Effect of Glutamine Supplementation on Splanchnic Metabolism in Lactating Dairy Cows

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ABSTRACT

The suggestion that glutamine (Gln) might become conditionally essential postpartum in dairy cows has been examined through increased postruminal supply of Gln. Net nutrient flux through the splanchnic tissues and mammary gland was measured in 7 multiparous Holstein cows receiving abomasal infusions of water or 300 g/d of Gln for 21 d in a crossover design. Milk yield increased significantly (by 3%) in response to Gln supplementation, but the 2.4% increase in milk protein yield was not statistically significant. Glutamine treatment had no effect on portal or hepatic venous blood flows. Net portal appearance of Gln and Glu was increased by Gln supplementation, accounting for 83% of the infused dose with, therefore, only limited amounts available to provide additional energy to fuel metabolism of the portal-drained viscera. The extra net portal appearance of Gln was offset, however, by a corresponding increase in hepatic removal such that net Gln splanchnic release was not different between treatments. Nonetheless, the Gln treatment resulted in a 43% increase in plasma Gln concentration. Infusions of Gln did not affect splanchnic flux of other nonessential amino acids or of essential amino acids. Glutamine supplementation increased plasma urea-N concentration and tended to increase net hepatic urea flux, with a numerical increase in liver hepatic O₂ consumption. There were no effects on glucose in terms of plasma concentration, net portal appearance, net liver release, or postliver supply, suggesting that Gln supplementation had no sparing effect on glucose metabolism. Furthermore, mammary uptake of glucose and amino acids, including Gln, was not affected by Gln supplementation. In conclusion, this study did not support the hy-

pothesis that supplemental Gln would reduce glucose utilization across the gut or increase liver gluconeogenesis or mammary glutamine uptake to increase milk protein synthesis.

Key words: glutamine, amino acid, splanchnic, nutrient flux

INTRODUCTION

Glutamine (Gln) is a nonessential amino acid (NEAA) as it can be synthesized by mammalian tissues. Nonetheless, under situations of high metabolic demand, such as postsurgery or during infection, exogenous Gln has been shown to exert beneficial effects (Newsholme, 2001; Wilmore, 2001; Ziegler, 2001). Therefore, Gln may be considered conditionally essential during periods of physiological stress. Such a scenario may exist for the dairy cow during early lactation, when there may be a considerably larger demand for Gln than can be met from absorption, endogenous stores, and synthesis *de novo*. First, milk production is a large drain for Gln, with Gln and Glu together comprising 20% of milk protein AA (Jensen, 1995). Second, Gln is an important energy source for the gut in monogastrics: in piglets, Gln and Glu catabolism may account for 50% of the CO₂ produced by the gut (Reeds et al., 2000), comparable to the contribution from glucose (van der Schoor et al., 2001). In the immediate postpartum period, when the cow has a glucose deficiency of approximately 500 g/d (Bell, 1995), the gut may rely heavily on Gln as an energy source. Additional Gln supply may spare glucose catabolism by the gut, thereby increasing supply to peripheral tissues. As a glucogenic AA, Gln may also increase postsplanchnic glucose supply by enhancing hepatic gluconeogenesis. Third, Gln provides N to support nucleic acid synthesis (Gate et al., 1999) during the proliferation of the gut and liver, which increase their mass by 19 and 12%, respectively, during the first 8 wk postpartum (Gibb et al., 1992).

That Gln supply may be limited in early lactation is suggested by the observation that plasma Gln concen-

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trations are depressed and not restored to precalving levels even by 56 d into lactation, unlike other AA (Meijer et al., 1995b; Doepel et al., 2002). Although plasma concentration is not a direct reflection of whole-body flux, concentration depression over an extended period would suggest that dietary supply plus de novo synthesis of Gln are insufficient to meet the increased multiple demands during the postpartum period. Indeed, the output of Gln and Glu in milk protein is often less than their uptake by the mammary gland (MG; Guinard and Rulquin, 1994). Meijer et al. (1993) suggested that, at low plasma Gln concentrations, supply to the MG becomes a restriction that might be overcome by supplementation because MG uptake would increase in response to an increment in arterial Gln concentration.

Potentially, Gln supplementation may benefit a number of important processes in the early postpartum cow. Therefore, we hypothesized that Gln supplementation might improve milk and milk protein yields through 3 means: 1) sparing glucose utilization by gut tissue and increasing liver synthesis of glucose, 2) increasing hepatic release of Gln and thus peripheral tissue supply, and 3) increasing mammary uptake of Gln through increased arterial supply, thereby removing a limitation on milk protein secretion. Our objectives, therefore, were to determine the effect of postruminal Gln supplementation in early lactation cows on the net flux of Gln and other nitrogenous and energetic compounds across the gut, liver, and mammary gland, and on milk production and composition.

MATERIALS AND METHODS

Animals and Treatments

Eight multiparous Holstein cows with an average BW of 733 ± 51 kg were surgically implanted with abomasal catheters (Doepel et al., 2006) and with chronic indwelling catheters in the mesenteric, portal, and hepatic veins and the caudal aorta, via a mesenteric artery (Huntington et al., 1989; Blouin et al., 2002). The right carotid artery was surgically raised to a subcutaneous position to allow access to arterial blood in the event that the aortic catheter failed. Surgeries were performed a minimum of 6 wk before calving.

Continuous abomasal infusions were administered to the cows according to a crossover design with 21-d periods. The 2 treatments were 10 L/d water (control) or 300 g/d (85.6 mmol/h) of L-Gln delivered in 10 L of water (Gln). Infusions were initiated within 48 h following parturition and administered continuously using a peristaltic pump. Fresh L-Gln infusion solutions were prepared daily. The infusion rate of 300 g/d of Gln was based on observations of Gln metabolism in

previous studies. In sheep, Gln irreversible loss rate is approximately 1 mmol/h per kg^{0.75} (Hoskin et al., 2001). If scaled to a 700-kg dairy cow, this would amount to 3.2 mol/d (470 g/d). The decrease in Gln plasma concentration prepartum to 21 d postpartum is approximately 40% (Doepel et al., 2002). If this decrease represents a change in apparent net needs it would amount to approximately 190 g/d. The cow may actually synthesize more Gln to reduce the decrease in plasma concentration making the actual net flows of Gln to support metabolism during the transition period even greater; therefore, an additional 50% was added to the supplement.

Cows were fed a diet as described previously (Doepel et al., 2006). Briefly, a TMR that supplied 30.6 Mcal of NE_L and 2,067 g/d of MP at 18 kg of DMI/d was fed *ad libitum* twice daily at 0800 and 1600 h from d 1 to 17, and in 12 equal meals per day delivered at 2-h intervals by automatic feeders from d 18 to 21 of each experimental period. The TMR consisted of 38% corn silage, 20% grass hay, 19% high moisture corn, and 23% protein supplement consisting of 49% soybean meal, 29% Soyplus (West Central Soy, Ralston, IA), 5% Megalac (Church & Dwight Co. Inc., Princeton, NJ), and 17% mineral and vitamin supplements. The cows also received 20 g of rumen-protected methionine (Mepron 85, Degussa, Burlington, Ontario, Canada) and 2 kg of long alfalfa hay (11.9% CP, 32.9% ADF, and 56.1% NDF) once daily in the morning before the TMR was offered. Orts were recorded daily. Moisture content of the silages was determined weekly and used to make ration adjustments. Cows were given free access to fresh water. Cows were milked twice a day, at 0830 and 1930 h, and milk yield was recorded at each milking. Milk was sampled at each milking from d 15 to 21 of each period. The experimental protocol was approved by the Institutional Committee for Animal Care of the Lennoxville Research Centre, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Blood Sampling

On d 21 of each period, blood samples were collected into heparinized tubes simultaneously from the arterial, hepatic venous, and hepatic portal catheters every 45 min for 4 h (6 samples), covering 2 cycles of feeding. Blood samples were also obtained from the subcutaneous abdominal vein by venipuncture following the same 45-min sampling schedule. To determine plasma flow across the splanchnic tissues, para aminohippuric acid (pAH; 10% wt/vol) was infused into a mesenteric vein catheter (Huntington et al., 1989). A priming dose of 2 g was given a minimum of 40 min before the first blood

sample was obtained, followed immediately by a continuous infusion of 14.4 g/h. Immediately after collection, blood was placed on ice. Packed cell volume (PCV) was determined by the microhematocrit method. For lactate analyses, 1 mL of fresh blood was mixed with 0.9 mL of water and 0.1 mL of 6 N perchloric acid and left on ice for 1 h before centrifugation and collection of the supernatant. The remainder of the blood was centrifuged (15 min, $1,800 \times g$ at 4°C) within 30 min of collection to yield plasma. Urea-N and pAH were analyzed on fresh plasma samples. For AA analysis, 1 g of plasma was added to 0.2 g of an internal standard of AA labeled with stable isotopes and the processed samples were frozen at -80°C . The internal standard solution was prepared with labeled AA diluted in water with the following concentration (μM): DL-His- α - ^{15}N (180), L-Ile- ^{15}N (708), L-Leu- ^{13}C (864), DL-Lys-2- ^{15}N - ^2HCl (486), DL-Met-1- ^{13}C (86), L-Phe-1- ^{13}C (247), L-Thr- ^{15}N (375), L-Val- ^{15}N (835), L-1- ^{13}C -Ala (1,060), L- ^{15}N -Cys (227), L-1- ^{13}C -Glu (273), L-1- ^{13}C -Gln (949), L-1- ^{13}C -Gly (1,131), L-1- ^{13}C -Ser (491), and L-Tyr- ^{15}N (245). Labeled AA (95 to 99 atom %) were supplied by CDN isotopes (Montreal, Quebec, Canada) for His, Leu, Lys, Met, and Phe, and by Cambridge Isotope Lab (Andover, MA) for others. The remainder of the plasma was stored at -20°C for subsequent analysis of glucose. Additional samples of arterial, portal, hepatic, and mammary blood (2 mL) were collected into a blood-gas collection device (Monovette, Sarstedt, Aktiegesellschaft and Co., Germany) for the determination of pH and oxygen.

Laboratory Analyses

Milk fat was measured according to the Röse-Gottlieb method (AOAC, 1996), and milk nitrogen content (protein = $\text{N} \times 6.38$) was determined by combustion (Nitrogen Determinator, model FP-428, Leco, St. Joseph, MI). Milk true protein, whey, NPN, and casein contents were determined as described by Raggio et al. (2004), and AA concentrations were measured by the isotopic dilution method (Calder et al., 1999) after hydrolysis as described by Borucki Castro et al. (2007). Milk lactose was calculated as milk DM% - (fat% + protein% + ash%).

Partial pressure of oxygen and pH were determined in fresh blood using a pH/blood gas analyzer (model IL 1306, Instrument Laboratory, Lexington, MA). Plasma concentrations of urea-N and pAH were determined with an automatic analyzer (Technicon Autoanalyser II, Technicon Instruments Corporation, Tarrytown, NY) as previously described (Huntington, 1984), on the day of the sampling. Plasma concentrations of ammonia were determined within a week of sampling on samples that were frozen using an enzymatic reaction (glutamate dehydrogenase), as described by Bergmeyer and

Beutler (1985). Plasma glucose concentrations were determined colorimetrically using an enzymatic (glucose oxidase/peroxidase) reaction (Boehringer Mannheim, Dorval, Quebec, Canada). Plasma amino acid concentrations were measured by isotope dilution using GC-MS (Calder et al., 1999; Raggio et al., 2004). A spectrophotometric method using lactate dehydrogenase was used to measure blood L-lactate concentration (Benson et al., 2002). Blood hemoglobin was determined colorimetrically using cyanmethemoglobin as the standard.

Calculations

Plasma flow across the splanchnic tissues was calculated from downstream dilution of the infused pAH (Katz and Bergman, 1969). Milk AA output was calculated using milk protein yield measured during the last 3 d of each period, with a 3.5% correction for bloodborne proteins, and reported AA composition (Jensen, 1995), except for Phe and Tyr, which were analyzed. Mammary plasma flow was estimated according to the Fick principle using Phe and Tyr as internal markers again with allowance for a 3.5% contribution from bloodborne proteins (Cant et al., 1993): mammary plasma flow = $[(\text{milk Phe} + \text{Tyr}) \times 0.965] \div (\text{arterio-venous difference Phe} + \text{Tyr})$. The net fluxes of AA and glucose across the portal-drained viscera (PDV), liver, total splanchnic tissues (TSP), and mammary gland were calculated for each cow-period as the product of the average plasma venous-arterial concentration difference and the average plasma flow. Ammonia and lactate net fluxes were calculated using plasma and blood concentrations, respectively, and blood flow, whereas fluxes of urea were calculated using plasma water concentrations [plasma concentration / (1 - DM of plasma)] and blood water flows. Blood flow was calculated as plasma flow divided by (1 - PCV). Blood water flow was calculated as blood flow multiplied by (1 - DM) of blood (Milano et al., 2000). A negative flux indicates utilization or removal, whereas a positive flux indicates net production or release of the nutrient across the tissue. Concentrations of O_2 in blood were calculated using measured partial pressures of O_2 , pH, and hemoglobin concentrations (Bartels and Harms, 1959).

Statistical Analysis

Before statistical analysis, metabolite concentrations and net flux data were averaged over the 6 sampling times on d 21 of each period, and DMI, milk yield, and milk composition were averaged over the last 7 d of each period. All data were statistically analyzed using the GLM procedure of SAS (SAS Institute, 1999) with treatment, period, and cow as the main effects ac-

Table 1. Dry matter intake and milk production during the last 7 d of treatment of cows infused with water (control) or 300 g/d of Gln¹

Item	Treatment		SEM	P-value
	Control	Gln		
DMI, kg/d	18.6	18.1	0.19	0.10
Yield				
Milk, kg/d	39.3	40.5	0.30	0.04
CP, g/d	1,200	1,229	24.6	0.44
True protein, g/d	1,149	1,169	25.5	0.61
Casein, g/d	984	1,004	21.6	0.55
Whey, g/d	165	165	7.4	0.99
NPN, g/d	51	61	2.0	0.02
Fat, g/d	1,485	1,485	16.8	0.98
Lactose, g/d	1,838	1,903	37.3	0.27
Milk composition				
CP, %	3.08	3.05	0.06	0.72
True protein, % of CP	95.8	95.1	0.22	0.07
Casein, % of CP	82.2	81.5	0.49	0.40
Whey, % of CP	13.6	13.5	0.48	0.86
Fat, %	3.81	3.69	0.04	0.06
Lactose, %	4.73	4.68	0.10	0.76

¹Least squares means $\pm$ SEM; n = 7.

cording to the crossover design. Treatment differences were considered significant if $P \leq 0.10$ and as a trend for $0.10 < P \leq 0.15$. All data are reported as least squares means with pooled standard errors (SEM).

RESULTS AND DISCUSSION

DMI and Milk Yield

Dry matter intake was 0.5 kg/d lower ($P = 0.10$) in the Gln-supplemented cows than in the control cows (Table 1). Conversely, postprandial Gln supplementation increased ($P = 0.04$) milk yield by 1.2 kg/d over that of the control cows (Table 1). Within this same study, a larger number of animals also showed a milk yield increase of 1.9 kg/d during the first 3 wk postcalving in response to Gln supplementation (Doepel et al., 2006). In an earlier report, Meijer et al. (1995a) observed an increment of 1.3 kg/d in cows receiving 300 g of Gln/d compared with unsupplemented cows. The calculated milk to feed ratio was 2.11 for the control cows and 2.24 for the Gln-treated cows, which indicated that the latter were more efficient converters of feed to milk or that they mobilized greater amounts of body tissue in support of milk synthesis. Milk CP yield, although 29 g/d higher with the Gln treatment, was not statistically different between the 2 treatments (Table 1). Casein and whey concentrations, as a percentage of CP, were also not different between treatments. In contrast, NPN content as a percentage of milk CP increased ($P = 0.07$) with Gln supplementation. The 20% increase in milk NPN yield paralleled the 23% increase in plasma urea-N and was likely related to increased MUN. Milk fat concentration was lower ($P = 0.06$) by

Table 2. Blood and plasma flow and net flux of O₂ across the splanchnic tissues and mammary gland in Holstein cows infused with water (control) or 300 g/d of Gln¹

	Treatment		SEM	P-value
	Control	Gln		
Plasma flow, L/h				
Portal	1,531	1,570	59	0.66
Hepatic	1,843	1,963	87	0.38
Mammary	748	779	19	0.30
Blood flow, L/h				
Portal	2,132	2,198	89	0.63
Hepatic	2,569	2,749	129	0.37
Mammary	1,047	1,092	29	0.33
O ₂ flux, ² mmol/h				
Portal-drained viscera	-3,140	-3,142	161	0.99
Hepatic	-3,923	-4,107	162	0.46
Total splanchnic tissues	-7,068	-7,227	253	0.68

¹Least squares means $\pm$ SEM; n = 7.

²Negative values indicate net removal, whereas positive values indicate net release across the tissue.

0.12% units with the Gln treatment but total milk fat yield was unaltered because of the higher milk yield. Decreases in milk fat content can be attributed to a dilution effect associated with increases in milk volume.

There was a period effect for several parameters. Dry matter intake was 1.9 kg higher in period 2 than in period 1 ($P < 0.001$) with an associated increase ($P = 0.09$) in milk yield: 40.5 vs. 39.5 (SEM = 0.30) kg/d. Period effects were expected because the cows approached peak lactation in period 2. Both milk fat and protein content decreased from period 1 to period 2 (data not shown) as usually observed when lactation progresses.

Plasma Flow and Oxygen Consumption

Gln supplementation had no effect on portal and hepatic plasma flows (Table 2), which averaged 1,550 and 1,903 L/h, respectively. Previous studies have shown no effect of increased protein supply on portal and hepatic plasma flow in dairy cows (Bach et al., 2000; Blouin et al., 2002; Raggio et al., 2004). Mammary blood flow averaged 1,070 L/h, and was not affected by treatment.

Oxygen consumption by the PDV, liver, and TSP was not significantly affected by Gln supplementation (Table 2). Nonetheless, the numerical increase in liver O₂ consumption was twice that needed theoretically to cover the observed increase in ureagenesis (4 ATP or 0.66 mol of O₂ per mol of urea synthesized: 87 mmol/h). This is in agreement with other studies (e.g., Milano et al., 2000), in which increases in O₂ consumption, greater by 2 to 8 times the theoretical need, have been associated with elevated ureagenesis.

Table 3. Effect of postprandial Gln supply on plasma arterial AA concentrations (μM) in Holstein cows infused with water (control) or 300 g/d of Gln¹

Amino acid	Treatment		SEM	P-value
	Control	Gln		
Essential AA				
Histidine	51.3	49.4	3.3	0.71
Isoleucine	172.8	160.3	15.5	0.60
Leucine	169.1	162.2	14.5	0.75
Lysine	73.8	68.5	3.9	0.38
Methionine	22.2	19.9	0.9	0.13
Phenylalanine	43.7	41.3	1.4	0.31
Threonine	83.6	66.9	4.8	0.06
Tryptophan	47.8	38.0	2.4	0.05
Valine	260.0	241.9	23.4	0.61
Nonessential AA				
Alanine	198.8	160.6	12.8	0.09
Cysteine	99.1	87.5	5.5	0.20
Glutamate	51.7	55.7	2.3	0.28
Glutamine	218.2	312.9	20.1	0.02
Glycine	325.0	266.0	9.8	<0.01
Serine	65.9	58.2	4.5	0.29
Tyrosine	46.1	35.9	1.6	<0.01

¹Least squares means $\pm$ SEM; n = 7, except for Trp where n = 6.

Splanchnic and Mammary Flux of AA

Plasma arterial AA concentrations are shown in Table 3. Several recent studies have shown that net fluxes are not different across the mammary gland (Pacheco-Rios et al., 1998; Thivierge et al., 2002) or the TSP tissues (Lobley et al., 2001) when measured with either blood or plasma. Plasma analysis of AA concentrations was selected for this study because of the additional advantage that the coefficient of variation is lower compared with that in blood. In response to supplementation, the concentration of Gln increased by 43%, whereas concentrations decreased for all other AA except Glu, the effect being significant only for Thr, Trp, Ala, Gly, and Tyr. These slight decreases may reflect the lower DMI with equivalent milk protein output in the Gln-treated cows. Overall, arterial concentrations of AA were not affected ($P > 0.05$) by period; however, there was a tendency ($P < 0.10$) for higher Trp (38.4 vs. 47.4 μM) but decreased Glu (57.3 vs. 50.1 μM) and Gly (310.6 vs. 280.5 μM) concentrations in the second period.

Net fluxes of AA were considered both for individual AA and the main groups of AA based on their MG metabolism as described by Mephram (1982). This classification has been reinforced recently based on their corresponding behavior across the splanchnic tissues (Lapierre et al., 2005). The group 1 AA have net stoichiometric transfers of their mammary uptake into milk protein, and include His, Met, Phe (+ Tyr), Trp, and Thr. These AA also have a high rate of hepatic removal, with postliver supply matching mammary up-

Table 4. Effect of postprandial Gln supply on net fluxes of nonessential amino acids (mmol/h) in Holstein cows infused with water (control) or 300 g/d of Gln¹

Amino acid	Tissue ²	Treatment		SEM	P-value
		Control	Gln		
Alanine	PDV	84.6	86.9	3.8	0.69
	Liver	-64.8	-76.0	8.3	0.39
	TSP	19.7	10.8	6.3	0.37
	MG	-30.4	-23.7	1.6	0.03
Cysteine	Milk ³	17.6	17.4	0.2	0.43
	PDV	3.9	2.9	0.8	0.43
	Liver	-6.0	-6.3	1.7	0.93
	TSP	-2.2	-3.3	1.6	0.62
Glutamate	MG	-4.9	-3.7	1.4	0.58
	Milk	2.7	2.7	0.03	0.43
	PDV	9.4	13.6	0.9	0.03
	Liver	31.4	27.9	2.1	0.28
Glutamine	TSP	40.8	41.4	2.1	0.85
	MG	-21.3	-22.9	0.7	0.17
	Milk	39.1	38.6	0.4	0.43
	PDV	4.2	70.6	5.3	<0.01
Glycine	Liver	3.1	-65.5	5.7	<0.01
	TSP	7.2	5.1	2.8	0.62
	MG	-27.2	-19.9	4.5	0.31
	Milk	30.6	30.2	0.3	0.43
Serine	PDV	43.2	40.6	1.6	0.31
	Liver	-61.2	-65.1	4.9	0.61
	TSP	-18.0	-24.5	5.1	0.42
	MG	-6.5	-1.9	3.2	0.36
Tyrosine	Milk	11.4	11.3	0.1	0.43
	PDV	40.7	41.2	1.7	0.85
	Liver	-29.7	-33.9	2.8	0.34
	TSP	11.0	7.3	2.0	0.26
Total AA-N ⁴	MG	-19.1	-16.0	1.4	0.18
	Milk	28.5	28.1	0.3	0.43
	PDV	21.4	22.0	1.1	0.72
	Liver	-8.7	-10.3	1.9	0.55
Total AA-N ⁴	TSP	12.7	11.6	1.7	0.68
	MG	-13.4	-13.4	0.2	0.99
	Milk	14.0	13.8	0.2	0.43
	PDV	502.1	487.7	24.4	0.69
Total AA-N ⁴	Liver	-162.6	-197.1	27.0	0.41
	TSP	339.5	290.6	23.5	0.20
	MG	-334.9	-312.9	18.4	0.44
	Milk	323.4	318.9	3.7	0.43

¹Least squares means $\pm$ SEM; n = 7; negative values indicate net removal, whereas positive values indicate net release across the tissue.

²Tissues: PDV = portal-drained viscera, TSP = total splanchnic tissues, MG = mammary gland.

³Calculated as milk protein yield $\times$ 0.965 $\times$ AA composition (Jensen, 1995) except for Phe and Tyr, which were analyzed.

⁴Excluding Gln.

take (Lapierre et al., 2005). The group 2 AA includes other EAA in which mammary uptake exceeds milk protein output (Mephram, 1982). These AA show little, if any, net hepatic removal, and include the branched-chain AA (Ile, Leu, and Val) plus Lys.

Net portal appearances of Gln and Glu were increased by Gln supplementation (Table 4). For Glu, net portal appearance increased by 4.2 mmol/h (45%; $P = 0.03$), whereas for Gln, the increase was approximately

16-fold (66.4 mmol/h; $P < 0.001$). Together, these represented 83% of the infused dose of Gln. On the control treatment, the net portal absorption of Gln and Glu was close to zero, as observed previously for dairy cows (Berthiaume et al., 2001, 2006; Blouin et al., 2002), indicating that both Gln and Glu supplied by RUP and microbial protein are largely used by the PDV. This is similar to observations in sheep in which net absorption of Gln is either zero (Nieto et al., 2002) or negative (El-Kadi et al., 2006). In milk-fed piglets, the PDV not only removes all dietary Glu and Gln, but also extracts both from the arterial supply to leave a negative net portal absorption, with this being more substantial for Gln than Glu. Indeed, catabolism of these AA by the porcine PDV is sufficiently extensive to account for approximately 50% of the tissue energy requirements (Stoll et al., 1999; van der Schoor et al., 2001). When provided with additional protein supply, piglets show minimal additional appearance of Gln in the portal vein (van der Schoor et al., 2001), indicating that the majority of the dietary supply is catabolized. Such data contrast with the high recovery of the infused Gln across the PDV in the current study. This may relate to species differences because work in vitro (Oba et al., 2004) has suggested that ovine enterocytes might rely less on Gln as an energy source than nonruminants, although glucose oxidation was still suppressed by supplementation of the medium with Gln or Glu. Furthermore, provision of Gln to a culture medium incubating enterocytes from early lactation dairy cows did not alter glucose uptake, although there was a decrease in glucose oxidation and elevated Glu release (Okine et al., 1995). Data from in vivo studies with other ruminants do not clarify this issue. In dairy cows, abomasal infusions of incremental doses of casein increased net portal absorption of Gln with recoveries from 0 to 100% (Hanigan et al., 2004). In sheep, nonsignificant improvements in absorption of Gln into the mesenteric vein were observed although recoveries ranged numerically from 0 to 80% (El-Kadi et al., 2006). Another possibility is that, in the current study, the maximal ability of the PDV to metabolize Gln in the high-yielding cow was met by the amount supplied in the basal diet. Alternatively, the requirements for Gln to either provide energy or synthesize metabolites, such as nucleic acids (Gate et al., 1999), might have been met by that available from the basal diet. Finally, requirements for Gln elsewhere in the body may have resulted in a sparing of use by the digestive tract. Although the last reason is very attractive in terms of the hypotheses under examination, nearly all of the additional Gln absorbed from the supplement was removed by the liver (Table 4). Within the limits of the procedures used, this meant that net splanchnic release was not different between treatments.

Portal absorption of the other NEAA was not affected by treatment (Table 4). Likewise, hepatic uptake of the NEAA (other than Gln) and splanchnic flux did not change with Gln supplementation. In general, net portal appearance of the EAA was not influenced by treatment (Table 5), with only Trp PDV appearance reduced ($P = 0.04$) by Gln supplementation. As previously reported (Lapierre et al., 2005), there was a substantial removal of group 1 AA across the liver and this was not affected by Gln infusion, with postliver supply averaging 63% of net portal appearance. With regard to the group 2 AA, hepatic uptake of Lys tended ($P = 0.11$) to be greater in the Gln-treated cows and together, hepatic removal of group 2 AA increased ($P = 0.05$) with Gln infusion, resulting in a tendency ($P = 0.12$) for reduced postliver supply of these AA.

Contrary to the suggestion advanced by Meijer et al. (1993), increased arterial concentrations of Gln did not enhance mammary uptake of Gln (Table 4). Otherwise, of the NEAA, only Ala MG uptake was affected by treatment ($P = 0.03$), being 22% lower for the Gln treatment. In both treatments, however, Ala uptake was in excess of milk protein requirements, which would provide C-skeletons for glucose or galactose synthesis (Bequette et al., 2006) or act as an energy source with, in both cases, the N available to meet any shortfall in the other NEAA. For the group 2 AA, Lys had a lower ($P = 0.07$) mammary uptake with Gln infusion but the total of group 2 AA was not affected by treatment (Table 5). There was also no difference in mammary gland uptake of group 1 AA, either individually or overall.

Splanchnic and Mammary Flux of Metabolites

Glutamine infusion increased ($P = 0.02$) the arterial concentration of urea-N but had no effect on concentrations of ammonia, lactate, or glucose (Table 6). Increased concentrations of urea resulted from a trend ($P = 0.15$) toward elevated hepatic ureagenesis, with the increment (262 mmol of urea-N/h) more than sufficient to account for the increase in hepatic removal of Gln-N (137 mmol/h), other AA-N (35 mmol/h), and ammonia (50 mmol/h). The small N shortfall might arise from increased removal of other AA not measured in this study (e.g., Arg, Asn, Cit). Based on correlation analyses between ureagenesis and hepatic removal of AA, Reynolds (1992) suggested that liver detoxification of ammonia to urea involves increased AA catabolism. Metabolism of Gln in the mitochondria of periportal hepatocytes involves release of the amido-N as ammonia (Meijer, 1985), a key element in maintaining synthesis of carbamoyl phosphate, an intermediate in the ornithine cycle. Although the extreme penalties on N metabolism predicted for ammonia detoxification are

Table 5. Effect of postruminal Gln supply on net fluxes of essential amino acids (mmol/h) in Holstein cows infused with water (control) or 300 g/d of Gln¹

Amino acid	Tissue ²	Treatment		SEM	P-value
		Control	Gln		
Histidine	PDV	9.7	9.5	0.4	0.75
	Liver	-2.9	-3.8	0.9	0.49
	TSP	6.8	5.8	0.9	0.43
	MG	-9.4	-8.8	0.6	0.47
	Milk ³	8.3	8.2	0.1	0.43
Isoleucine	PDV	28.4	29.8	1.7	0.59
	Liver	3.0	-2.1	2.4	0.19
	TSP	31.3	27.6	2.8	0.40
	MG	-25.7	-25.2	0.9	0.72
	Milk	21.4	21.1	0.2	0.43
Leucine	PDV	44.0	46.5	2.9	0.57
	Liver	3.0	-2.6	2.8	0.22
	TSP	47.0	43.9	4.0	0.61
	MG	-38.4	-38.1	1.0	0.84
	Milk	35.6	35.1	0.4	0.43
Lysine	PDV	35.8	36.1	2.0	0.92
	Liver	4.0	-2.8	2.5	0.11
	TSP	39.8	33.3	2.8	0.16
	MG	-35.2	-31.0	1.3	0.07
	Milk	27.0	26.6	0.3	0.43
Methionine	PDV	14.0	12.6	2.0	0.64
	Liver	-4.4	-4.8	1.1	0.79
	TSP	9.6	7.8	1.3	0.37
	MG	-8.4	-7.7	0.3	0.18
	Milk	8.9	8.8	0.1	0.43
Phenylalanine	PDV	25.4	25.5	1.5	0.94
	Liver	-10.1	-11.1	1.5	0.64
	TSP	15.3	14.4	1.2	0.63
	MG	-14.9	-14.5	0.2	0.22
	Milk	14.1	13.9	0.2	0.43
Threonine	PDV	26.5	26.5	1.1	0.99
	Liver	-7.0	-10.1	2.4	0.40
	TSP	19.5	16.4	2.0	0.32
	MG	-19.1	-18.1	0.6	0.23
	Milk	17.2	16.9	0.2	0.43
Tryptophan	PDV	7.4	5.7	0.4	0.04
	Liver	-3.6	-3.3	1.0	0.84
	TSP	3.8	2.5	0.7	0.28
	MG	-4.7	-3.6	0.8	0.37
	Milk	3.5	3.5	0.1	0.43
Valine	PDV	32.6	36.0	3.2	0.50
	Liver	2.1	-7.4	4.4	0.19
	TSP	34.8	28.6	4.1	0.33
	MG	-31.6	-29.4	1.3	0.30
	Milk	26.8	26.4	0.3	0.43
Group 1 AA-N ⁴	PDV	135.2	123.4	7.0	0.29
	Liver	-47.3	-48.4	8.9	0.94
	TSP	87.9	75.0	7.3	0.27
	MG	-90.8	-86.7	4.4	0.54
	Milk	86.1	85.0	1.0	0.43
Group 2 AA-N ⁵	PDV	181.6	185.5	9.8	0.79
	Liver	14.9	-18.8	9.4	0.05
	TSP	196.5	166.7	11.1	0.12
	MG	-166.0	-156.4	5.8	0.30
	Milk	137.8	135.9	1.6	0.43

¹Least squares means $\pm$ SEM; $n = 7$, except for Trp where $n = 6$; negative values indicate net removal, whereas positive values indicate net release across the tissue.

²Tissues: PDV = portal-drained viscera, TSP = total splanchnic tissues, MG = mammary gland.

³Calculated as milk protein yield $\times 0.965 \times$ AA composition (Jensen, 1995), except for Phe and Tyr, which were measured.

⁴Group 1 includes His, Met, Phe, Thr, Trp, and Tyr.

⁵Group 2 includes Ile, Leu, Lys, and Val.

no longer considered valid, there is evidence that small rates of AA catabolism may be necessary (Milano et al., 2000). The current observations would be compatible with this concept, with the increased ureagenesis due to the hepatic removal of Gln resulting in increased hepatic removal of group 2 AA (Table 5). This is a particularly interesting observation because removal of extra group 1 AA would penalize milk protein production whereas the group 2 AA are supplied beyond the liver in amounts in excess of uptake by the MG and needed for milk protein synthesis. Therefore, posthepatic tissues other than the MG are also involved in catabolism of these AA (see Lobley and Lapierre, 2003). If degradation in these sites were reduced to accommodate the additional losses in the liver, then milk protein synthesis would not be compromised. Indeed, peripheral tissue utilization of group 2 AA (postliver supply minus mammary uptake) decreased numerically from 31 ± 1 mmol of AA-N/h, whereas mammary gland uptake (166 ± 6 mmol of AA-N/h) and mammary gland removal not used for milk (28 ± 5 mmol of AA-N/h) were unchanged. Such changes in peripheral catabolism of AA to deficiency of Leu (Bequette et al., 1996) or Lys (Lapierre et al., 2003), both group 2 AA, has been demonstrated previously.

Our data do not support a sparing effect on glucose across the PDV with increased Gln supply, because net portal appearance was not increased with Gln supplementation (Table 6). With only 17% of the infused Gln not recovered across the gut, this would, at best, spare only an additional 7 mmol/h of glucose, far from the required hepatic gluconeogenesis. There was also no indication that the increased hepatic Gln removal was used toward gluconeogenesis.

In conclusion, the results of this study did not support our hypotheses that Gln supplementation would improve milk and milk protein yields via effects on Gln and glucose availability and metabolism. There was no apparent sparing of glucose across the PDV (indeed, little of the supplemental Gln was metabolized by the PDV), nor was there increased hepatic gluconeogenesis. Furthermore, although the arterial concentration of Gln was increased, this did not result in increased MG uptake. The majority of the infused Gln-N was converted to urea or otherwise catabolized by the liver. This study suggests that strategies specifically designed to enhance Gln supply during early lactation are unlikely to increase milk protein yield, if a diet comparable in quality to that provided in this study is offered. The reason why arterial Gln concentrations take longer than other AA to return to precalving concentrations during early lactation remains unknown, but this might reflect a successful mechanism to cope with the increased demand when both endogenous and exogenous

Table 6. Effect of postruminal Gln supply on metabolite arterial concentrations and net fluxes in Holstein cows infused with water (control) or 300 g/d of Gln¹

Metabolite	Treatment		SEM	P-value
	Control	Gln		
Ammonia concentration, mM	0.047	0.042	0.015	0.81
Ammonia flux, mmol/h				
PDV	683	711	46	0.68
Liver	-668	-718	57	0.56
TSP	15	-7	18	0.44
Urea-N concentration, mM	9.9	12.2	0.50	0.02
Urea-N flux, mmol/h				
PDV	-429	-430	43	0.99
Liver	1,015	1,277	109	0.15
TSP	586	847	119	0.18
Lactate concentration, mM	0.39	0.39	0.01	0.90
Lactate flux, mmol/h				
PDV	163	174	5.4	0.22
Liver	-292	-296	22.5	0.91
TSP	-129	-122	21.0	0.83
Glucose concentration, mM	2.88	2.73	0.10	0.35
Glucose flux, mmol/h				
PDV	17.2	0.9	34.1	0.75
Liver	676	652	39.0	0.68
TSP	693	653	17.5	0.17
MG	-566	-528	24.0	0.31

¹Least squares means $\pm$ SEM; n = 7; negative values indicate net removal, whereas positive values indicate net release across the tissue.

²Tissues: PDV = portal-drained viscera, TSP = total splanchnic tissues, MG = mammary gland.

supplies are insufficient without penalizing vital processes.

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