Effect of Starch on the Bioavailability of Glutamine and Leucine in the Dairy Cow

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ABSTRACT

This experiment was designed to quantify changes in utilization of Gln and Leu by the gut wall as a result of changes in the starch supply to the duodenum. Four dairy cows fitted with cannulas in the rumen and the distal duodenum were adapted for 3 wk to starch infusion, either into the rumen (600 g/d of flaked maize) or into the duodenum (300 g/d of flaked maize plus 300 g/d of maize meal), in a 2 × 2 crossover design. Absorption and elimination kinetics and the relative bioavailability of Gln and Leu were measured during wk 4 and 5. After infusion of 50 g of Gln or 10 g of Leu into the duodenum or jugular vein, blood samples were taken from the jugular vein at 0.5-h intervals up to 4 h after infusion. Concentrations of Gln and Leu in plasma fitted best to an open, one-compartment model (duodenal infusion) or to an open, two-compartment model (i.v. infusion). Both amino acids were rapidly absorbed; half-life times were less than 20 min. The amount of Gln trapped in the splanchnic bed was higher than the amount of Leu trapped in the splanchnic bed. Site of starch infusion did not affect the relative bioavailability of amino acids.

(**Key words**: glutamine, leucine, absorption kinetics, starch)

Abbreviation key: **AUC** = area under the curve.

INTRODUCTION

The maximum possible amount of milk N produced per gram of N consumed in the dairy cow has been estimated to be 40 to 50% (18). Under practical conditions in The Netherlands, N utilization varies between 17 and 30% (17). Amino acids that disappear from the lumen of the small intestine are prone to metabolism in the gut wall and in the liver, which decreases their availability for milk protein synthesis.

Utilization of AA by the gut wall has been established both in nonruminants and ruminants (2, 20). In a study using sheep, only 30 to 80% of the AA that disappeared from the small intestine were recovered in portal blood, suggesting a high rate of metabolism in mucosal cells of the gut (15). Particularly, Gln, Glu, and Asp are extensively metabolized in intestinal tissues, and Gln is assumed to be the major energy source for these tissues (20). Generally, Gln is characterized by negative net absorption across portal-drained viscera (5, 12). Data from the studies of Heitmann and Bergman (2) and Reynolds and Huntington (12) have shown that the net absorption of Gln in vivo was influenced by the energy content of the feed. In vitro, the oxidation of Gln by gut mucosal tissue is decreased as concentrations of glucose in the medium are increased (1). The relevance of this observation for the in vivo situation is questionable, however.

Ten to 20% of glucose that is produced by the liver is derived from AA (3), implying that about 30% of the AA flux to the liver is converted to glucose (H. de Visser, 1994, personal communication). There are few data on the variability of AA utilization for gluconeogenesis in the liver in relation to the supply of other glucogenic precursors to the liver in dairy cows.

Bypass starch has been suggested to improve N utilization by decreasing AA utilization in portal-drained viscera and in the liver, because bypass starch may directly supply glucose to these tissues. This study aimed to reveal whether the loss of AA in splanchnic tissues can be reduced by increasing the amount of bypass starch in the diet. The rate of absorption and elimination and the relative bioavailability of Gln and Leu was measured for lactating dairy cows at two amounts of starch available in the duodenum.

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MATERIALS AND METHODS

Experimental Design

The experimental design was a 2 × 2 crossover with four cows and two treatments. Treatments were the infusion of starch, either into the rumen (control) or into the duodenum. Starch was infused continuously (600 g/d) during the 32 d of each experimental period. The starch solution that was infused into the duodenum consisted of 300 g of corn meal and 300 g of flaked corn meal suspended in 15 L of tap water. As a control treatment, 600 g of flaked corn meal solution were infused through the rumen cannula. All cows received 15 L/d of tap water through the cannula that was not used for starch infusion. Starch solutions were prepared weekly and stored at 4°C. The cows were allowed to adapt to the starch infusion for 3 wk (d 1 to 21) before measurements of absorption and elimination kinetics of AA took place.

Cows and Diets

Four multiparous dairy cows (Holstein Friesian × Dutch Friesian) were equipped with a large rumen cannula (i.d., 100 mm; Bar Diamond, Parma, ID) and a T-shaped cannula (i.d., 19 mm) in the distal duodenum. Cows were tethered in tie stalls and had free access to block salt and water. Cows received a diet consisting of 33% artificially dried grass and 67% pelleted concentrate (DM basis). Gross chemical composition and feeding value of the feed components and diet were measured and calculated as described previously (7) and are presented in Table 1.

Of the total daily ration, 40% was offered at 0800 h, and 60% was offered at 2000 h. Orts were placed in the rumen via the cannula at 0900 h. The diet was calculated to meet 95% of the protein requirements and 105% of the energy requirements to maximize the uptake of AA in milk protein. The amount fed was adapted to the actual production between both experimental periods. At the onset of the experiment, cows were 26 to 46 wk into lactation. At each milking, milk production was measured, and samples were taken for the analysis of milk composition (Multispec infrared analyzer; Foss Electric, Hillerød, Denmark).

AA Infusions

On d 19, a catheter (i.d., 1.0 mm; o.d., 1.8 mm; Tygon®; Norton, Akron, OH) was inserted into the left jugular vein after local anesthesia. The catheter was sutured to the skin, and the external part was taped around the neck. Catheters were rinsed daily with physiological saline containing heparin (250 IU/ml). Bolus infusions of physiological saline, Gln, and Leu were carried out in the following sequence: d 22, physiological saline (1500 ml) into the jugular vein; d 23, Gln solution into the duodenum; d 25, Gln solution into the jugular vein; d 30, Leu solution into the duodenum; and d 32, Leu solution into the jugular vein. L-Glutamine (50 g) or L-Leu (10 g) (both from Merck, Darmstad, Germany) were dissolved in 1500 ml of physiological saline directly before starting the infusions. Solutions used for the i.v. infusions were sterilized over a cellulose acetate microfilter (pore size, 0.2 μm; Nalge Co., Rochester, NY). Just before the i.v. infusions started, the AA and saline solutions were warmed to body temperature (ca. 35°C). Intravenous infusions were carried out through a needle (8 cm × 2.10 mm) inserted in the right jugular vein, which was connected via Silastic® tubes (i.d., 4 mm; Dow Corning Co., Midland, MI) to a peristaltic pump. Infusions into the duodenum were performed via the intestinal cannula. Infusions started 2 h after the morning feeding, and the infusion rate was 5 ml/s.

TABLE 1. Chemical composition and feeding value of the concentrate, artificially dried grass, and diet.[1]

	Concentrate[2]	Dried grass	Diet
DM, g/kg	911	920	912
	(g/kg of DM)		
OM	924	877	908
CP	93	227	137
Crude fat	116	33	89
Starch	104	5	71
NDF	323	545	396
Magnesium oxide	4	. . .	3
Minerals[3]	10	. . .	7
Vitamins A and D_3[4]	trace	. . .	trace
Digestibility of OM, %	81	76	79
NE_L, kJ/kg of DM	8747	6193	7904
Digestible protein supply,[5] g/d	83.8	85.6	84.4

[1]Diet consisted of 33% artificially dried grass and 67% concentrates. Values do not account for infused starch (600 g/d) into the rumen or duodenum.

[2]Concentrate consisted of 34% sugar beet pulp, 34% soybean hulls, 18% tapioca, 10% animal fat, 2% beef molasses, 1% vitamins and minerals (premix from Pre-Mervo, Utrecht, The Netherlands. Composition of vitamins and minerals is given in footnotes 3 and 4 to Table 1), and 0.4% MgO.

[3]Minerals included 15.5% Ca, 30.0% Mg, 0.25% Cu, 0.2% Mn, 0.2% Zn, 0.006% Co, 0.012% I, and 0.005% Se.

[4]Contained 153 IU/d of vitamin A and 37 IU/d of vitamin D_3 per cow.

[5]In the intestine. Included digestible RUP and microbial protein according to the Dutch protein evaluation system (16).

Blood Sampling and AA Analysis

Blood samples were drawn from the jugular vein directly before and directly after the infusion of AA or

saline and subsequently at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h after infusion. Samples were collected in heparinized tubes stored in melting ice. After centrifugation at 3750 × *g* at 0°C for 10 min, plasma was deproteinized using sulfosalicylic acid (final concentration = 4%, wt/vol), and concentrations of AA were determined using ion-exchange column chromatography with lithium buffers as described previously (7). On the days when saline was infused, approximately 4 ml of plasma were stored at 4°C and analyzed within 26 h for glucose and urea using enzymatic methods (both from Boehringer, Mannheim, Germany).

Kinetic Parameters and Statistical Analysis

Kinetic parameters of absorption, distribution, and elimination of the AA were calculated using PKCALC (14). Concentrations of Gln and Leu in plasma were corrected for baseline values that were measured after the saline infusions. Curves for concentration versus time after duodenal infusion (n = 16) fitted best to an open, one-compartment model for extravascular application; an absorption phase and an elimination phase were differentiated (13). The explained variance was over 96% with four exceptions (r^2 = 0.51, 0.62, 0.86, and 0.94) and one missing case. In two cases of duodenal infusion of Leu, the absorption rate could not be estimated accurately because the half-life of absorption was less than 5 min. Curves for concentration versus time after i.v. infusion (n = 16) fitted best to an open, two-compartment model describing a distribution phase and an elimination phase (13). The model explained the variance in the data for over 99% except in four cases (r^2 = 0.93, 0.95, 0.98, and 0.98); one case was missing.

Area under the curve ($\mathbf{AUC}_{0\rightarrow t}$) was calculated using the linear method (i.e., through interpolating subsequent concentrations of AA where 0 represents the moment immediately after infusion and t is the time (minutes) of the last utilized sample). The $AUC_{0\rightarrow\infty}$ was calculated as $AUC_{0\rightarrow t} + C_t\lambda$ where C_t is the estimated concentration at the time of the last utilized sample and λ is the biological half-life. The relative bioavailability (F) was calculated as the AUC after duodenal infusion divided by the AUC measured after i.v. infusion. Plasma clearance was calculated as (F × dose)/$AUC_{0\rightarrow\infty}$.

All data were statistically analyzed according to the model $y = \mu + \alpha + \beta + \tau + \epsilon$ where μ = overall mean, α = effect of cow, β = effect of period, τ = effect of site of starch infusion, and ϵ = random error. Cow and period were used as blocking terms. Analysis was performed using the general procedures of Genstat 5 (11).

RESULTS

The site of starch infusion did not affect DMI; calculated nutrient intake; concentrations of glucose, urea, or AA in plasma; or production of milk and milk components (Table 2).

Glutamine that was infused into the duodenum was rapidly absorbed. Peak concentrations (700 to 1100 μmol/L) were measured 50 min after infusion (Figure 1). The kinetics of Gln absorption and elimination were not affected by the site of starch infusion (Figure 1 and Table 3), although the half-life of absorption tended to be shorter when starch was infused into the duodenum. When the same dose of Gln was applied i.v., peak values (1500 to 3000 μmol/L) were measured immediately after infusion. Again, there was no effect of site of starch infusion on distribution and elimination kinetics. The relative bioavailability of Gln was not significantly affected by site of starch infusion and was 103 and 82% when starch was infused into the rumen or into the duodenum, respectively. Although the difference in the distribution volumes after duodenal or i.v. infusion suggested that 7 to 44% of the Gln that was infused into the duodenum was initially trapped in splanchnic tissues, most of this trapped Gln was released unaltered over time, resulting in an AUC that was close to that observed after i.v. infusion. Infusion of Gln evoked a small, nonsignificant rise in the concentration of Glu

TABLE 2. Effect of site of starch infusion on DMI, calculated nutrient intake,[1] blood plasma metabolites, and production of milk and milk components.

	Site of starch infusion			
	Rumen	Duodenum	SED	*P*
Intake				
DMI, kg/d	16.2	15.7	0.7	0.58
Digestible protein supply,[2] g/d	1363	1285	55	0.29
NE_L, MJ/d	127	124	5	0.60
Blood plasma				
Glucose, mmol/L	3.71	3.86	0.13	0.37
Urea, mmol/L	1.56	1.77	0.12	0.22
Total AA, μmol/L	2146	2039	147	0.54
Essential AA, μmol/L	899	791	59	0.21
Nonessential AA, μmol/L	1248	1248	89	0.99
Milk				
Production, kg/d	16.4	15.9	0.2	0.28
Fat, g/d	683	666	38	0.73
Protein, g/d	595	621	9	0.21
Lactose, g/d	695	695	12	0.99

[1]Including 600 g/d of infused starch.

[2]In the intestine. Included digestible RUP and microbial protein according to the Dutch protein evaluation system (16).

in plasma (20 to 40 μmol/L), which lasted for less than 150 min.

Leucine that was infused into the duodenum was absorbed more rapidly than Gln. Peak values (350 to 650 μmol/L) were measured 20 min after infusion (Figure 2). Half-life of absorption was short and was less than 5 min in two cows (Table 3). As for Gln, absorption and elimination of Leu that was infused into the duodenum were not affected by the site of starch infusion. Also, rate of distribution and elimination after i.v. infusion were not affected. When infused i.v., the half-life of terminal elimination of Leu tended to be longer than that after duodenal infusion (94 vs. 66 min, respectively, $P = 0.18$). However, because of rapid distribution (half-life, 5.6 to 8.7 min), the AUC after i.v. infusion was lower than that after duodenal infusion, resulting in a relative bioavailability of over 100% (Table 3). No other AA concentrations were affected by the infusion of Leu.

DISCUSSION

Glutamine and Leu were chosen to evaluate the effect of the availability of duodenal starch on the utilization of AA by splanchnic tissue. Glutamine is known for its high utilization in splanchnic tissues (20), but Leu is metabolized predominantly in peripheral tissues (8), which was reflected in our results. Although Leu was dosed five times less than Gln, the AUC after duodenal infusion of Leu was about half that of Gln.

The extent to which the small intestine of the ruminant is capable of starch digestion is still controversial (4, 6, 9, 10). We have no measure of the amount of starch digested or the amount of glucose that appeared in the portal vein in both treatments. Peripheral glucose concentrations were not changed but were probably not indicative of the portal appearance of glucose (4). Assuming that 10% of the starch escaped rumen degradation, then approximately 175

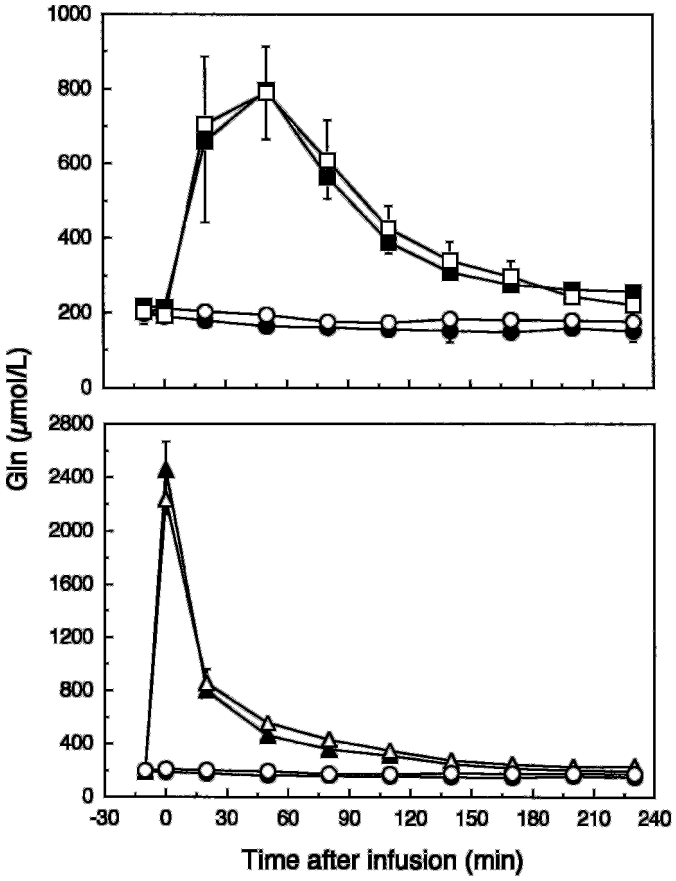

Figure 1. Plasma concentrations of Gln after infusion of saline (○, ●) and after duodenal (□, ■) or i.v. (△, ▲) infusion of 50 g of Gln. Cows were treated with an infusion of 600 g/d of starch into the rumen (open symbols) or into the duodenum (closed symbols). Error bars represent the standard errors of the means.

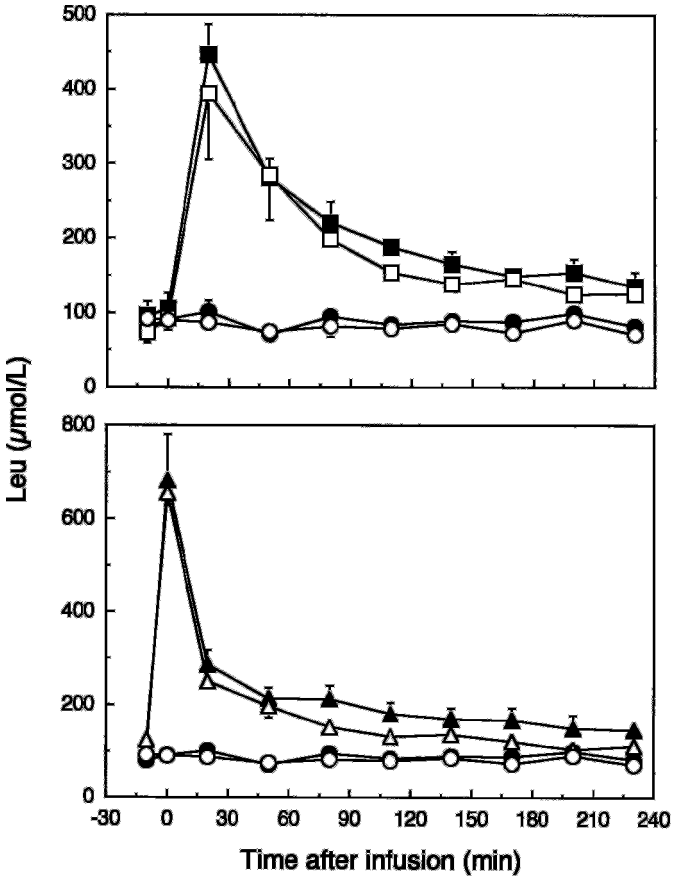

Figure 2. Plasma concentrations of Leu after infusion of saline (○, ●) and after duodenal (□, ■) or i.v. (△, ▲) infusion of 10 g of Leu. Cows were treated with an infusion of 600 g/d of starch into the rumen (open symbols) or into the duodenum (closed symbols). Error bars represent the standard errors of the means.

TABLE 3. Effect of site of starch infusion on kinetic parameters and relative bioavailability of Gln and Leu after duodenal or i.v. infusion.

Infusion and bioavailability	Site of starch infusion		SED	*P*
	Rumen	Duodenum		
Gln				
Duodenum				
Half-life of absorption, min	16.6	12.0	1.4	0.08
Half-life of elimination, min	43.1	38.8	7.3	0.61
Distribution volume, L	396	314	72	0.38
Area under the curve, mmol/L × min	58.0	54.4	6.3	0.63
Relative bioavailability, %	103	82	14	0.26
i.v.				
Half-life of distribution, min	5.7	8.3	0.8	0.08
Half-life elimination, min	38.8	47.7	4.5	0.19
Distribution volume, L	222	291	14	0.04
Area under the curve, mmol/L × min	57.5	67.4	4.8	0.18
Clearance, L/min	6.1	5.1	0.5	0.18
Leu				
Duodenum				
Half-life of absorption, min	9.4	7.0	. . .[1]	. . .
Half-life of elimination, min	68.3	63.5	15.7	0.79
Distribution volume, L	204	232	. . .[1]	. . .
Area under the curve, mmol/L × min	30.3	29.5	2.0	0.72
Relative bioavailability, %	106	143	51	0.60
i.v.				
Half-life of distribution, min	5.6	8.7	1.2	0.24
Half-life of elimination, min	98	89	20	0.74
Distribution of volume, L	272	276	13	0.81
Area under the curve, mmol/L × min	29.9	24.5	9.5	0.67
Clearance, L/min	2.5	3.1	1.0	0.64

[1]Statistics could not be estimated for the half-life of absorption and the distribution volume of Leu because of too few data.

g/d of starch should have been available in the intestine of cows that had starch infused into the rumen. Thus, the infusion of starch into the duodenum (600 g/d) increased the starch supply to the small intestine considerably. Both amounts seem to be within the capacity for starch digestion in ruminants [less than 960 g/d; (6)]; therefore, extra glucose should have been available to the intestine when starch was infused into the duodenum. Site of starch infusion, however, affected neither the elimination kinetics nor the relative bioavailability of Gln and Leu. The only parameters that were affected were the half-lives of absorption of Gln and Leu, which were always shorter when starch was infused into the duodenum (six of six observations). We have no explanation for this result, which was contradictory to our expectation, because transport of sugars and AA over the brush border membrane is known to be competitive (19).

Typically for Leu, but also in some individual cases for Gln, the AUC after duodenal infusion was larger than the AUC after i.v. infusion, resulting in a relative bioavailability of over 100%. This result was related to the rapid elimination of AA from peripheral plasma directly after i.v. infusion, which might have been an artifact related to the high initial concentrations after i.v. infusions. Nevertheless, the AUC after infusion of Gln or Leu into the duodenum did not increase when starch was infused into the duodenum. Thus, utilization of Gln and Leu by the splanchnic tissue was not influenced by starch supply to the duodenum. Because of the technique used (i.e., measurement of peripheral concentrations of AA), the results did not discriminate between utilization of AA by the intestinal wall or by the liver. Effects at the site of the gut wall might have been compensated by liver metabolism of AA. Also, it should be noticed that both energy and protein were fed above requirements because of decreasing production during the course of the experiments. Thus, AA catabolism might have been stimulated in the cows and might have been relatively insensitive to extra postruminal energy supply.

CONCLUSIONS

In dairy cows, differences in the absorption and elimination kinetics between Gln and Leu were in-

dicative of the difference in the utilization of these AA by the splanchnic tissue. Under the experimental conditions reported in which supply was at or above energy and protein requirements, extra starch supply to the duodenum did not affect utilization of Gln or Leu by the splanchnic tissue.

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