Glutamine Is a Potentially Limiting Amino Acid for Milk Production in Dairy Cows: A Hypothesis

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Recently, extensive research has been focused on glutamine because of its key position between energy and protein metabolism. Evidence is growing that glutamine is essential in situations of metabolic stress; practical application of this knowledge can already be found in parenteral nutrition of severely ill patients. Furthermore, glutamine is claimed to increase muscle protein synthesis. Glutamine and its counterpart, glutamic acid, are the most abundant amino acids in milk protein. Nevertheless, the role of nonessential amino acids (NEAA) in milk protein synthesis in high-yielding dairy cows has been practically neglected during the past 20 years. Evaluating current literature on glutamine metabolism in ruminants with emphasis on data related to milk protein production, we conclude the following: (1) Ruminants have a relatively low glutamine synthetase capacity compared with monogastric species, reflected in relatively low plasma glutamine levels; (2) The uptake of glutamine by the mammary gland is effectively 100% of the arterial supply; (3) Milk production in high-yielding dairy cows represents a metabolic stress comparable to fasting or acidosis; and (4) Responses of plasma and tissue glutamine pools in conditions of "metabolic stress," including high milk production, resemble those of most essential amino acids (EAA). Therefore we hypothesize that glutamine, although regarded as a NEAA, limits milk protein synthesis in high-yielding dairy cows.

STUDIES ON LIMITING amino acids for milk production have focused mainly on the essential amino acids (EAA).[1-15] It is now generally accepted that methionine and, to a lesser extent, lysine are the first limiting amino acids for milk production in lactating cows.[5,10,11,16] Yet results from studies in which methionine was supplemented in a form that is protected against degradation during passage through the rumen are not concordant. Illg et al[13] found higher milk yield and milk protein production, while others reported only increased milk protein production with no effect on overall milk yield.[3,14] However, in most studies using methionine in a form that passes unchanged through the rumen, either no effect or only a slight effect on milk production was found.[1,9,12,16-18] These different findings have led to the conclusion that together with methionine a larger group of five to 10 EAA are colimiting for milk production, with the colimiting EAA thus masking the effect of supplemented methionine and/or lysine. But as Mepham[11] stated, "with over 20 amino acids involved, the possible number of permutations of order and degree of rate-limiting seems effectively infinite."

Halfpenny et al.[19] put forward the hypothesis that some nonessential amino acids (NEAA), particularly glutamic acid and proline, might limit synthesis of milk proteins, after having observed an increase in plasma glutamic acid level and milk protein production in response to ruminal infusion of propionic acid in two cows. Their study was criticized by Mepham and Linzell,[20] who could not confirm their findings infusing a mixture of NEAA (including glutamic acid and glutamine) in the mammary artery of goats. The possibility of NEAA being (co)limiting for milk protein synthesis has received little attention since then. Part of the reason for this may be found in the assumptions made in the definition of a limiting amino acid. For instance, Clark et al[5,7] assume that a limiting amino acid has a relatively low plasma concentration combined with a high extraction rate by the mammary gland; this approach excludes most (if not all) NEAA. However, results from Derrig et al[4] indicate that the extraction rate of glutamic acid by the mammary gland (70% to 74%) is the highest of all amino acids. From their data it can be calculated that glutamic acid output in milk is 244% of mammary arterial uptake. This means that 85 g/d glutamic acid is synthesized in the mammary gland, which is approximately ten-fold the amount of most other NEAA. Apparently, glutamic acid synthesis rate by the mammary gland was not considered to be limiting for milk production by these investigators, since the conclusion was made that methionine and lysine were first limiting.[4] Similarly, in a recent study by Seymour et al[14] it was found that abomasal infusion of methionine and lysine increased the plasma ratio of EAA to NEAA while glutamine concentration decreased sharply. The investigators concluded that "the demand for NEAA appears to have been increased by infusion of methionine and lysine," yet the NEAA were not considered to be potentially colimiting.

Recently, the indispensability of some NEAA in clinical nutrition and animal feeding has attracted renewed attention.[21,22] Heger[21] found that optimum protein utilization in growing rats is influenced by the presence of proline, asparagine, and glutamic acid. During the last decade, the physiological importance of glutamine has been recognized in humans and other nonruminant mammalian species, and has been reviewed extensively by Lacey and Wilmore[22] and Bulus et al.[23] It has been known since the 1950s that glutamine is an essential substrate for bacterial and tissue culture growth.[24-27] Other studies have shown that supplementation of glutamine in parenteral nutrition in clinical settings improves intestinal function (decreases necrosis)

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and immune system function[28-30] and results in a faster recovery of the patient.[31,32] Glutamine is an obligate substrate for several tissues characterized by a high protein turnover such as tumor cells,[25,26] cells of the immune system,[27] and intestinal mucosa of several species[33-35] including ruminants.[36,37] In these organs, glutamine not only serves as an energy source, but also as a precursor for nucleotides (adenosine triphosphate [ATP], guanosine triphosphate [GTP], and nicotinamide adenine dinucleotide [NAD]), purines, and pyrimidines, thus providing essential components for cell replication.[38] Glutamine plays an important role in the acid-base balance of the body by being the major NH_3 donor to the kidney for neutralization of urine during acidosis.[39-42]

Glutamine is the most abundant amino acid in the plasma of humans (500 to 650 μmol/L)[43] and several other monogastric species including the rat (600 to 1,000 μmol/L)[33,35] and the dog (600 to 750 μmol/L).[44] Muscle cells show even higher free-glutamine levels—about 30 times plasma levels[45]—and are considered to function as a glutamine reserve.[37,39,40,46] Some investigators suggest a direct relationship between muscle free-glutamine concentration and muscle protein synthesis.[47-49]

The metabolic pathways and functions of glutamine in different organs are summarized in Fig 1. It is apparent that glutamine occupies a key position between energy and protein metabolism. Furthermore, glutamine seems to be indispensable in cases of metabolic stress.[22,50] The high rate of protein synthesis in the lactating mammary gland suggests an essential role for glutamine, yet little is known about the role of glutamine in the process of milk protein synthesis. This review aims to summarize and evaluate data on glutamine and glutamic acid in relation to lactation in the dairy cow.

PLASMA VALUES OF GLUTAMINE AND GLUTAMIC ACID IN RUMINANTS

The amide of glutamine is readily hydrolyzed in the presence of glutaminase and glutamine is chemically degraded to ammonia and pyrrolidonecarboxylic acid at low pH, especially if the temperature is above 4°C; therefore precautions must be taken during sample preparation and analysis to obtain reliable values for both glutamine and glutamic acid.[22,51-56] Due to this instability of glutamine together with the inability of several widely used chromatographic techniques to separate glutamine from glutamic acid, both amino acids are frequently reported as a summation value or are not reported at all. Moreover, when only glutamic acid is reported (which also occurs frequently), high values may be suspect due to contamination from glutamine.

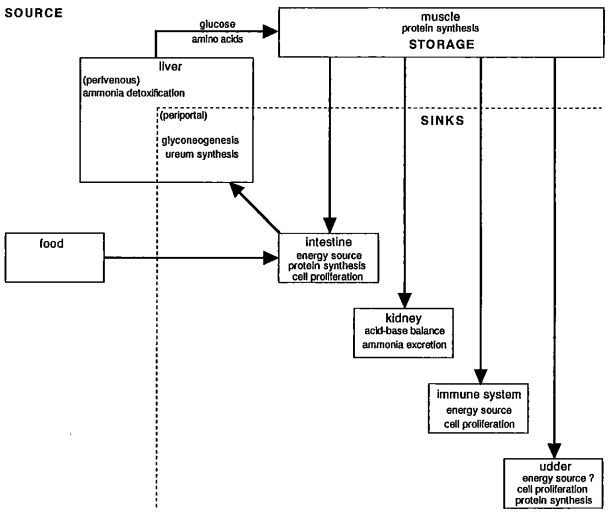

Fig 1. Schematic view of the metabolic pathway of glutamine.

Table 1. Average Values of Arterial and/or Venous Plasma Concentrations of Glutamic Acid and Glutamine in Ruminants (μmol/L)

	Glutamic Acid			Glutamine				Reference
	Min	Mean	Max	Min	Mean	Max	A/V	No.
Sheep*	56		59	173		225	A	59
Calves*	41		76	174		214	A	58
			73			400-500	V	63
Lactating cows	42		88		—		V	1
	49		69		—		V	2
	8		131	50		160	V	3
		52			—		V	4
		60			—		A	7
		39			—		V	12
	35		77		—		V/A†	13
	49		57	184		342	V	14
	149		180		—		V	15
	22		59			297-449	V/A†	16
		44			—		V	17
	62		73	177		243	V	60
		55			258		V	61
		75			—		V	81
Beef steers		83			255		A	62
	83		85			520-550	A	64
	146		150	195		237	A	57

NOTE. When studies comprised different treatments (dietary, infusions, etc) with an effect on plasma concentrations, minimum and maximum values are given, ie, average value in treatment with lowest level *v* average value in treatment with highest level. Otherwise, mean values are given.

Abbreviations: A, arterial; V, venous.

*Minimum values for fasted animals; maximum value for fed animals.

†Minimum value measured in venous plasma; maximum value measured in arterial plasma.

Plasma free-glutamine values in dairy cows are relatively low and show large variations in comparison to those in monogastric species.[34,43] Table 1 summarizes arterial and venous plasma levels of glutamic acid and glutamine as reported in different studies. Both values for plasma glutamic acid and minimum values for plasma glutamine show very good agreement, despite the fact that the results have been obtained in different laboratories over a period of 25 years. Plasma glutamic acid values range from approximately 40 to 80 μmol/L. The extreme values for plasma glutamic acid reported by Fisher,[3] Whitelaw et al,[15] and Reynolds and Huntington[57] may possibly be explained by inadequate precautions having been taken during analysis.

The lowest values for glutamic acid observed in lactating cows are in agreement with values observed in fasted calves[58] and sheep.[59] Similarly, the lowest values of plasma glutamine observed in lactating cows (170 to 190 μmol/L) are concordant with values in fasted calves and sheep. However, maximum values of glutamine show more variation in calves, lactating cows, and beef steers. Values of 200 to 300 μmol/L are observed,[14,57-62] but concentrations of 400 to 600 μmol/L also have been reported.[16,63,64] This difference is independent of sampling site.

Because the size of the plasma free–amino acid pool is very small compared with the total amino acid content of the body, it has been suggested that plasma amino acid levels should readily reflect uptake by tissues and transfer between pools, and therefore should be sensitive indicators of inadequate or excessive supply.[65] Plasma glutamine level in the ruminant shows quite a large range, with an absolute minimum of 170 to 190 μmol/L. This minimum was found in starved animals,[58,59] in animals with acute acidosis,[59] and in animals on different treatments aimed at providing a higher intake of casein or EAA. Although the decrease of plasma NEAA level after infusion of protein or EAA is generally recognized,[5,11] it should be stressed that this decrease is mostly due to a sharp decrease of the glutamine level to minimum values. This suggests that high milk production is a metabolic stress leading to inadequate supply of glutamine to the udder.

INTESTINAL ABSORPTION AND UTILIZATION OF GLUTAMINE AND GLUTAMIC ACID IN RUMINANTS

Glutamine has been shown to be the major energy source for intestinal tissues in a variety of monogastric species.[33-35] Glutamine and glucose may be used by the intestinal mucosa at equimolar rates. However, CO_2 production from glutamine is approximately fivefold that from glucose.[32] Glutamine accounts for 30% to 46% of total CO_2 production, whereas CO_2 derived from glucose adds only 6% to 10%.[33-35] High availability of glucose in the intestinal lumen has not been observed to reduce glutamine oxidation in the intestine.[32] More than 50% of available glutamine is fully oxidized,[32,33] while only 3% of available glucose is oxidized to CO_2.[32]

In ruminants, the significance of glutamine as an energy source for the intestine is less well understood. Bergman[66] showed that volatile fatty acids (VFA), glucose, and glutamic acid account for 41%, 53%, and 6% (on a molar basis), respectively, of intestinal substrate utilization in sheep. Other measurements of net utilization of energy-yielding substrates by the splanchnic bed in ruminants suggest that VFA are the most important energy source (81 to 142 mmol/h),[67] followed by glucose (17 to 123 mmol/h),[57,68,69] glutamine (11 to 20 mmol/h),[57,70] and glutamic acid (0.5 to 6.0 mmol/h).[57,70,71] In a study by Harmon,[72] glucose uptake by rumen papillae of Holstein steers in vitro was approximately twofold that of glutamine, whereas CO_2 production from glucose was sevenfold that from glutamine and the oxidation of glutamine could be decreased by addition of butyrate and glucose.

Although extensive metabolism of glutamine in the intestinal wall of ruminants has been shown,[73] all available data suggest that glutamine is not the predominant respiratory fuel for the intestine in ruminants, as it is in monogastric species. In contrast to data from monogastrics, intestinal utilization of glutamine in ruminants decreases with increasing availability of other energy substrates.[57,72] Concordantly, oxidation of peripherally infused ^{14}C-labeled glutamine to CO_2 in lactating dairy cows is slow and delayed, as it is for most EAA.[74] This suggests that in the cow both glutamine and EAA are conserved and hence remain available for tissue and milk protein synthesis.

GLUTAMINE METABOLISM IN LIVER, KIDNEY, AND MUSCLE

The liver may act as a glutamine source or sink. Regulation of arterial plasma levels of glutamine by the liver is nicely illustrated by the data of Reynolds and Huntington[57] (Fig. 2). Net absorption of glutamine over portal-drained viscera was negative on a Lucerne diet in beef steers. Despite this negative net absorption, the arterial plasma level of glutamine increased significantly after the meal. However, despite positive absorption of glutamine from the gut on meal-fed concentrate (78% ground corn), peripheral plasma concentration was not affected by the meal. In periportal hepatocytes, urea cycle enzymes and glutaminase are present and active, while glutamine synthetase is found in perivenous hepatocytes.[75] Perivenous glutamine synthetase functions as a scavenger for the ammonia that has escaped periportal urea synthesis[76]; thus the blood is detoxified before entering the peripheral tissues. This pathway is very important during acidosis when urea synthesis is slowing down to spare bicarbonate ions and more ammonia is reaching the perivenous part of the liver. During acidosis, the kidney shifts from glutamine release to glutamine uptake.[40,41,59] Glutamine is deamidated and deaminated and ammonia is secreted into the tubular lumen, neutralizing the acid urine. Both the liver and muscle cells supply glutamine to the circulation during acidosis.[39] However, in sheep glutamine release from the hindquarters was not increased during acidosis (Table 2), suggesting that most of the extra glutamine supply to the kidney originated in the liver. In this study by Heitmann and Bergman,[59]

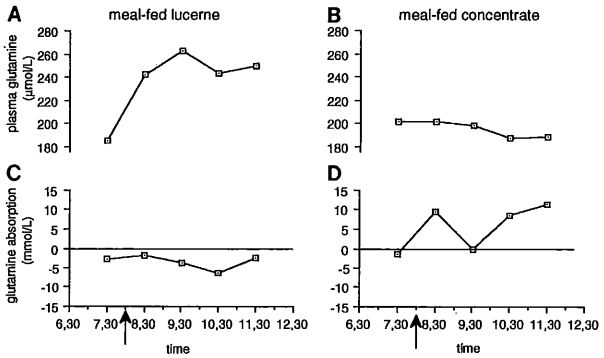

Fig 2. Arterial plasma concentration (A and B) and net absorption (C and D) of glutamine in beef steers on meal-fed lucerne (A and C) and meal-fed concentrate (B and D). The arrow indicates the morning feeding. (Data from Reynolds and Huntington.[57])

Table 2. Glutamine Uptake (−) and Release (+) From PDV, Liver, Kidney, and Hindquarters of Sheep (mmol/h)

	Fed	Fasted	Acidotic
PDV	−1.09	−1.81	−1.35
Liver	−2.15	−1.82	−0.96
Kidney	+0.66	−0.30	−1.75
Hindquarters	+1.07	+1.68	+0.90
Balance	−1.51	−2.25	−3.16

Data from Heitmann and Bergman.[57]

glutamine balance over three vascular beds (splanchnic, renal, and hindquarters) was found to be increasingly negative in, respectively, the fed, fasted, and acidotic state, suggesting that glutamine release from the liver and/or muscle is underestimated or, to quote Welbourne,[40] "the major [glutamine] source is as yet unidentified."

ROLE OF GLUTAMINE AND GLUTAMIC ACID IN MILK PROTEIN SYNTHESIS

Glutamine and its counterpart, glutamic acid, together make up 18% to 23% of milk protein–bound amino acids,[4,77] and are therefore quantitatively most abundant in milk protein. The uptake of glutamic acid by the mammary gland ranges from 49% to 74% of the arterial supply[4,5,78] and is generally found to be higher than that for any other amino acid. Glutamic acid uptake by the mammary gland was 59 g/d, which is three times the uptake by the intestine.[72] Similarly, uptake of glutamine as a percentage of the arterial supply is high and comparable to that of lysine, methionine, and other EAA,[14] whereas for most other NEAA much lower extraction rates are reported. In addition to the high uptakes of glutamic acid and glutamine, 85 g/d glutamic acid is synthesized in the mammary gland, which is 10-fold the production of other individual NEAA.[4]

Data from Seymour et al,[14] who measured arteriovenous differences over the mammary gland in cows on different dietary treatments, allow a closer look at the uptake of glutamine by the mammary gland. Plotting glutamine uptake by the mammary gland versus arterial plasma glutamine level reveals a linear relationship (Fig 3). For other amino acids, such a relationship is not evident from their data. The slope of the regression line almost equals 1, and the X-intercept is a plasma value of 185 μmol/L. From these data it may be concluded that (as we stated before) 170 to 190 μmol/L is a minimum plasma glutamine level in dairy cows. Below this level, net uptake of glutamine by the mammary gland does not occur. However, above this level, uptake of glutamine by the mammary gland equals 99%, suggesting that the mammary gland shows a high preference for taking up glutamine above synthesis of glutamine or glutamic acid. Changes in glutamine uptake by the mammary gland explained 54% of the variance in milk protein production ($P < .05$), whereas changes in plasma total EAA level explained 42%. No other significant correlations between amino acid plasma levels or uptake with milk protein production could be found in their data.[14]

Most plasma amino acid levels tend to decrease at about

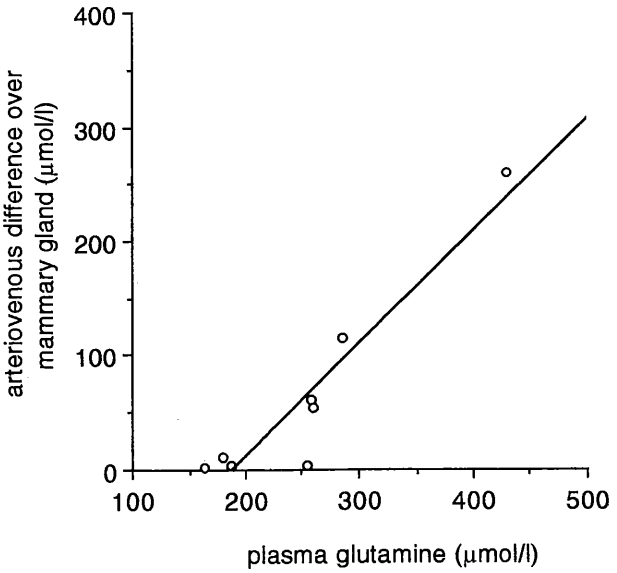

Fig 3. Relationship between arterial plasma glutamine concentration and mammary uptake of glutamine. Y = 185 + 0.99×; r^2 = .89, $P < .05$. (Calculated from data of Seymour et al.[14])

the time of parturition, but generally show a rapid return to normal levels after a few days.[60] However, glutamine and alanine levels showed a second decrease 2 days after the onset of lactation; plasma glycine levels increased simultaneously, suggesting muscle catabolism.[60] Our unpublished observations show that low glutamine levels persist during 15 weeks of lactation (Fig 4). It is noteworthy that in our experiments and in the study by Verbeke et al[60] the lowest plasma glutamine levels were approximately 180 μmol/L.

HYPOTHESIS

Although data relating glutamic acid and especially glutamine to milk production in dairy cows are scarce, some general conclusions can be drawn from current literature. A large range of normal plasma free-glutamine values has been observed; however, there is consistency in the data regarding the minimum value of 170 to 190 μmol/L. The high uptake of glutamic acid by the mammary gland was already observed during the 1960s[79] and has been con-

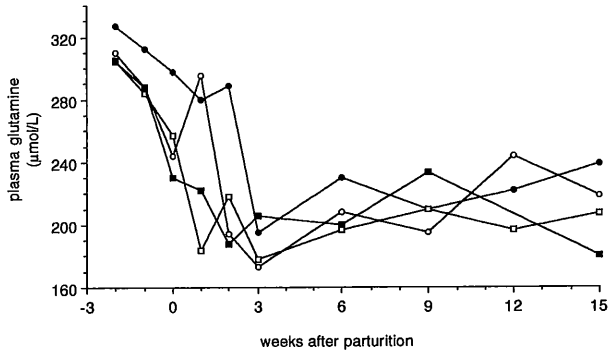

Fig 4. Venous plasma concentration of glutamine in four dairy cows from 2 to 15 weeks after parturition (Meijer et al, unpublished observations).

firmed by numerous studies.[5] As we have shown above, uptake of glutamine by the mammary gland appears to be 99% of the arterial supply, taking into account the minimum plasma level of 185 μmol/L. These data suggest that glutamine and glutamic acid are of great importance to the mammary gland, in agreement with the high quantitative output of both amino acids in milk protein. In addition to the high uptake of both amino acids, the production of glutamic acid in the mammary gland is massive when compared with other NEAA.[4] It thus seems likely that glutamic acid taken up by the mammary gland is not used for other purposes than being a direct precursor for milk protein. The glutamic acid produced may originate directly from glutamine, since the uptake ratio of glutamine to glutamic acid is 2:1[14] and the output ratio in milk appears to be 1:2.[77] Regarding milk protein synthesis, it would appear that glutamic acid is in fact more important to the udder than glutamine, but glutamine is the form in which the amino acid is transported across the mammary cell membrane. This situation would be analogous to glutamic acid/glutamine exchange across the erythrocyte membrane[80] and would explain the extremely high uptake of glutamine by the udder. It could also explain the lack of effect on protein synthesis observed by Mepham and Linzell[20] after infusion of glutamic acid into the mammary gland.

In conclusion, we believe that the udder of a high-yielding dairy cow stakes a large claim on plasma glutamine, resulting in plasma values comparable to those in starved or acidotic animals. This suggests that there is an upper limit to glutamine production in liver, muscle, and udder. Therefore, using the words of Halfpenny et al,[19] we hypothesize that the "glutamic acid pool" in the mammary gland is potentially limiting for milk protein production in dairy cows, with glutamine being the most important arterial supply for this pool.

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