REVIEW

Review: Do Horses Receive Optimum Amounts of Glutamine in Their Diets?

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ARTICLE INFO	ABSTRACT
	In some species of growing mammals glutamine is an essential amino acid that, if inadequate in the diet, is needed for normal growth and development. It is thus sometimes considered to be a conditionally essential amino acid in some species. A review of studies that have measured L-glutamine concentrations ([glutamine]) in horses demonstrates that plasma [glutamine] has routinely been reported to be much lower (~330 µmol/L) than in other mammals (> 600 µmol/L). Plasma [glutamine] represents the balance between intestinal transport into the blood after hepatic first pass, tissue synthesis and cellular extraction. The hypothesis is proposed that sustained low plasma [glutamine] represents a chronic state of sub-optimal glutamine intake and glutamine synthesis that does not meet the requirements for optimum health. While this may be without serious consequence in feral and sedentary horses, there is evidence that provision of supplemental dietary glutamine ameliorates a number of health consequences, particularly in horses with elevated metabolic demands. The present review provides evidence that glutamine is very important (and perhaps essential) for intestinal epithelial cells in mammals including horses, that horses with low plasma [glutamine] represents a sub-optimal state of well-being, and that horses supplemented with glutamine exhibit physiological and health benefits.

1. Introduction

Glutamine is an important metabolite involved in gluconeogenesis, lipolysis, antioxidant defense, the production of nitric oxide, the secretion of peptides (e.g. glucagon-like peptide 1, GLP-1), neuromediators, the regulation of cell growth, regulation of cellular function and cell / tissue regeneration [1-5]. The importance of glutamine in numerous cellular processes, and the fact that plasma concentrations ([glutamine]) appears to be quite low (by ~50%) in most horses studied compared to other mammals, suggests that dietary glutamine may

be inadequate in many horses. Unfortunately, the dietary requirement for glutamine has been considered only with respect to protein synthesis. However dietary glutamine requirements also need to be considered with respect to its numerous other functions in the body. For example, in other species inadequate glutamine is associated with increased incidence and severity of respiratory disease [6], brain disorders [7], type 1 diabetes [5], slowed muscle glycogen synthesis [4], impaired gut morphology and health [8-11] and impaired immune health [12,13].

The purposes of this review are to report the concentrations of glutamine in equine plasma and muscle in studies

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published in the past 50 years and to use a comparative physiology approach to demonstrate that dietary glutamine ingestion appears to be low in most horses studied, and perhaps in the general horse population. Some of the equine studies reviewed herein have already suggested that horses may benefit from dietary glutamine supplementation. Many amino acids, including glutamine, are ingested in proteins and peptides as well as free amino acids as part of the normal diet. Some amino acids (glutamine for example) can be synthesized within the body, while others cannot. The ability to synthesize certain amino acids varies by species and developmental stage. In horses, except for lysine, the dietary requirements for amino acids have not been defined. The recommended protein intake [14] may provide adequate amino acids for protein synthesis related to growth and development, however recent studies have reported that the dietary supply of some amino acids may be in inadequate. With respect to protein synthesis, these amino acids now include lysine [15], threonine [15,16] and methionine [17].

Under normal conditions, the body appears to attempt to meet glutamine demand primarily by *de novo* synthesis (Figure 1) from endogenous glutamate and branched-chain amino acids within tissues such as skeletal muscle [18-21], liver [18] and placenta [19]. Plasma [glutamine] is therefore the result primarily of tissue glutamine synthesis and tissue glutamine extraction [3,20], and secondarily of dietary intake especially in the first few hours after meal ingestion [22-24]. During periods of increased metabolism (exercise, gestation, lactation) a state of protein catabolism often occurs [18,19,22,25]. The sustained or increased tissue glutamine demand and metabolism during these states are associated with reduced plasma [glutamine] (Table 1). Under these conditions glutamine has been termed a "conditionally essential" amino acid [2,3,8,12,26,27].

2. Importance of glutamine

The term glutamine will be used to refer specifically to L-glutamine, one of twenty amino acids that are used to build proteins under the guidance of the genetic codes. Glutamine exists in two zwitterionic forms, L-glutamine and D-glutamine. Because both the amino and carboxyl groups are attached to the first (alpha, α) carbon, glutamine is classified as an α-amino acid. Glutamine is also neutral, i.e., it possesses no electrical charge. Glutamine is additionally the amide of glutamic acid, another naturally occurring amino acid, and is involved in various metabolic activities including the formation of glutamate, and the synthesis of proteins, nucleotides and amino sugars.

The total amount of glutamine in the body is approximately 400 g in adult horses [18]. Glutamine plays important roles within intestinal tissues and skeletal muscle, and the body as a whole, including regulation of cellular gene expression, neuronal excitability, protein turnover, cellular metabolism, immunity and acid-base balance [27-29]. The amino acids glutamine and glutamate make up 10-20% of dietary protein, and both are extensively metabolized in the small intestine of most mammals. Watford [30] asserts that, with normal levels of dietary intake (5 - 10 g of glutamine daily for humans), there is no net small-intestinal absorption of glutamine or glutamate into the blood, such that body's glutamine pool results from de novo synthesis, primarily within skeletal muscle. In various mammals, including humans, about 20% of dietary glutamine may end up in the systemic circulation, but this is dependent on the amount of glutamine ingested and the metabolic state. Therefore the high requirement for glutamine by intestinal enterocyte and immune cells result in considerably less glutamine entering the systemic circulation than what is ingested.

There is now good evidence that glutamine, and some other non-essential amino acids, are not synthesized in sufficient amounts during periods of increased metabolic rate to support fetal development, neonatal growth, growth during lactation and as needed to maintain optimal vascular health, intestinal health and immune function in adult animals [27]. These amino acids have therefore been re-classified as "conditionally essential" [2,31] or "functional" amino acids (FAAs) because of their inadequacy in the diet, particularly in young and gestating mammals and during normal periods of increased metabolism such as during exercise and physical training. Inadequate intake of FAAs leads to functional deficits due to impairments in the regulation of key metabolic pathways involved in health, growth, development, reproduction and lactation [10,28,30,32]. Fürst et al. [33] characterized glutamine as a "conditionally indispensable amino acid during stress", where stress is the commonly-used physiological term to describe the normal physiological responses that result in elevated metabolism.

Glutamine should be considered to be a "conditionally essential" α-amino acid that is nutritionally important for many animals including horses and humans, particularly during periods in which the metabolic state of the animal is normally elevated, such as during normal exercise, gestation, lactation, growth and development [2]. Supplementation of FAAs such as glutamine, in amounts adequate to meet nutritional and metabolic requirements, has been proposed [2,8] as a nutritional strategy to maintain or improve health, growth and development and to prevent diseases. Xi et al. [28] reported that adequate, high concentrations of intracellular and extracellular glutamine are

associated with marked reductions in infection, sepsis, severe burn, cancer, and other pathologies. For example, oral glutamine supplementation in healthy humans performing moderate intensity exercise prevented the exercise-induced increase in intestinal permeability [34], thus maintaining integrity of the intestinal - immune system during periods of elevated metabolism.

Skeletal muscle is the major tissue that synthesizes glutamine by virtue of its mass in the body. The enzyme glutamine synthetase catalyzes the synthesis of glutamine from ammonia and glutamate. Mammalian skeletal muscle comprises approximately 40% of lean body mass, and intramuscular glutamine serves as a regulator of the anabolic state of this tissue. In nourished mammals, skeletal muscle releases glutamine into the circulation at a rate of 40 - 60 mmol/h [32,35]. When dietary intake of glutamine is low (e.g., as a result of typical horse forage) circulating concentrations of glutamine are low, and about 13-60% of that found when dietary glutamine is high (Table 1). The circulatory system provides a means of transporting glutamine to those cells that require it and that are not capable of synthesizing adequate amounts to meet their demands (Figure 1).

The liver, like skeletal muscle, both synthesizes and consumes glutamine. The enzymes for each process are compartmentalized to different hepatic cell systems [30]. The liver normally produces a small amount of glutamine and plays a role in fine-tuning plasma [glutamine].

In the kidneys, glutamine serves as the major substrate for ammoniagenesis, the process of removing nitrogen from the body, and in whole-body acid-base balance [30]. The glomeruli filter glutamine, but it is nearly completely resorbed by the renal tubules. During extended periods of increased metabolism (prolonged exercise, physical training, pregnancy, lactation, some diseases, including some cancers) resulting in net whole-body catabolism, there is a large increase in immune and intestinal cellular glutamine utilization, as well as increased hepatic extraction where glutamine is used for acute phase protein synthesis and glucose production. Exocrine signals acting on skeletal muscle result in a net proteolysis within muscle cells and increased net glutamine synthesis. This is often accompanied by decreased intestinal glutamine utilization [30], with consequent impairment of intestinal and immune function [28].

Glutamine is converted to glutamate in the brain and serves important roles in neurotransmitter regulation. In particular, glutamate regulates the neurotransmitter gamma-aminobutyric acid (GABA), which is required for brain functioning and mental activity. Glutamine newly synthesized from ammonia and glutamate by astrocytes

within the brain is extracted by neurons. Enzymes then hydrolyze the intracellular glutamine back to glutamate, some of which is decarboxylated to produce GABA, or transaminated to aspartate [10].
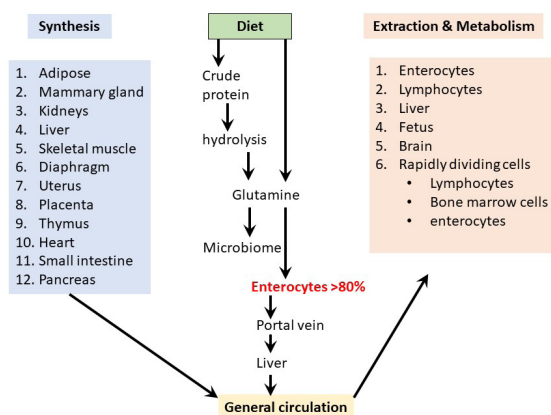

Figure 1. An overview of the tissues involved in the synthesis, transport and metabolism of glutamine. Most circulating glutamine is produced by glutamine-synthesizing tissues. Within the small intestine, greater than 80% of glutamine is extracted by and metabolized by enterocytes. Very little ingested glutamine enters the general circulation

Glutamine is essential for lymphocyte proliferation (which are unable to synthesize glutamine) and other rapidly dividing cells, such as gut mucosa and bone marrow (mesenchymal) stem cells; inadequate glutamine supply is associated with impaired function in these cellular systems [12,13,35]. High rates of leukocyte (particularly lymphocyte) glutamine extraction and utilization has led to the classification of glutamine as an immunostimulant.

Glutamine serves as a metabolic precursor for other important amino acids such as arginine, citrulline and proline. Within the intestinal system (splanchnic bed), unoxidized glutamine and proline serve as important precursors for citrulline synthesis, which is then converted to arginine in the kidney [36]. Arginine is a semi-indispensable amino acid in neonates and serves numerous metabolic roles in young and adult mammals [3,12].

Glutamine also plays important roles in whole-body biochemical and energy regulation. For example, it serves as a substrate for several amidotransferases that synthesize purines, pyrimidines, NAD, glucosamine and asparagine [30,32]. Most of the body's glutamine is hydrolyzed to glutamate and ammonia via the action of glutaminase. Glutamate can, in turn, be converted into glutathione, proline, ornithine and arginine, it can also be catalyzed to produce glucose, or it can be oxidized to produce ATP. The carbon is excreted as carbon dioxide and the nitrogen is excreted

as ammonia and urea.

Amino acids, through a large variety of inhibitory mechanisms and signaling pathways, act to regulate gene expression in numerous cell types within the body. Transcription factors mediate these effects, including specific regulatory sequences, such as amino acid response elements that are sensitive to changes in amino acid concentration [37]. In particular, glutamine, at appropriate concentrations, enhances numerous cell functions by activating various transcription factors. Some of the better-understood functions include the inflammatory response, cell proliferation, cell differentiation and survival, and several metabolic functions.

In summary, Ruth and Field [38] identified the following metabolic functions for glutamine:

(1) serves as a precursor and energy substrate for immune and epithelial cells;

(2) is important for intestinal development and function and for maintaining the integrity of the gut barrier, the structure of the intestinal mucosa, and redox homeostasis;

(3) supports proliferative rates and reduces enterocyte apoptosis;

(4) protects against pathogenic bacterial damage to intestinal structure and barrier function;

(5) lowers inflammatory response and increases immunoregulatory cytokine production; and improves the proliferative responses and numbers of intestinal immune cells.

2.1 The Small Intestine - Immune System Relationship

The present understanding of the intimate relationship between the gut (entire gastro-intestinal system) and the immune system has recently been presented by Ruth and Field [38] and Miron and Cristea [39]. There is considerable agreement amongst mammalian studies across species, and it is highly likely that the key elements observed in other mammals are transferable to horses:

(1) the intestine is the main site of nutrient absorption and amino acid metabolism, and the gut-associated lymphoid tissue (GALT) is also the largest immune system organ in the body.

(2) the enterocytes play multiple roles with respect to immune function and maintenance of immune health, including protection against oral pathogens, inducing oral tolerance to food stuffs, and maintaining a healthy interaction with commensal bacteria.

(3) the enterocytes also maintain barrier function between luminal contents (external environment) and the internal environment of the body (also reference [40]). This barrier function is dependent on dietary glutamine availability (also reference [34]).

(4) dietary amino acids (in particular, glutamine, glutamate, arginine, and perhaps methionine, cysteine and threonine) are essential to optimize the enterocytes' and intestinal immune cells' immune functions (i.e., dendritic cells, beta cells, macrophages, T cells). Each has unique properties essential for maintaining the intestine's integrity, growth and function, and for regulating local tissue and organ immune responses.

Glutamine supplementation has been shown to be effective in maintaining a normal intestinal barrier against pathogens and preserve mucosal integrity [8,34,38,39,41-43]. Using rodent models of intestinal mucosal obstruction and injury (similar to an equine obstructive small intestinal "colic"), glutamine supplementation prevented the large increases in intestinal permeability and bacterial translocation seen in non-supplemented animals [42,43].

3. Are Horses Deficient in Glutamine?

Compared to other amino acids, the concentrations of glutamine are relatively high in plasma (0.30 - 2.0 mmol/L depending on species and stage of development) and skeletal muscle (up to 3 mmol/L or approximately 60 mmol/kg wet weight). These concentrations provide an indication of the importance of glutamine within the body. With low to normal amounts of dietary glutamine, up to 100% of the glutamine ingested with protein is utilized by cells of the small intestine. In this typical situation, none of the dietary glutamine enters the systemic circulation [30]. Instead, plasma concentrations of glutamine are maintained by de novo synthesis from metabolic precursors. In this sense glutamine is considered to be non-essential.

In horses, the pre-feeding or fasting tissue glutamine concentrations reported in most studies (Table 1) are considerably lower than those reported in well-fed, healthy humans [44] (500 - 700 µmol/L) and rats [45,46] (700 - 1,000 µmol/L), and horses [47] (900 - 1,000 µmol/L). The fact that research horses receiving a near-optimum diet have average plasma [glutamine] ranging from 880 to 1,020 µmol/L [47] lends further support to the theory that dietary glutamine may be physiologically limiting in many horses.

3.1 Horses at Rest

In adult horses a number of studies published over a nearly 50-year period have reported an average plasma glutamine concentration of about 300 µmol/L (Table 1). The lowest values appear to be 150 µmol/L [48] and the highest values about 1,000 µmol/L [47]. In foals peak values occur at 2 weeks, and decline to values seen in adults by time of

Table 1. Fasting or pre-feeding plasma and muscle glutamine concentrations in horses

Study	Breed (number)	Age	Tissue	Concentration[1]
Johnson and Hart 1974 [72]	Mixed (12)	adult	Plasma	293 ± 21
Rogers et al. 1984 [22]	QH mares (10)	10 - 12 months gestation and 1st 3 weeks lactation	Mare plasma Mare plasma Mare plasma Foal plasma	300 (12 w prepartum) 600 (1-2 d postpartum) 511 (3 w postpartum) 736 (3 w postpartum)
Russell et al. 1986 [73]	QH (6)	22 months	Plasma	15^2
Miller & Lawrence 1988 [64]	QH (6)	adult	Plasma Plasma	493 ± 48 (low protein) 393 ± 18 (high protein)
Miller-Graber et al. 1990 98	unknown (4)	adult	Muscle	$\sim 450 \pm 40$
Jahn et al. 1991 [54]	TB (24)	2 - 4 years	Plasma	227 ± 14
Duckworth et al. 1992 [75]	Mixed (7)	adult	Plasma	572 ± 24
Silver et al. 1994 [50]	Pony mares (12)	At 235 - 308 days of gestation	Maternal plasma Fetal plasma Maternal at 36 h fast	370 ± 20 510 ± 50 247 ± 31
Zicker et al. 1994 [25]	Mixed (6)	45 - 47 week gestation mares	Facial artery Uterine vein Umbilical artery Umbilical vein	268 ± 10 270 ± 14 682 ± 42 723 ± 27
King & Suleiman 1998 [69]	TB (6)	adult	Plasma	367 ± 19
Routledge et al. 1999 [53]	Mixed (19)	6 - 12 years	Plasma	470 ± 15 310 ± 20
Robson et al. 2003 [55]	Endurance traineda	9.4 ± 2.2 years	Plasma	279 ± 16
Harris et al. 2006 [66]	TB (6)	5 - 9 years	Plasma	320 ± 30 280 ± 20 250 ± 25
Hackl et al. 2006 [24]	Various (10) 40 day trial	9 - 14 months	Plasma	304 ± 9 (start study) 345 ± 15 (end study)
Hackl et al. 2009 [62]	SB trotters (36)	2 - 10 years	Plasma	385 ± 16*
Manso Filho et al 2008 [18]	SB (3)	1., 10 & 30 years	Skeletal muscle	3,000 to 5,000
Manso Filho et al. 2008 [19]	SB (8)	Pregnant mares	Plasma Plasma Plasma Skeletal muscle	290 ± 20, 7 w prepartum 510 ± 20, parturition 290 ± 15, 8 w postpart. $7,000 \pm 700$ (all times)
Manso Filho et al. 2009 [20]	SB (8)	Mares at birth	Amniotic fluid Placenta	310 ± 25 $2,800 \pm 2,100$
Urschel et al. 2010 [48]	TB (6)	4 - 8 years	Plasma	149 ± 21 155 ± 20
Van den Hoven et al. 2010 [21]	SB (10)	2.5 - 6 years	Plasma Skeletal muscle Skeletal muscle	403 (209 - 663) $1,800 \pm 1,100$ $2,500 \pm 1,200$
Westermann et al. 2011 [61]	SB (10)	20 + 2 months	Plasma	392 ± 62*
Nostell et al. 2012 [63]	SB trotters (12)	4 - 9 years	Plasma	255 ± 30
Urschel et al. 2012 [47]	Arabian (12)	9 - 22 years	Plasma	$1,060 \pm 60$
Peters et al. 2013 [60]	Warmblood mares (6)	12 + 3 years	Plasma	281 ± 40
Tanner et al. 2014 [16]	Not stated (6)	6 mo weanlings	Plasma	561 ± 24
Mastellar et al. 2016 [23]	TB (6 & 6)	176 + 30 days Adult mares	Plasma Plasma	610 ± 20 424 ± 19
Mastellar et al. 2016 [97]	TB (6)	1 year	Plasma	223 ± 42 $\sim 1,000 \pm 110$

Notes: Values are mean $\pm$ standard error. [1] Plasma: μmol/L; Muscle, placenta: μmol/kg wet weight. [2] reported values in this paper are 10-fold less than likely, which would make this ~ 150 μmol/L. * Significant decrease with exercise. QH = quarter horse; SB = Standardbred; TB = Thoroughbred.

weaning [49]. Skeletal muscle [glutamine] is typically an order of magnitude greater than that of plasma, but also declines rapidly (within 1 month) in foals [49].

Manso Filho et al. [49] examined muscle and plasma [glutamine] during the first year of life in Standardbred foals. Glutamine was one of the most abundant free α-amino acids in skeletal muscle at birth. The concentration at 7 days declined by more than 50% by 6 months with no change thereafter. The initially high glutamine concentrations can in part be explained by provision of glutamine in mother's milk, and occurring at a time of elevated glutamine synthesis in mares that doubles plasma [glutamine] at the time of parturition [19,22] and mirrors elevated umbilical plasma vs maternal plasma [glutamine] [25,50]. As solid foods are introduced and the horse is weaned, then plasma [glutamine] decreases to reflect the balance between tissue requirements (related to metabolic activity) and dietary intake. One interpretation is that low plasma glutamine concentrations reflects a high demand for glutamine, thus the prevalent plasma [glutamine] of ~300 µmol/L may indicate a state of suboptimal glutamine synthesis and provision.

The fact that so many equine studies report relatively low plasma glutamine concentrations, while there is evidence that plasma glutamine in horses can be as high as normally seen in humans, raises the possibility of chronic hypoglutaminemia in the general horse population. It is proposed that a chronic hypoglutaminemia would be due to inadequate dietary supply of glutamine [2,27] combined with inadequate rates of endogenous glutamine synthesis from metabolic precursors. From these results it is concluded that the low tissue glutamine concentrations reported in most equine studies reflect typical equine diets that are low in dietary sources of glutamine such that dietary supplementation of glutamine may be appropriate.

3.2 Horses with Elevated Metabolic Needs

3.2.1 Pregnancy and Lactation

During pregnancy, the fetus extracts glutamine from the placental circulation [49] and umbilical [25] plasma [glutamine] is more than double that of mares' general circulation [25]. There is a large increase (approx. doubling) of plasma [glutamine] between 2 weeks pre-partum and the first few days post-partum [22]. Lactation is also very metabolically demanding period: the mammary glands extract glutamine during lactation and [glutamine] is abundant in the milk of lactating mares [19], although. After peaking at 1 - 2 weeks of lactation, by 3 months of lactation in mares' plasma and milk [glutamine] had decreased by more than 50% which, together with loss of lean body mass, is indic-

ative of a mild catabolic state [49]. The authors concluded that the decrease in circulating [glutamine] during lactation, when large amounts of glutamine are being extracted by the mammary gland, "means that glutamine availability for maternal organs, such as the small intestine and immune cells, may be limiting as lactation proceeds".

Despite the capacity of key tissues, predominantly skeletal muscle, to synthesize glutamine, these data, though limited, suggest that increased dietary glutamine intake would be needed in order to maintain adequate (> 500 µmol/L) plasma [glutamine]. Plasma [glutamine] averaged 360 µmol/L pre-fasting in seven lactating mares, but fell to 247 µmol/L after 36 h of fasting and recovered to only 318 µmol/L 6 h after additional feeding [50]. While fetal plasma [glutamine] were nearly double those of their mares [22,49], the fetal plasma [glutamine] similarly decreased after fasting [22]. This, in part, reflects the high requirement of the developing fetus for glutamine, despite the high capacity of the placenta to synthesize glutamine [20].

Dietary composition also has pronounced effects on plasma glutamine concentrations in the transition (peripartum through to beginning of lactation) mare; the dietary provision of even non-glutamine-containing supplements added to forage more than doubled plasma [glutamine] [22]. This provides evidence that the provision of other nutrients to glutamine-producing cells and tissues, increases the production and release of glutamine by these cells / tissues into the blood at a crucial time when glutamine is in high demand by other cells / tissues. It is also an indication that whole body glutamine demands have not been adequately met prior to provision of additional nutrients. In horses in a catabolic state, dietary supplementation of glutamine can help minimize or prevent the catabolic state and be used to maintain steady [glutamine] essential for intestinal and immune function and health [51].

Consistent with the equine studies cited above, Wu [52] considers dietary glutamine to be "substantially inadequate" to meet the requirements for protein synthesis in extra-intestinal tissues in growing pigs. By extension, this author infers that such is the case for mammals during periods of elevated metabolism (exercise, lactation, active growth and development [27]). A typical diet does not provide sufficient arginine, proline, aspartate, glutamate, glutamine, or glycine for optimum protein accretion in growing pigs [52].

3.2.2 Exercise

Exercise, whether of long-term low intensity or short-term high intensity, imposes significant increases in cellular and whole body metabolism often associated with increased

skeletal muscle proteolysis [35,53]. As a result the intramuscular and plasma concentrations of some amino acids and ammonia increase. Plasma [glutamine] also rises in part as a result of proteolysis and in part due to an increased requirement to detoxify ammonia [54].

In horses, humans and rodents, moderate to high intensity or duration exercise results in immune function suppression [35,55-57]. Exercise typically, but not always [54], depresses plasma glutamine [35,57], but this result also needs to be considered in the context of intensity of exercise and timing of post-exercising sampling. Glutamine supplementation also enhances the immune response to intense exercise, effects that appear to be mediated by intestinal/immune system interaction [35,58,59]. Glutamine supplementation prevents the increase in intestinal permeability that occurs during moderate intensity exercise [34]. Neutrophils, which comprise 50-60% of the total leukocyte count, elicit some of the beneficial effects seen with glutamine supplementation [56].

Supplemented, exercise-conditioned rats performed one hour of exercise at 85% of peak VO_2 [56]. In one group of rats, glutamine was supplemented by oral gavage one hour before exercise. Compared to the control group that did not receive glutamine, the supplemented rats' neutrophils had significantly increased phagocytic capacity. The supplemented rats also showed a smaller decrease in nitric oxide production than normally seen with intense exercise and higher production of reactive oxygen species.

In the equine hindlimb, glutamine appears not to be utilized by muscle as a fuel source during exercise [60] and only contributed to about 1.3% of the VO_2 at rest. Post-exercise, average hindlimb venous plasma [glutamine] was slightly greater than arterial. This may indicate net synthesis and release by muscle in order to meet glutamine demand of other tissues in the body, but definitive research remains to be performed.

In horses performing a constant speed, 20-minute duration, high intensity (about 80% of peak VO_2) exercise test [61], and with high-intensity maximal speed exercise [62], plasma [glutamine] decreased significantly immediately after exercise and did not recover.

Horses performing a very high intensity (about 115% of peak VO_2) exercise test had decreased plasma [glutamine] 5 minutes after exercise, and a significant recovery peaking at 30 to 60 minutes, followed by a gradual decline to typical post-prandial steady-state values [53]. These authors and Jahn et al. [54] attributed the post-exercise glutamine increase to ammonia detoxification associated with the increased intramuscular ammonia production. Similar results were reported with horses completing high intensity field exercise testing [63].

The decrease in plasma [glutamine] associated with relatively high intensity exercise contrasts with the increase seen by Jahn et al. [54] and during constant-speed moderate-intensity exercise [64] and was of a magnitude similar to the osmotic loss of plasma fluid (decrease in plasma volume [65] suggesting no net addition or loss of glutamine during this type of exercise. Plasma [glutamine] returned gradually to pre-exercise values over a 30-minute period.

When Harris et al. [66] supplemented dietary glutamine (single feeding and 10 days of supplementation at 30 and 60 mg/kg body mass; equal to about 15 and 30 grams, respectively) in athletically-worked horses, they found that even this relatively low amount of supplementation nearly doubled plasma [glutamine]. They concluded that increasing plasma [glutamine] through the diet has "benefit in the athletically worked horse with lowered plasma glutamine concentrations". A recent study in horses supplemented with a dietary protein / amino acid mixture within the first hour of completing high intensity exercise concluded that supplementation directly after training decreases post-exercise skeletal muscle proteolysis [67].

When Matsui et al. [68] infused radio-labeled phenylalanine (for calculating amino acid kinetics in horse muscle) they showed that intravenous administration of an amino acid mixture shortly after heavy exercise decreased the rate of muscle proteolysis and increased the rate of protein synthesis in the hind limb. van den Hoven et al. [21] reported that oral administration of amino acids to horses within 1 hour after exercise increased the intramuscular amino acid concentrations. Using exercise trained horses, van den Hoven et al. [21] supplemented the diet with amino acids for 6 weeks. High intensity exercise resulted in a 16% decrease in muscle [glutamine], followed by a 30% increase in muscle [glutamine] 4 hours after completion of exercise. This was associated with a 25% increase in post-exercise plasma [glutamine] when the amino acid supplement was offered during the first hour post-exercise. By 18 hours after exercise, plasma and muscle values had returned to pre-exercise baseline values. Both of these studies indicate a benefit, if not a need, for supplementary dietary glutamine, as well as some other amino acids, as a result of exercise, even in horses receiving daily supplements of amino acids.

Robson et al. [55] examined the effects of long-term endurance exercise (80 km endurance race) on plasma [glutamine] and immune function parameters. Pre-race plasma [glutamine] were low (279 $\pm$ 16 µmol/L). That there was no decrease immediately post-race, one hour post-race, and one-day and three-days post-race may be attributed to these very low starting values. In the post-race period, the horses experienced decreased neutrophil oxidative burst

activity and up to a three-fold decrease in circulating lymphocytes that was not fully recovered by three days post-race (impaired immune response). In these athletic horses, it appears that low plasma [glutamine] contributed to the severity of the observed immune depression. The results also indicate that these endurance horses did not receive adequate dietary glutamine.

A 16-week, regular exercise training program for Thoroughbred horses [69], and 4 to 16-week training periods of varying intensities using Standardbred horses [61], had no effect on plasma [glutamine] pre- versus post-training, which remained between 300 and 500 µmol/L. There do not appear to be studies that have examined the effect of standard race-training programs on glutamine concentrations and tissue stores.

A viral challenge (equine influenza virus) of six horses resulted in a gradual and progressive ~30% decrease in plasma [glutamine] over a six-day period, and [glutamine] remained depressed for at least an additional eight days [53]. The study authors attributed this result to an increased requirement for glutamine by immune system cells. A sustained decrease in circulating [glutamine] was suggested to impair the horses' ability to mount an effective immune response.

In summary, the evidence provided in this section indicates that dietary glutamine, and perhaps other amino acids that influence tissue glutamine synthesis, are not provided in adequate amounts to maintain optimum health. Intestinal health and immune health appear to be the best studied with respect to deficiency of glutamine, but effects on other systems may come to light as research continues.

4. Dietary and Supplemented Glutamine

The main sources of glutamine in the equine diet are plant proteins from forage and from supplemented grains [14]. The crude protein (CP) content of forages ranges from very low - with timothy at about 8% and alfalfa as high as 25% [70,71]. For horses, there is no glutamine recommended dietary allowance (RDA). The NRC [14] states that the daily protein requirement is 0.49 - 0.68 g /kg body mass (compared to 0.6 - 0.8 g/kg in humans). For horses in light to moderate work, this translates to 250 g of CP per day for a 450 kg horse, which provides up to 40 g of glutamine daily based on the typical proportions of amino acid in equine diets [14]. The recommendation increases to approximately 320 g CP/day for 450 kg horses in heavy work, which translates to 51 g of glutamine daily. A recent study using isotopically labeled amino acids compared two protein-supplemented diets in weanling horses, with horses receiving either 3.1 g or 4.1 g CP/kg body weight/day [16]. Compared to horses receiving the lower amount of

crude protein, horses receiving the higher amount of crude protein showed time-dependent increases in plasma amino acid concentrations, including glutamine, and that these horses had a higher rate of whole body net protein synthesis. Tanner et al. [16] concluded that, in the lower CP group, provision of at least one amino acid potentially limited the rate at which protein synthesis occurred.

In horses, as with other animals that consume dietary protein, plasma amino acid concentrations depend on feed composition, time of blood sampling relative to meals and tissue amino acid turnover [24,66,72,73]. When Miller and Lawrence [64] fed diets containing 12.9% versus 18.5% crude protein for two weeks, plasma [glutamine] was actually greater on the control diet (493 ± 18 µmol/L) compared to two weeks on the high protein diet (393 ± 18 µmol/L). While the amino acid profile of the diets had not been determined one interpretation of these results is that provision of additional amino acids in the high protein diet may have resulted in increased demand for glutamine or reduced synthesis of glutamine.

In response to the consumption of single meals, plasma [glutamine] typically increases with a peak occurring three to five hours after feeding [22-24,53,73]. The increase in plasma [glutamine] can be explained by absorption of glutamine into the blood from the intestinal system (mainly small intestine) and/or from de novo synthesis of glutamine. This distinction must be made because studies that have directly examined intestinal glutamine transport have reported little to no glutamine entry into the circulation [74-76]. The lion's share of intestinal (luminal) glutamine is taken up by enterocytes [76] and metabolized [74]. Of the glutamine that does enter the portal circulation, the portal drained viscera extracts about two-thirds of the circulating glutamine [75]. Thus ingested glutamine poorly accounts for the observed plasma glutamine concentrations. At stated by Manso Filho et al. [18] the great majority of glutamine within the body of the horse must be synthesized de novo through the action of glutamine synthetase. This conclusion is supported by the inverse relationship between tissue glutamine concentration and glutamine synthase protein expression in horses [18].

In the post-feeding period if food is withheld, then between 32 and 48 hours after feeding plasma [glutamine] rises somewhat for several hours [73]. This sustained elevation during the fasting period reflects release of synthesized glutamine into the circulation and indicates the importance of maintaining elevated plasma [glutamine].

In all mammals, and, indeed, most vertebrates, the primary functions of the small intestine are to absorb both water and low molecular weight nutrients exiting from the stomach. These nutrients include mono- and di-saccha-

rides (i.e. glucose, fructose), amino acids and dipeptides, free fatty acids, monovalent and divalent cations and anions (electrolytes), vitamins and trace minerals. These functions in the horse are similar to that of other mammals [77-82].

Many nutrients are transported into the blood by the intestinal system via the portal circulation (i.e., the blood supply to the liver from the intestinal system). In contrast, many amino acids enter the small intestine but do not enter the portal circulation, and thus do not make their way to the rest of the body. The intestines use 20% of the extracted amino acids for intestinal mucosal protein synthesis and the remainder for many other metabolic processes, including providing oxidative energy. For example, two-thirds of the glutamine, one-third of the proline and nearly all of the glutamate and aspartate are catabolized within swine small intestines rather than absorbed into the circulatory system [52]. Enterocytes are the major site of glutamine extraction and oxidative ATP production, particularly the absorptive columnar epithelial cells of the small intestine [30]. While one-third of glutamine not extracted by epithelial cells lining the intestinal lumen enters the portal circulation, cells of the small intestine also absorb some of this glutamine from the arterial circulation. This basal membrane route of glutamine entry into enterocytes appears to be important for the maintenance of gut health and immune function. In contrast to glutamine, most EAAs entering the small intestine are not extensively metabolized within enterocytes.

Enterocytes along the length of the equine small intestine are well endowed with a variety of transporters for neutral (e.g. glutamine) and cationic amino acids [83], although there are some modest differences in the amino acid transporter rates and affinities between horses and omnivores [84]. The large capacity for glutamine transport results in the majority of ingested glutamine being transported into enterocytes along the entire length of the intestinal system, with most of the uptake occurring from the small intestine [76,85].

Salloum et al. [76] studied the transport of glutamine into equine luminal enterocytes isolated from the jejunum. Similar to that of other mammals, the system B sodium-dependent transporter accounted for about 80% of the total transport. In a complementary study using anesthetized adult horses, Duckworth et al. [75] measured the capacity of the small intestine to extract glutamine from the arterial circulation. The extraction of glutamine by the equine jejunum in vivo more than doubled when the arterial concentration of glutamine was increased by bolus infusion, and jejunal extraction of glutamine was greater than that in the large intestine.

5. Safety of Supplemented Glutamine

Only one peer-reviewed scientific study has examined safety of orally supplemented glutamine (very low amount) in horses and no adverse events were reported [86]. Therefore the peer-reviewed scientific literature on other species is used to provide an indication of safety in horses, keeping in mind the similarities between horses and other mammals with respect to small-intestinal and immune-system functions. The capacity of the intestinal system, skeletal muscle, liver and kidneys to extract glutamine and glutamate is high, and Bertolo and Burrin [36] found that diets rich in glutamine or glutamate have little effect on circulating concentrations and low potential for toxicity.

In humans there is no defined Tolerable Upper Intake Level for dietary protein and 35% percent of total energy intake from protein is considered safe [87]. Within this context, and based on the absence of adverse effects, the Observed Safety Limit of glutamine supplementation (i.e., the highest amount one can consume that will not cause side effects) was identified to be 14 g/d (20 mg/kg body mass) in supplemental form above normal food intake in normal healthy adults [88]. This equates to 100 g/day for a 500 kg horse. Higher dietary intake levels have been tested in humans and shown to be well tolerated (for review see Wischmeyer [89], Watford [30]. In humans and other animals, ingestion of approximately 0.75 g glutamine/kg body mass (in the range of 40 - 60 grams per day) may increase plasma ammonia concentrations above the tolerated safety limit [90]. When orally supplemented below 40 gram per day in humans (~0.5 g/kg body mass) no adverse effects were reported [91,92]. Holecek [92] reported that intake levels of 40 grams or more consumed per day, glutamine: (1) may impair amino acid transport and distribution among tissues because it competes with other amino acids for transport systems [76], such that individuals with reduced kidney function should carefully consider glutamine requirements; (2) may impair synthesis of endogenous glutamine and enhance glutamate and ammonia production; (3) may impair ammonia detoxification; and (4) may result in an abnormal balance of amino acids in the body. Intake levels as high as 2.0 g/kg body mass in rats caused only an approximate 30% increase in brain striatal glutamine concentrations and only a 13% increase in striatal fluid GABA concentrations [93]. Fifty human subjects aged 17 - 65 years old ingested a carbohydrate/glutamine (50 grams of glutamine) supplement less than 20 hours prior to elective bowel surgery and no adverse effects were observed. The authors concluded that this amount of acute glutamine supplementation was safe during pre-op-

erative "fasting" and subsequent surgery [94]. Elderly men and women (69 $\pm$ 8.8 years) ingesting 0.5 g/kg supplemental glutamine had no increase in plasma ammonia levels, although these subjects did have increased serum urea and creatinine (within the normal range) that were deemed not clinically relevant [95]. In critically ill children, several studies have shown that glutamine supplementation was safe and did not cause toxic levels of ammonia or glutamate that could be suggestive of neurotoxicity (reviewed by Albrecht et al. [29]. Single oral doses of glutamine of 20 - 22 g/kg, 8 - 11 g/kg, and 19 g/kg were lethal in mice, rats, and rabbits, respectively [96].

Based on these data, and considering the relevant similarities between species with respect to glutamine metabolism, it can be concluded that supplementing dietary glutamine at up to 0.4 g/kg body mass above that provided in normal diets (13% crude protein) is safe for horses with healthy renal function in the long term. It is also indicated that short periods (one to two days) using dosages at high as 0.6 g/kg body mass may be safe when dealing with horses that have exceptionally high glutamine demands (late gestation, lactation, post-surgery, and after very stressful exercise or transport).

6. Summary and Conclusions

In summary, tissue glutamine concentrations are lower in most horses studied than in other mammals, reflecting diet composition, tissue glutamine requirements and possible dietary inadequacy. During normal periods of increased metabolic activity (lactation, growth and development of young, exercise and training), glutamine requirements are increased and glutamine availability appears to often not be adequate to meet requirements for optimum health. Dietary provision of glutamine has utility in minimizing or preventing catabolic states associated with periods of increased metabolic rate (exercise, lactation). Diets deficient in glutamine do not provide sufficient glutamine to enterocytes and or other body systems. Dietary glutamine supplementation resulted in significant increases in horses' systemic glutamine concentrations. Glutamine supplementation can help athletic horses increase plasma glutamine concentrations. All athletic horses tested have had low plasma glutamine concentrations, typically half that of well-fed healthy horses and healthy humans. A sustained decrease in plasma glutamine impairs a horse's ability to mount an effective immune response. During periods of inadequate dietary intake, inadequate tissue concentrations of glutamine are associated with impaired health, growth, development, intestinal function and immunity.

In conclusion, it is proposed that supplementary dietary glutamine will support intestinal cell nutrition, the immune system, and the general health of horses. Consideration of the numerous benefits afforded by adequate dietary intake of glutamine has led to the animal feeds and supplements industries to develop, produce and market numerous glutamine-containing dietary supplements for horses, cattle, sheep, humans, swine, poultry and fish. As stated by Wu and coworkers [8,27,28,52] supplementing conventional diets with glutamine can optimize growth in young animals and help maintain health in animals and humans.

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