of saline or AA for subsequent determination of casein content. The sample was thawed, and the fat was scraped off following centrifugation at 800 × g for 10 min at 10°C; the casein fraction was precipitated by adjusting the pH to 4.5 using 1.0 M HCl. The washed pellet was

TABLE 1. Concentrate formulation and analysis of experimental feeds for ash, NDF, starch, and CP (2).

Composition	Concentrate	Silage	Total diet
	(kg/tonne of DM)	(g/kg of DM)	(kg/tonne of DM)
Ingredient			
Barley, ground	538		323
Wheat, milled	92		55
Corn, meal	110		66
Molassed sugar beet pulp	103		62
Molassine meal[1]	65		39
Straw and cassava[2]	92		55
Silage			400
Analysis			
Ash	100	109	104
NDF	203	474	311
Starch	338	ND[3]	203
CP	142	149	145

[1]Molasses-impregnated peat (Rumenco, Burton on Trent, Staffordshire, England).

[2]Alkali-treated straw (Nutritionally Improved Straw;, Unitrition Selby, North Yorkshire, England) mixed with cassava 1:1 (wt/wt).

[3]Not determined.

TABLE 2. Rate of L-AA infusion for treatments of nonessential AA (NEAA) and essential AA (EAA).

EAA	Infusion	TAA	Infusion
	(g/d)		(g/d)
Met	10.7	Gly	6.5
Tyr	0.4	Ala	12.4
Phe	36.7	Pro	37.6
His	10.2	Ser	23.7
Trp	5.5	Cys	2.9
Thr	16.5	Asn	17.6
Val	24.9	Asp	13.0
Ile	22.4	Gln	35.1
Leu	36.7	Glu	43.4
Lys	31.0		
Arg	12.8		
Total	207.8		192.2

freeze-dried overnight, and the N content of this pellet was determined using an organic nitrogen-protein analyzer [model FP228; Leco Instruments (UK) Ltd., Stockport, Cheshire, England]. The casein content of the original sample was then calculated as N × 6.38.

Statistical Analysis

Statistical design was a simple crossover, and all data were adjusted for the corresponding covariate to allow comparison of the treatment effects by analysis of variance using Genstat (11). Cows and periods were the blocking factors. Two degrees of freedom existed for the error term for significance testing of treatment effects using the F distribution. Effects of infusion were determined using a paired t test to compare the period of AA infusion with the covariate period.

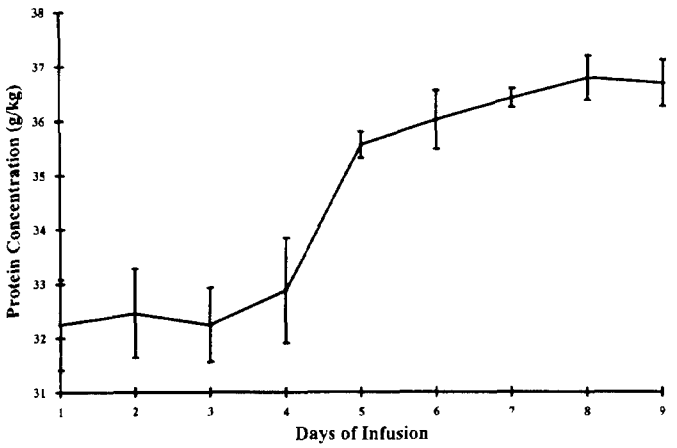

Figure 1. Daily changes in mean (±SEM) protein concentration in milk during jugular infusions of saline or essential AA mixtures in four lactating dairy cows. Days 1 to 4, saline infusion; d 5 to 9, infusion of 208 g/d of the essential AA mixture.

RESULTS AND DISCUSSION

Mean milk production (Table 3) for the covariate periods was 23.8 and 22.4 kg/d for TAA and EAA, respectively. Both AA infusions caused small but nonsignificant increases in milk production that were equivalent to 0.63 and 1.08 kg/d for TAA and EAA, respectively. Changes in fat yield were small and inconsistent, and fat concentration fell by 2.5 g/kg ($P < 0.1$) with TAA but not with EAA.

The decrease in lactose concentration for both treatments, although only statistically significant for the TAA infusion, was similar to that previously observed with abomasal infusions of casein (5). Because little or no change occurred in yield of fat or lactose, the mechanism that stimulated milk protein synthesis may not affect synthesis of fat or lactose.

Infusion of TAA increased milk protein concentration from 32.4 to 35.0 g/kg ($P < 0.01$; Table 3) compared with that of the covariate period, and the response in milk protein concentration to EAA (32.5 to 36.9 g/kg) was higher ($P < 0.05$). Milk protein yield increased by 87 g/d (11.4%; $P < 0.01$) with TAA and by 143 g/d (19.7%; $P < 0.05$) with EAA infusions. We estimated that about 70% of this increase was due to the higher concentration of protein, because only 30% of the increased protein yield would have been observed if the concentration had not changed. The increase in milk protein output for cows receiving either the EAA or TAA infusions was reflected mainly by an increase in casein output as measured on the final day of infusion (Table 3). Comparison of the data on casein yields with total milk protein output indicated that approximately 100 and 69% of the increment in milk protein yield that was due to TAA and EAA, respectively, could be accounted for as increased casein synthesis.

The large and consistent increases in milk protein concentration that were obtained during this experiment were the first indications that protein concentration could be manipulated by intravascular infusion of a mixture of AA based on the composition of milk protein. Figure 1 shows that the changes in milk protein concentration occurred very rapidly; over 90% of the increase occurred within 24 h of the start of the infusion. These changes occurred even though cows were fed approximately 104% of the metabolizable protein and 106% of the metabolizable energy requirements. Indeed, inclusion of AA infusions in the metabolizable protein calculation indicated that the metabolizable protein requirement of the cows was fully met during the AA infusions (120% for TAA and 108% for EAA), suggesting that any limitation during infusion might be due to the supply of metabolizable energy. Diets in some other trials in which AA were infused were more deficient in N, only supplying 75% (18) to 85% (10) of NRC (16) requirements.

In a similar study conducted using goats in early lactation (3), jugular infusions of EAA did not increase milk production or milk protein yield. However, the lack of response might have been due to reduced feed intake during infusion. In the current study, no differences were observed in feed intake between the covariate and the infusion periods.

Extra milk N output represented 22% of the extra AA N that was infused during the TAA treatment and was typical of the efficiencies observed with abomasal infusions of casein [(7); 18 to 33%]. However, corresponding calculations for the EAA infusate indi-

cated a proportional response of 69%, although this value was reduced to 36% when the transfer of EAA into milk EAA was calculated. This value (69%) was similar to the 63% obtained by Schwab et al. (18) who reported that infusion of EAA into the abomasum increased milk protein yield but not the concentration of milk protein.

These higher recoveries might have been due to the relative concentrations of the infused AA that were available to the mammary gland in the correct profile for milk protein synthesis, because the composition was not subject to metabolic alterations by the gut and liver, as occurs with abomasal infusion. The overall efficiency with which AA are used for milk protein must have increased with the EAA infusion compared with the TAA infusion, because NEAA derived from the diet were utilized for milk protein on EAA treatment in addition to the supplied EAA. The lack of change in urea concentration of milk suggested that the infused AA were used with high efficiency within the cow, because infusion of casein usually increases plasma concentrations of urea (3, 19), and urea concentration in milk directly reflects plasma concentration of urea (13).

Why the recovery of infused AA N as milk N was not significantly increased was unclear because additional NEAA would have been required on EAA infusion, and these must ultimately be derived from the diet or by transamination of EAA in the mammary gland. This latter process would decrease the overall efficiency of transfer of the EAA into milk protein. However, until the mammary extraction of AA and their subsequent metabolic fate have been determined for these treatments, the reasons for these apparent differences must remain unknown. The fact that a mixture of only 10 EAA stimulated milk protein concentration as much as did 10 EAA and 10 NEAA could be used in diet formulation.

CONCLUSIONS

Milk protein concentration can be stimulated on a basal diet of concentrates and grass silage by intravascular infusion of mixtures of free AA based on the composition of milk protein or just the EAA from this mixture. The efficiency with which these infused AA are converted into milk protein is high compared with conversion when AA are administered by dietary supplementation. This result may not only be related to the route of administration but also to the composition of the mixture of AA supplied.

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TABLE 3. Comparison of milk production, component output, and composition in response to AA infusions.[1]

	TAA[2]			EAA		
	Control	Infused	SED[3]	Control	Infused	SED
Milk production, kg/d	23.8	24.4	0.29	22.4	23.5	0.49
Composition, g/kg						
Fat	46.0	43.5†	1.09	46.9	46.5	0.43
Protein	32.4	35.0**	0.29	32.5	36.9*	0.88
Lactose	48.4	47.2*	0.20	48.2	46.5	0.49
Urea, mg/kg	256	241	15.1	209	231	26.4
Component output, g/d						
Fat	1066	1046	29.4	1037	1078	19.0
Protein	765	852**	14.1	726	869*	37.1
Lactose	1156	1162	14.2	1084	1094	29.3
Milk casein determined on last days of infusion						
Concentration, g/kg	25.4	27.5	1.20	27.6	30.4†	1.06
Output, g/d	584	671	31.3	607	705	33.5

[1]Casein was determined on a.m. and p.m. milk samples taken on the last day of saline or AA infusion. Significance was determined by paired *t* test.

[2]TAA = Total AA (essential and nonessential); EAA = essential AA.

[3]Standard error of difference.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

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