Infusion of Glucose Directs Circulating Amino Acids to the Mammary Gland in Well-Fed Dairy Cows

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ABSTRACT

The effect of intestinal glucose supply on mammary utilization of amino acids (AA) was studied in four lactating dairy cows. Glucose (0, 443, 963, and 2398 g/d) was continuously infused in the duodenum over 14-d periods using a Latin square design. A grass silage-based diet was formulated so that treatments (diet + infusions) were isoenergetic and isonitrogenous and met 100 and 110% of energy and protein requirements, respectively. Mammary AA uptake was determined by arteriovenous difference and continuous blood flow measurement. The milk protein yield tended to be quadratically increased (to +88 g/d for 963 g of glucose) by glucose infusion, but milk protein content was not significantly affected. Treatments did not change significantly arterial concentrations of urea and glucogenic AA. Mammary arterial fluxes of essential AA increased linearly with glucose infusion, whereas fluxes of nonessential and glucogenic AA were not significantly affected. Mammary arteriovenous differences and extraction rates were roughly unchanged by treatments. Mammary uptake of all essential AA, excluding Arg and Val, increased linearly with increasing supply of glucose. Ratio of blood AA uptake to milk protein output increased significantly for His, Met, and Leu. For the highest infused dose of glucose, all AA except for His were taken up in excess relative to their secretion in milk. Based on evolution of extraction rate and ratio of uptake to output, His and Leu could have limited the milk protein yield response to glucose infusions.

(Key words: glucose infusion, mammary metabolism, amino acids, dairy cow)

Abbreviation key: BCAA = branched-chain amino acids, EAA = essential amino acids, G0 = 0 g/d of glucose

infused in the duodenum, G1 = 443 g/d of glucose infused in the duodenum, G2 = 963 g/d of glucose infused in the duodenum, G3 = 2398 g/d of glucose infused in the duodenum, NEAA = nonessential amino acids, PDI = protein truly digested in the small intestine.

INTRODUCTION

There is a substantial interest in milk protein because of its high nutritional value and its technological properties for the dairy and nondairy industry. Many countries already promote milk protein production through differential payment of milk protein and milk fat. For example, in France, the ratio of the price of true protein to that of fat changed from 0.8 to 2.5 during the last 10 yr. Different technical means exist to improve milk protein yield through nutrition. The oldest is to increase energy supply by adding a nutrient other than fat (see the review by Sutton and Morant, 1989; DePeters and Cant, 1992). Another solution is to provide limiting AA (see the review by NRC, 2001; Rulquin et al., 2001). Enhancing specific energy sources such as propionic acid in the rumen or glucose postruminally is another solution (Thomas and Chamberlain, 1988). In these cases, the main mechanism evoked to explain a milk protein increase is a sparing effect of energy on glucogenic AA; the spared AA being available at mammary level for the synthesis on milk protein. Recently, our group obtained increases of milk protein (7 to 10%) by substituting VFA produced in the rumen for glucose absorbed in the small intestine of cow fed a grass silage-based diet (Hurtaud et al., 2000). Such substitution increased glucose entry rate by 38%, with only a 13% decrease of gluconeogenesis (Rigout et al., 2002b). In this case, it was interesting to see whether the decrease in gluconeogenesis is at the origin of the increase of milk protein synthesis.

Therefore, we decided to examine the effects of an increased glucose supply on AA mammary metabolism. The objective of this study was to analyze the effect of

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graded doses of glucose infused into the duodenum on the milk protein yield and mammary metabolism of AA in well-fed dairy cows consuming grass silage-based diets.

MATERIALS AND METHODS

Treatments and Experimental Design

Experimental details were described previously by Rigout et al. (2002b). Briefly, the experiment was conducted according to a 4×4 Latin square design over 14-d periods. There was no transition time between periods. Treatments consisted of continuous duodenal infusions of 0, 443, 963, and 2398 g/d of glucose (**G0**, **G1**, **G2**, and **G3**, respectively) with equal supplies of NE_L and protein truly digested in the small intestine (**PDI**). The combinations of diets plus infusions were formulated to provide 100 and 110% of energy and protein requirements, respectively (INRA, 1989). To avoid the confounding effect of an increasing supply of energy with glucose, and to keep energy and protein constant among treatments, we replaced 0.46 kg of grass silage and 1.62 kg of energy concentrate with 1.0 kg of glucose and 0.50 kg of soybean meal, assuming that 1 kg of glucose provided 2.75 Mcal of NE_L and 0 g of **PDI**. The percentage of the DMI provided as duodenal glucose was 0, 2.5, 5.1, and 14.2% for the treatments G0, G1, G2, and G3, respectively.

Feeding and Infusions

Four multiparous Holstein cows (635 ± 52 kg of BW; 81 ± 21 DIM; 32 ± 2 kg/d of milk yield) were managed in individual tie stalls and were fed grass silage immediately after calving. Starting 2 wk before the start of infusions, cows were adapted to the basal diet (63.9% grass silage, 7.1% formaldehyde-treated soybean meal, 27.7% energy concentrate; DM basis) supplemented with minerals and vitamins (300 g/d) and with L-Lys HCl (11 g/d; Ajinomoto Co. Inc., Tokyo, Japan) and DL-Met (16 g/d; Rhône-Poulenc, Commentry, France) according to the recommendations of Rulquin et al. (2001).

Grass silage was allocated three times per day (25% at 0700 h, 25% at 1300 h, and 50% at 1900 h) and concentrate in eight equal portions per day. To reach a steady state required for isotopic dilution measurements described in a companion paper (Rigout, 2002b), access to feed was daily limited to 1 h every 3 h from 0700 h onwards. The amount of feed offered and refusals were weighed daily. The DM content of grass silage was determined daily to adjust offered amounts.

Appropriate daily doses of monohydrated glucose (Dextrose, Rocquette Frères, Lestrem, France), L-Lys

HCl and DL-Met were weighed every 7 d, chilled at 4°C, and dissolved daily in 20 kg of water. The solution was then infused continuously in the duodenum with a peristaltic Ismatec pump (Bioblock Scientific, Illkirch, France). This solution was replaced every 24 h.

Surgical Preparation

The surgical preparations were reviewed and approved by the animal care committee of the French Ministry of Agriculture. At 6 mo before calving, the four cows were fitted with a proximal T-shaped duodenal cannula placed 10 to 15 cm from the pylorus. At 2 or 3 mo before the beginning of the experiment, cows were surgically prepared to estimate the net mammary balance of nutrients according to the methods described by Guinard et al. (1994). Two indwelling catheters were placed in the left carotid artery and in the left subcutaneous abdominal vein to measure mammary arteriovenous differences. A transit time ultrasonic flow probe was positioned around the left external pudic artery before the S-shape to measure mammary blood flow. Catheter maintenance was as described by Guinard et al. (1994).

Measurements, Sampling, and Analyses

To allow adaptation, all reported measurements were made only during wk 2 of each 2-wk period. The mammary blood flow was continuously measured throughout the duration of the experiment. For the determination of urea and AA plasma concentrations, 7.5 mL of blood was sampled on d 13 at 0.5, 2.5, 4.5, 6.5, 8.5, and 10.5 h after morning milking. Blood samples were collected simultaneously from arterial and venous catheters with heparinized syringes (Sarstedt, Nümbrecht, Germany). Blood samples were kept on ice and centrifuged at $2500 \times g$ for 10 min at 4°C. Plasma urea and AA were determined from a pooled sample from each sampling session with a multiparameter analyzer (KONE Instruments Corporation, Espoo, Finland) using a KONE kit for urea and AA as previously described (Pisulewski et al., 1996).

Cows were milked twice daily at 0630 and 1830 h. As blood flow was measured only on one half udder, each half udder was milked separately, and milk yield for each half was recorded at each milking. Milk true protein was analyzed by infrared analysis (Milkoscan, Foss Electric, Hillerød, Denmark) at each milking. On d 14, 2 L of milk was taken from the left udder of each cow at morning milking, and samples were prepared for the determination of casein, whey protein, and NPN as described by Hurtaud et al. (2000). Composition of whey proteins was determined by chromatography

Table 1. Effects of duodenal glucose infusion on milk yield, arterial, and mammary uptake of glucose, insulin concentration, and mammary plasma flow (MPF).

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
						<i>P</i>		
Milk yield, kg/d	25.5	26.6	27.8	25.0	1.48	NS ³	0.043	NS
Glucose								
Arterial concentration, mg/100mL	59.1	64.8	65.9	69.3	2.81	0.006	0.073	NS
Mammary uptake, g/h	32.1	34.6	38.6	34.8	0.267	NS	0.006	NS
Arterial insulin, ng/mL	0.912	1.183	1.172	1.129	0.187	NS	0.120	NS
Mammary plasma flow, L/min	3.96	4.89	5.26	5.88	0.416	0.002	0.039	NS

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³NS = Not significant, $P > 0.15$.

(Fast Protein Liquid Chromatography, Amersham Pharmacia Biotech, Saclay, France) on whey with Tris buffer.

Calculations and Statistical Analysis

Lactational performances were averaged for the last 7 d of each period. The net mammary uptake of AA (g/h) was calculated with mammary plasma flow estimated from the mammary blood flow (recorded on d 13 from 0630 to 1830 h) averaged and corrected for hematocrit.

To evaluate the efficiency of AA utilization for protein synthesis, the ratio of net uptake to output for AA was calculated from the net mammary AA uptake estimated over 12 h on one half udder and from the corresponding yield of AA in milk protein as described by Guinard et al. (1994). The data were processed by ANOVA using the general linear model procedure of (SAS, 1987); cow, period, treatment, and residual effects were the sources of variation. Differences in treatments were divided into orthogonal polynomial contrasts of linear, quadratic, and cubic effects of increasing amounts of glucose (Rigout et al., 2002b). Results were expressed as least squares means with root mean-squares errors because of missing values relating to one period for one cow.

RESULTS

Effects of glucose infusions on energy metabolism were described in companion papers (Rigout et al., 2002a, 2002b). Some of these data are necessary to discuss the effect on protein metabolism and are summarized in Table 1. Milk yield increased quadratically ($P < 0.05$) with increasing duodenal infusions of glucose. Arterial glucose concentrations increased linearly, whereas insulin concentrations were not affected by treatments. Mammary uptake of glucose increased qua-

dratically ($P < 0.01$) to reach a maximum with the G2 treatment. Mammary plasma flow increased curvilinearly with treatments by up to 48% between G0 and G3 (Table 1).

Cows were overfed protein beyond the planned levels. Instead of receiving 110% of their requirements, cows consumed an average of 123% of their requirements (Table 2). The tendency ($P = 0.066$) of glucose infusions to increase the protein balance was mainly due to the high value obtained with the G3 treatment.

Milk Protein

Glucose infusions tended ($P = 0.057$) to quadratically increase milk true protein yield. Indeed, the protein yield gradually increased to +12% with the G2 treatment, but the protein yield was no different than the initial value with the G3 treatment (Table 2). Milk true protein content was not significantly affected by glucose infusion. However, casein concentration linearly increased ($P = 0.02$) with glucose infusions. The yield and concentration of milk-soluble proteins were not significantly affected by the treatments. Concentration of milk NPN was doubled by glucose infusion and followed a cubic response with the graded doses.

Arterial Concentrations

Arterial urea concentrations were not affected by treatments (Table 3). Arterial concentrations of total, essential (EAA), nonessential (NEAA), and glucogenic AA were not affected by the graded infusions of glucose. Among the individual AA, only His and Tau concentrations tended ($P = 0.072$ and 0.076 , respectively) to be significantly modified by treatments (Table 3). Concentrations of His gradually dropped up to -48% with the G2 treatment and then returned to the control value. By contrast, Tau concentrations increased up to +23%

Table 2. Effect of duodenal infusions of glucose on protein balance and milk protein content and yield.

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
						<i>P</i>		
PDI ³ intake, g/d	2066	2076	2157	2071	106.6	NS ⁴	NS	NS
PDI balance, %	122	121	120	128	4.0	0.066	NS	NS
True protein								
%	2.97	2.96	3.03	3.05	0.129	NS	NS	NS
g/d	749	776	837	756	52.2	NS	0.057	NS
NPN								
%	0.10	0.29	0.20	0.27	0.064	0.058	0.095	0.033
g	5.1	18.3	13.3	15.5	5.71	NS	0.114	0.101
Soluble proteins, g/kg	5.5	5.5	5.4	5.6	0.30	NS	NS	NS
α-Lactalbumin, %	24.9	25.8	26.3	23.9	2.45	NS	NS	NS
β-Lactoglobulin, %	75.1	74.2	73.7	76.1	2.45	NS	NS	NS
Casein, g/kg	23.6	24.5	24.4	25.5	0.72	0.020	NS	NS

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³Proteins truly digested in the small intestine (INRA, 1989).

⁴NS = Not significant, *P* > 0.15.

with the G2 treatment and then returned to the control value.

only NEAA whose arteriovenous difference decreased linearly with increasing doses of glucose infusions.

Mammary Arteriovenous Differences

Arteriovenous differences of EAA were not significantly affected by treatment (Table 4). Cysteine was the

Mammary Extraction Rates

The mammary extraction rate decreased linearly (*P* < 0.05) only for Lys and Cys (Table 5). Extraction rates

Table 3. Effect of duodenal infusions of glucose on arterial urea and AA concentrations (mg/100 mL).

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
						<i>P</i>		
Urea	20.7	23.7	22.9	19.5	2.89	NS ³	NS	NS
Lys	1.18	1.05	1.07	1.19	0.253	NS	NS	NS
His	0.52	0.38	0.27	0.50	0.174	NS	0.072	NS
Arg	1.38	1.72	1.25	1.45	0.461	NS	NS	NS
Thr	0.98	1.26	1.16	1.06	0.246	NS	NS	NS
Val	1.76	1.51	1.54	1.69	0.638	NS	NS	NS
Met	0.71	0.72	0.69	0.72	0.034	NS	NS	NS
Ile	1.23	0.99	1.07	1.16	0.510	NS	NS	NS
Leu	0.99	0.80	0.85	0.95	0.319	NS	NS	NS
Phe	0.72	0.69	0.70	0.78	0.068	NS	NS	NS
Asp + Asn	1.29	1.60	1.38	1.45	0.328	NS	NS	NS
Ser	0.82	0.89	0.84	0.86	0.273	NS	NS	NS
Glu + Gln	3.83	4.88	4.52	4.00	0.860	NS	NS	NS
Gly	2.32	2.23	2.32	2.13	0.675	NS	NS	NS
Ala	1.35	1.64	1.39	1.45	0.308	NS	NS	NS
Tyr	0.68	0.89	0.75	0.90	0.130	0.136	NS	0.135
Cys	0.50	0.57	0.50	0.48	0.099	NS	NS	NS
Orn	0.76	0.72	0.65	0.71	0.135	NS	NS	NS
Cit	1.38	1.38	1.51	1.41	0.205	NS	NS	NS
Tau	0.53	0.56	0.65	0.56	0.073	NS	0.076	NS

¹Duodenal infusions of glucose: G0 = 0% of the DMI, G1 = 2.5% of the DMI, G2 = 5.1% of the DMI, G3 = 14.2% of the DMI.

²Root mean squares error.

³NS = Not significant, *P* > 0.15.

Table 4. Effect of duodenal infusions of glucose on mammary arteriovenous differences of AA (mg/100 mL).

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
	<i>P</i>							
Lys	0.60	0.51	0.48	0.55	0.115	NS ³	NS	NS
His	0.15	0.13	0.13	0.13	0.024	NS	NS	NS
Arg	0.48	0.44	0.40	0.45	0.118	NS	NS	NS
Thr	0.26	0.23	0.26	0.24	0.058	NS	NS	NS
Val	0.46	0.39	0.39	0.41	0.131	NS	NS	NS
Met	0.16	0.13	0.14	0.14	0.026	NS	NS	NS
Ile	0.42	0.39	0.37	0.43	0.124	NS	NS	NS
Leu	0.61	0.53	0.56	0.59	0.110	NS	NS	NS
Phe	0.28	0.24	0.28	0.27	0.050	NS	NS	NS
Asp + Asn	0.35	0.37	0.31	0.34	0.093	NS	NS	NS
Ser	0.14	0.17	0.16	0.07	0.078	0.136	NS	NS
Glu + Gln	0.80	0.75	0.72	0.81	0.132	NS	NS	NS
Gly	0.03	−0.02	−0.02	−0.02	0.046	NS	NS	NS
Ala	0.22	0.30	0.20	0.18	0.104	NS	NS	NS
Tyr	0.25	0.27	0.23	0.24	0.089	NS	NS	NS
Cys	0.04	0.06	0.02	−0.02	0.035	0.034	NS	NS
Orn	0.27	0.24	0.15	0.19	0.066	NS	0.088	NS
Cit	0.14	0.18	0.14	0.16	0.041	NS	NS	NS
Tau	−0.004	−0.040	0.010	−0.002	0.015	NS	NS	0.012

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³NS = Not significant, $P > 0.15$.

Table 5. Effect of duodenal infusions of glucose on mammary extraction rates of AA (%).

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
	<i>P</i>							
Lys	52.2	48.6	45.7	45.6	3.03	0.048	0.073	NS ³
His	33.4	43.0	51.4	31.5	16.77	NS	0.137	NS
Arg	36.5	26.8	32.2	31.6	7.13	NS	NS	NS
Thr	32.9	18.0	22.7	28.8	9.91	NS	0.136	NS
Val	27.5	26.5	27.0	24.0	4.63	NS	NS	NS
Met	22.4	17.6	20.3	19.4	3.77	NS	NS	NS
Ile	38.6	39.6	36.9	36.2	8.32	NS	NS	NS
Leu	64.4	66.6	66.2	62.2	14.64	NS	NS	NS
Phe	39.2	35.9	39.7	35.9	7.63	NS	NS	NS
Asp + Asn	27.6	22.7	22.6	24.6	6.59	NS	NS	NS
Ser	19.1	18.7	19.1	8.4	11.20	NS	NS	NS
Glu + Gln	22.4	15.4	16.0	20.9	6.05	NS	0.136	NS
Gly	1.17	-1.03	-0.73	-1.47	2.81	NS	NS	NS
Ala	16.5	17.8	14.0	9.9	7.00	NS	NS	NS
Tyr	36.4	30.9	31.1	26.5	8.89	NS	NS	NS
Cys	8.09	10.38	3.08	-3.83	7.25	0.041	NS	NS
Orn	35.9	34.9	22.7	26.6	8.10	NS	0.147	NS
Cit	10.8	12.8	9.1	11.0	2.57	NS	NS	NS
Tau	-0.77	-7.64	1.99	-0.37	2.68	NS	NS	0.008

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³NS = Not significant, $P > 0.15$.

Table 6. Effect of duodenal infusions of glucose on mammary arterial flux of AA (g/h and per half udder).

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
						<i>P</i>		
EAA ³	22.02	26.58	27.24	33.24	4.92	0.029	NS ⁴	NS
NEAA ³	26.04	36.72	37.02	39.66	9.78	NS	NS	NS
TAA ³	48.12	63.36	64.32	72.90	12.3	0.059	NS	NS
Branched AA ³	8.94	9.54	10.92	13.26	3.36	0.112	NS	NS
Glucogenic AA ³	27.84	38.70	37.86	41.70	10.56	NS	NS	NS
Lys	2.76	3.12	3.42	4.14	0.66	0.033	NS	NS
His	1.32	1.20	0.84	1.80	0.66	NS	NS	NS
Arg	3.30	4.98	3.96	5.10	1.44	NS	NS	NS
Thr	2.34	3.60	3.66	3.66	1.02	NS	NS	NS
Val	3.96	4.32	4.86	5.88	1.50	0.121	NS	NS
Met	1.68	2.10	2.22	2.52	0.24	0.006	0.136	NS
Ile	2.76	2.88	3.36	4.08	1.14	0.148	NS	NS
Leu	2.22	2.34	2.70	3.30	0.72	0.069	NS	NS
Phe	1.68	2.04	2.22	2.76	0.18	<0.001	0.136	NS
Asp + Asn	3.12	4.62	4.38	5.10	1.44	NS	NS	NS
Ser	1.98	2.58	2.64	3.06	1.14	NS	NS	NS
Glu + Gln	9.42	14.04	14.28	14.04	3.30	NS	0.124	NS
Gly	5.58	6.54	7.32	7.56	2.82	NS	NS	NS
Ala	3.12	4.68	4.44	5.10	1.02	0.082	NS	NS
Tyr	1.68	2.58	2.40	3.18	0.48	0.010	NS	NS
Cys	1.20	1.68	1.62	1.68	0.36	NS	NS	NS
Orn	1.80	2.16	2.04	2.52	0.42	0.095	NS	NS
Cit	3.36	4.08	4.80	4.98	0.96	0.085	NS	NS
Tau	1.20	1.62	2.04	1.98	0.12	<0.001	0.001	NS

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³EAA = essential AA (Lys, His, Arg, Thr, Val, Met, Ile, Leu, Phe); NEAA = nonessential AA (Asp, Asn, Ser, Glu, Gln, Gly, Ala, Tyr, Cys); TAA = Total AA; branched-chain AA = Val, Leu, Ile; Glucogenic AA = NEAA + (His, Arg).

⁴NS = Not significant, $P > 0.15$.

of Val, Met, Ile, and Leu remained fairly constant among treatments. Extraction rate of His dramatically increased (+54%) to a maximum for the G2 treatment.

Arterial Mammary Fluxes

Arterial mammary fluxes of total AA tended to increase ($P = 0.059$) and EAA increased linearly ($P = 0.029$), whereas the flux of NEAA and glucogenic AA was not significantly affected (Table 6). Excluding His, all arterial EAA fluxes were increased with the graded glucose infusions. The increase was linear ($P < 0.05$) for Lys, Met, and Phe and tended to be linear ($P = 0.069$) for Leu. For the NEAA, Tyr increased ($P = 0.01$) linearly and Ala, Orn, and Cit tended ($P < 0.10$) to increase linearly. Fluxes of Tau increased to reach up to a maximum with the G2 treatment (Table 6).

Mammary Amino Acid Uptakes

The uptake of most EAA, excluding Arg and Val, increased (His, Met, and Leu) or tended to increase (P

< 0.1) (Lys, Thr, Ile, and Phe) linearly with the graded perfusions of glucose (Table 7). Among NEAA, only the uptake of Glu + Gln increased, and that of Cys decreased linearly.

Ratio of Mammary Uptake to Milk Output

Histidine was always removed by the mammary gland in equivalent amounts to its output in milk, whereas Lys, Val, and Arg were always taken up in excess relative to their milk output (Table 8). The number of AA taken up in excess increased with the amounts of glucose infused. For the G3 treatment, all AA, excluding His, were taken up in excess (Table 8).

DISCUSSION

Duodenal infusion of glucose resulted in increased glucose disposal for the dairy cow, as judged by arterial glucose concentration. Rigout et al. (2002b) estimated that between G3 and G0 a 38% increase of glucose disposal and only a 13% reduction of gluconeogenesis

Table 7. Effect of duodenal infusions of glucose on net mammary uptake of AA (g/h per half udder).

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
	<i>P</i>							
Lys	1.40	1.48	1.52	1.91	0.301	0.053	NS ³	NS
His	0.35	0.38	0.42	0.47	0.060	0.041	NS	NS
Arg	1.09	1.25	1.28	1.57	0.334	0.102	NS	NS
Thr	0.61	0.66	0.82	0.84	0.153	0.091	NS	NS
Val	1.05	1.09	1.23	1.42	0.330	NS	NS	NS
Met	0.37	0.36	0.44	0.49	0.043	0.007	NS	NS
Ile	0.97	1.12	1.19	1.47	0.306	0.069	NS	NS
Leu	1.39	1.52	1.75	2.05	0.292	0.022	NS	NS
Phe	0.66	0.69	0.87	0.93	0.164	0.058	NS	NS
Asp + Asn	0.80	1.07	0.98	1.20	0.283	NS	NS	NS
Ser	0.33	0.48	0.50	0.23	0.202	NS	0.136	NS
Glu + Gln	1.85	2.18	2.28	2.82	0.195	0.001	NS	NS
Gly	0.07	−0.04	−0.05	−0.06	0.137	NS	NS	NS
Ala	0.51	0.82	0.62	0.60	0.371	NS	NS	NS
Tyr	0.57	0.76	0.73	0.83	0.229	NS	NS	NS
Cys	0.10	0.18	0.05	−0.05	0.117	0.060	NS	NS
Orn	0.65	0.69	0.47	0.67	0.222	NS	NS	NS
Cit	0.34	0.52	0.44	0.56	0.169	NS	NS	NS
Tau	−0.012	−0.12	0.036	−0.011	0.047	NS	NS	0.013

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³NS = Not significant, $P > 0.15$.

occurred. Milk yield is closely related to the mammary glucose uptake.

Milk protein yield increased 12% through an increase in milk yield because the milk protein content was unchanged. The response of milk protein yield is in agreement with literature results of isoenergetic post-ruminal glucose infusion with grass silage-based diets (Figure 1). The decrease in milk protein yield with the highest dose of infused glucose confirmed the results of Hurtaud et al. (2000). In the current experiment, the

decrease seems related to an impairment of lactose synthesis with the G3 treatment (Riquot et al., 2002b).

The response curve presented a maximum protein yield for 8% of glucose digestible in the intestine per kilogram DMI. Thus with a grass silage-based diet, the metabolic rate of feed protein conversion into milk protein seems related to the glucose availability. In this experiment, the metabolic rate of conversion of PDI to milk protein was increased by 4% (0.49 to 0.51, data not in tables) by increasing glucose availability.

Table 8. Effect of duodenal infusions of glucose on the ratio of net mammary uptake to milk output of AA.

	Glucose ¹				RMSE ²	Effect		
	G0	G1	G2	G3		Linear	Quadratic	Cubic
	<i>P</i>							
Lys	1.34	1.38	1.24	1.73	0.309	0.104	NS ³	NS
His	0.98	1.02	1.00	1.25	0.193	0.093	NS	NS
Arg	2.36	2.65	2.46	3.22	0.706	0.144	NS	NS
Thr	1.04	1.07	1.19	1.34	0.301	NS	NS	NS
Val	1.20	1.21	1.23	1.55	0.414	NS	NS	NS
Met	1.13	1.10	1.15	1.41	0.197	0.068	NS	NS
Ile	1.28	1.45	1.38	1.88	0.448	0.112	NS	NS
Leu	1.07	1.15	1.18	1.52	0.247	0.043	NS	NS
Phe	1.01	1.02	1.14	1.33	0.264	0.122	NS	NS

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³NS = Not significant, $P > 0.15$.

Figure 1. Comparison of response curves of true milk protein yield to postruminal glucose infusions. Regression equation including all data of both trials: $y = 829 + 21.9x - 1.44x^2$; $R^2 = 0.86$; $SED = 13.3$.

Amino Acids and Gluconeogenesis

As cows were well fed in protein (23% above requirements), extra AA could have been used for gluconeogenesis with G0 as commonly proposed by Clark et al. (1977), and subsequent glucose infusion would spare glucogenic AA. However, this was not the case, because increasing glucose did not increase arterial fluxes of glucogenic AA but mainly fluxes of EAA. Black et al. (1990) demonstrated that EAA are poorly oxidized and mainly conserved for protein synthesis. Therefore, it is unlikely that the increase in fluxes of EAA resulted from a sparing effect of glucose on gluconeogenic AA. Moreover, in this experiment, plasma urea concentration was not affected by the treatments. If we take into account that the mammary plasma flow increased, then the urea flux increased, and this is not in accordance with a decrease of gluconeogenesis from amino acids.

Amino Acids and Protein Synthesis

In the present experiment, the increase of the net mammary uptake of AA did not result from any change in EAA concentrations. Effects of postruminal infusion of glucose on EAA concentration in systemic plasma are inconsistent. Infusions have resulted in small and nonsignificant variation as in this trial, and in Dhiman et al. (1993) and Kim et al. (2000). However, glucose infusions often resulted either in a decrease in branched-chain AA (BCAA) or in EAA concentration (Whitelaw et al., 1986; Dhiman et al., 1993); or in an increase in EAA (Hurtaud et al., 2000). Differences in insulin stimulation could explain a fraction of these

inconsistent responses. Indeed, in studies involving infusion of insulin, plasma concentrations of AA and especially those of BCAA are significantly reduced (Debras et al., 1989; McGuire et al., 1995; Griinari et al., 1997; Mackle et al., 1999; Mackle et al., 2000; Bequette et al., 2001). In the current experiment, the increase of insulin concentration (1.3-fold of basal concentration) might have been too small to elicit a significant decrease of plasma BCAA concentrations.

The rise of net mammary uptake of AA resulted to an increase in mammary plasma flow that was also observed by Mackle et al. (1999) and Bequette et al. (2001) during hyperinsulinemic-euglycemic clamps. The effect of infused glucose on mammary arterial supply of AA is in agreement with the concept of coordination of 'labile protein reserves' between body tissue and mammary use (Oldham, 1993). The considerable enhancement of partitioning of blood AA towards the mammary gland is similar to that observed during hyperinsulinemic-euglycemic clamps applied to dairy ruminants (Mackle et al., 2000; Bequette et al., 2001). The common element between clamp studies and this study is the increase of glucose disposal but not the insulin level (Rigout et al., 2002b). It is possible that glucose disposal plays a major role because during hyperinsulinemic-euglycemic clamps, Bequette et al. (2001) found that high mammary blood flow and thus high mammary AA supply are closely related to the glucose concentrations obtained during the clamp.

An increase of mammary arterial supply of AA resulting from duodenal glucose infusion failed to alter mammary arteriovenous difference and mammary extraction rate of AA. Similar results were obtained by Tesseraud et al. (1992), who infused medium doses of insulin (insulinemia was threefold the basal concentration) in conjunction with glucose and AA. A decrease in arteriovenous differences of AA in the mammary gland and an increase in the extraction rate of BCAA was obtained when insulin was administered with glucose alone (Laarveld et al., 1981) or when a high dose of insulin (insulinemia was sixfold the basal concentration) was used in conjunction with glucose and casein infusion (Mackle et al., 2000).

The increase in arterial AA supply plus the constancy of the mammary AA extraction rate resulted in an increase of mammary uptake of EAA with increasing doses of glucose duodenally infused. This enhancement of mammary AA uptake was sufficient to account for the increase of AA output in milk protein. Results obtained during hyperinsulinemic-euglycemic clamp studies were somewhat different. A decrease of mammary uptake was reported on goats (Bequette et al., 2001) but not on cows (Mackle et al., 2000). It is difficult to explain the large overconsumption of AA occurring with the

highest dose of glucose duodenally infused. An overestimation of the mammary blood flow was improbable because:

- the blood flow probes used (20 mm) were calibrated to measure flow average ranging from 2.5 up to 10 L/min;
- for this treatment, the ratio of uptake of glucose to lactose output (Rigout et al., 2002b) was within the published range (Bickerstaffe et al., 1974);
- the uptake of acetate and BHA was just sufficient to cover "de novo" synthesis of fatty acids (Rigout et al., 2002a).

A large exportation of AA previously removed by the mammary gland as free AA in milk would increase milk NPN concentrations, and this was not the case.

A possibility is the overestimation of arteriovenous difference of AA due to venous release of peptides that were not detected by our analytical technique. These peptides can originate from an excessive protein catabolism within the mammary gland (Oddy et al., 1988), a greater part of the synthesized proteins not being secreted as a result of the reduction of lactose secretion observed with the highest duodenal glucose infusion (Rigout et al., 2002b).

Limiting Amino Acids

The 12% increase of milk protein yield obtained in this experiment is close to the 15% obtained by Mackle et al. (2000) between a water-infused control and a hyperinsulinemic-euglycemic clamp. However, this response was far from the 17 to 20% increases obtained between a casein abomasally infused control and a hyperinsulinemic-euglycemic clamp coupled with the same casein infusion (Griinari et al., 1997; Mackle et al., 2000). The difference in the range of the milk protein response could result from the removal of a limitation in a specific AA by the casein infusion.

In the current study, despite the protein overfeeding of +23%, His and Leu may have limited the milk protein response. Indeed, the drop in arterial His concentrations as milk protein increased is characteristic of a limiting AA. The decrease of mammary arterial flux of His, whereas that of other amino acids increased, reinforced this conclusion. A His deficiency of grass silage-based diets is in agreement with the result of Kim et al. (1999), Vanhatalo et al. (1999), and Korhonen et al. (2000).

In the control treatment, Leu was taken up by the mammary gland in agreement with its milk output. Commonly, Leu is removed by the mammary gland in large excess relatively to its output (Guinard and Rulquin, 1994; Mackle et al., 2000) and is taken up in agreement with milk output only when Leu is limiting

(Bequette et al., 1996; Fulquin and Pisulewski, 2000). Leu has been proposed as a limiting AA for diets with low contents of corn (Rulquin et al., 2001), although there is no direct demonstration of this limitation (Kröber et al., 2001). It should be noted that the 20% milk protein response observed by Mackle et al. (2000) was obtained with a hyperinsulinemic-euglycemic clamp coupled with an abomasal casein infusion reinforced with BCAA.

CONCLUSIONS

This study demonstrates that a sparing effect of glucose on glucogenic AA is unlikely able to explain the increase of mammary protein synthesis obtained by infusing glucose into the duodenum. The origin of this increase resulted not in an improvement of mammary metabolism but in an increase of mammary arterial blood flow. Increase in milk protein yield obtained by substituting rumen VFA for postruminal glucose in cows fed grass silage-based diets is an example of the coordination of the use of nutrients between tissues. High AA supply and high glucose entry rate were necessary conditions to turn AA stored in labile protein reserves towards the mammary gland.

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