February 17, 2016

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Jeannie Perron, JD, DVM

Covington & Burling LLP
One CityCenter
850 Tenth Street, NW
Washington, DC 20001-4956
T +1 202 662 5687
jperron@cov.com

By Hand Delivery

February 17, 2016

Geoffrey Wong
Supervisory Biologist
Division of Animal Feeds (HFV-224)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Place
Rockville, MD 20855

Re: GRAS Notice for L-Glutamine as an Ingredient in an Equine Feed Supplement

Dear Geoff:

I am enclosing the following materials on behalf of my client, Freedom Health L.L.C., and its Notifier, Dr. Michael L. Lindinger, Ph.D., VP, The Nutraceutical Alliance:

One hard copy of:

- A GRAS Notice for L-Glutamine as an Ingredient in an Equine Feed Supplement;
- A GRAS Expert Panel Opinion on L-Glutamine as an Ingredient in an Equine Feed Supplement;
- Appendices to the GRAS Opinion; and
- All references cited in the GRAS Opinion.

Because the GRAS Opinion and Appendices contain confidential information that must not be produced to the public in response to a request under the Freedom of Information Act or for any other reason, we have designated such information as "Confidential" in the enclosed documents. We have also included a hard copy of the GRAS Opinion and Appendices redacted to mask the information for which we are making a confidentiality claim.

Finally, we have included on a CD an electronic version of all of the foregoing documents.

Please let me know if you have any questions. Per the information in the GRAS Notice, questions may also be directed to:

Michael L. Lindinger, Ph.D., VP
The Nutraceutical Alliance
Lindenfarne Horse Park,
10526 4th Line Nassagaweya
Campbellville, ON, Canada L0P 1B0
1 (519) 835-6351
(b) (6)

Many thanks.

Respectfully submitted,

Jeannie Perron, JD, DVM

Attachments

cc: Michael Lindinger, Ph.D.
The Nutraceutical Alliance
Patrick Warczak
Freedom Health, LLC

NUTRACEUTICAL ALLIANCE

Dr. Michael I. Lindinger, PhD

February 17, 2016

Division of Animal Feeds (HFV-224)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Place
Rockville, MD 20855

Re: GRAS Notice for L-Glutamine as an Ingredient in an Equine Feed Supplement

On behalf of Freedom Health, L.L.C. (Freedom Health), I am hereby submitting a Generally Recognized as Safe (GRAS) Notice directed to the use of L-Glutamine as an ingredient in an equine feed supplement for consumption by horses post-weaning at levels not to exceed 4 grams per day, or approximately 9 mg / kg body wt / day, above that already present in a horse's typical forage and grain diet. The intended nutritional effect is to support the cellular metabolism of intestinal epithelial cells. As the attached information reflects, Freedom Health has determined that L-Glutamine at these levels is generally recognized by qualified experts as safe for the intended use based on scientific procedures.

- The Notifier is Freedom Health. All correspondence pursuant to this Notice should be directed to Freedom Health's consultant, Michael L. Lindinger, Ph.D., at the following address:

Michael L. Lindinger, Ph.D., VP
The Nutraceutical Alliance
Lindenfarne Horse Park,
10526 4th Line Nassagaweya
Campbellville, ON, Canada L0P 1B0
1 (519) 835-6351
(b) (6)

- The notified substance is L-Glutamine.

- When used to support the cellular metabolism of intestinal epithelial cells in an equine feed supplement for post-weaning horses at levels not to exceed 4 grams per day, or approximately 9 mg / kg body wt / day, above that already present in a horse's typical forage and grain diet, L-Glutamine is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FDCA) because Freedom Health has determined that such use is GRAS.

- L-Glutamine's applicable conditions of use are described above. It will be used in a commercial equine supplement at the level stated in the previous bullet point.

NUTRACEUTICAL ALLIANCE

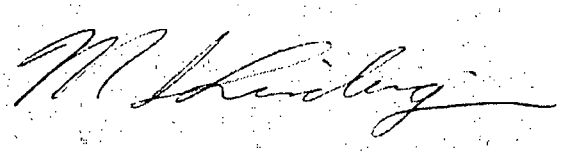
- The GRAS determination is based on scientific procedures.

- The data and information that form the basis for Freedom Health's GRAS determination are attached to this Notice and are hereby incorporated herein. Please note that the attached documents contain information that is exempt from disclosure pursuant to Exemption 4 of the Freedom of Information Act (FOIA) (5 U.S.C. § 552(b)(4)). Freedom Health will additionally submit a redacted version of the same documents in which the confidential information has been redacted. The redacted documents are intended for FDA's use in the event it ever receives a FOIA request to which such information would be responsive. We ask that the information marked as confidential in these documents and redacted from the redacted version of the documents be withheld as confidential business information under FOIA Exemption 4.

All other information required to be provided in this Notice is included in the attached document entitled, "Generally Recognized as Safe (GRAS) Exemption Claim for L-Glutamine as an Ingredient in an Equine Feed Supplement," which is hereby incorporated herein.

If you need any additional information, please do not hesitate to contact me.

Respectfully submitted,



Michael L. Lindinger, Ph.D.

Feb. 12, 2016

Attachment

Generally Recognized as Safe (GRAS) Exemption Claim

for

L-Glutamine as an Ingredient in an Equine Feed Supplement

Prepared for:
U.S. Food and Drug Administration
Center for Veterinary Medicine
Division of Animal Feeds (HFV-224)
7519 Standish Place
Rockville, MD 20855

Notifier:

Freedom Health, LLC
65 Aurora Industrial Pkwy
Aurora, OH 44202

February 14, 2016

Table of Contents

A. Introduction

On behalf of Freedom Health, LLC (the Notifier) the Nutraceutical Alliance submits this dossier of information in support of its notification to the Center for Veterinary Medicine (CVM) of the United States Food and Drug Administration (FDA) that the use of L-glutamine is Generally Recognized as Safe (GRAS) when used to supplement the normal feed of horses (the target species) at up to 4 grams per day above the L-glutamine levels present in horses' normal forage / grain diets.

At the request of the Notifier, a panel of independent scientists, qualified by their relevant experience and scientific training to evaluate the safety of feed ingredients (Expert Panel), was convened to evaluate the pertinent data and information. Their mandate was to determine whether, under the conditions of intended use as a supplement to the equine diet, with an intended benefit of supporting intestinal cell nutrition, the commonly occurring amino acid L-glutamine is GRAS based on scientific procedures.

The Expert Panel consisted of the qualified scientific experts:

Michael L. Lindinger, Ph.D., VP, The Nutraceutical Alliance, Campbellville, ON, Canada L0P 1B0

E. Murl Bailey, Jr., DVM, Ph.D., DABVT, Professor of Toxicology and Emergency Medicine, Department Of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843□4466; Email: (b) (6)

David A. Dzanis, DVM, Ph.D., DACVN, CEO of Regulatory Discretion, Inc. Santa Clarita, CA. Email: dave@regulatorydiscretion.com; phone: 661-251-3543

Curricula vitae evidencing the Expert Panel members' qualifications for evaluating the safety of feed ingredients are provided in **Appendix 1**.

A naturally occurring amino acid commonly found in foods, L-glutamine has been safely and effectively used for decades as a nutritional supplement for human health and performance and in the production livestock industries. The reported benefits include support of intestinal nutrition, immune function and general health.

Members of the Expert Panel independently and collectively critically examined a comprehensive package of publicly available scientific information and data pertinent to the safety and function of L-glutamine when provided as an equine feed supplement. Members of the Expert Panel independently and collectively also critically evaluated the data and information summarized herein. The Expert Panel concluded that the use of L-glutamine, as an ingredient of animal feeds and when produced in accordance with current good manufacturing practices, is Generally Recognized as Safe (GRAS) based on scientific procedures to supplement the diet of horses in an amount up to 4 grams per day above the levels of L-glutamine already present in normal equine diets.

The determination of GRAS status is on the basis of scientific procedures in accordance with 21 C.F.R. § 570.30(b) and conforms to the guidance provided by the Food and Drug Administration (FDA) in 62 Fed. Reg. 18938 (April 17, 1997) and FDA's Notice of Pilot Program: Substances Generally Regarded as Safe Added to Food for Animals, 75 Fed. Reg. 31806 (June 4, 2010). We have also consulted http://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/ucm050223.htm, which applies specifically to food additives in feeds, as published in 21 C.F.R. Part 570. Part 571 describes the kinds of data that should be submitted by the petitioner and the required format for the petition itself. We have also consulted the CVM Guidance for Industry 221, which provides recommendations for the preparation and submission of animal food additive petitions.

Accordingly, we submit information in each of the areas specified in the CVM Guidance for Industry 221, with the exception Section H (Proposed Tolerances for the Food Additive), which is not applicable in this case.

A.1 Availability of Information

The data and information that form the basis for the GRAS determination are provided as Appendices with this notification. The Notifier's Consultant will also retain copies of all of the data and information that support the GRAS determination. These data and information are available for CVM's review and copies will be sent to CVM upon request.

B. Administrative Information

B. 1. Name and Address of the Notifier

Mr. Patrick Warczak, Jr.
VP Marketing
PWarczak@freedomhealthllc.com
Freedom Health, LLC
65 Aurora Industrial Pkwy
Aurora, OH 44202
United States
Office: 330-562-0888

B. 2. Acknowledgment of Receipt of Notification and inquiries to be directed to the Notifier's Consultant:

Michael I. Lindinger, PhD
(b) (6)
The Nutraceutical Alliance
10526 4th Line Nassagaweya
Campbellville, ON, Canada
L0P 1B0
Office: 519 835 6351
Email: (b) (6)

A letter authorizing The Nutraceutical Alliance to serve as agent for the Notifier is included as **Appendix 2.**

C. Identity and Composition

C.1. Identity: Names and Identities of the Notified Substance

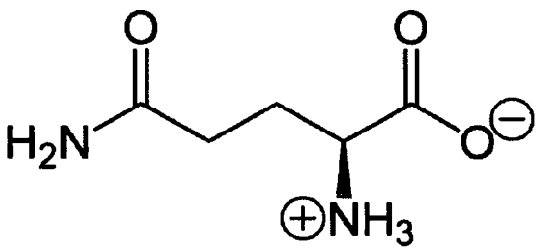
Source of information: PubChem:
http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5961

L-glutamine

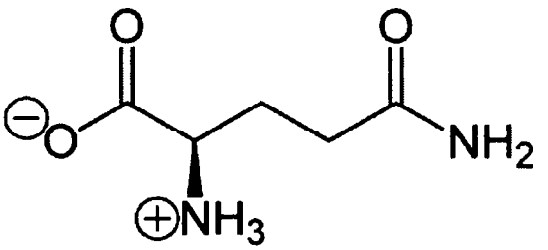
- Formal chemical name: (2S)-2,5-diamino-5-oxopentanoic acid (IUPAC)
- Common names, synonyms, or trade names
  L-glutamine,
  Levoglutamide,
  Cebrogen,
  Glavamin,

Stimulina,
Glumin,
Levoglutamid,
(Levo)glutamide
Glutamic acid amide,
L-(+)-Glutamine
2-amino-4-carbamoylbutanoic acid
(S)-2-aminoglutaramic acid
L-glutamic acid 5-amide
(S)-2,5-diamino-5-oxopentanoic acid

- Chemical Abstracts Service (CAS) registry number: 56-85-9.
- Empirical formula (Hill notation): $C_5H_{10}N_2O_3$.
- Molecular formula: $C_5H_{10}N_2O_3$.
- Structural formula: 2 dimensional, L and D isomers

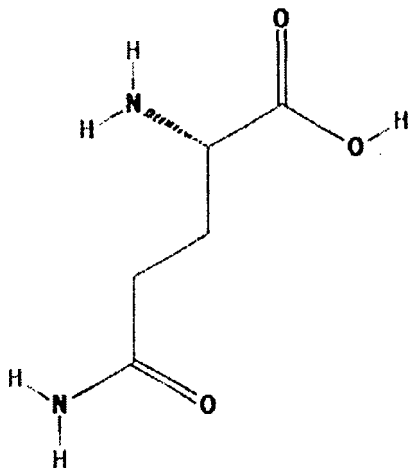


L-glutamine

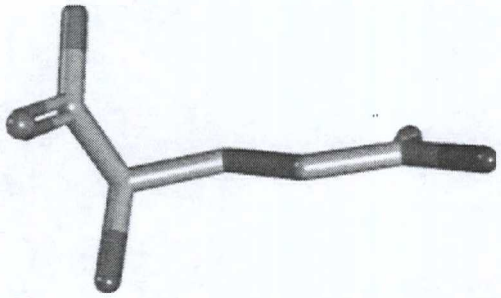


D-glutamine

- Structural formula: 2 dimensional L-glutamine



- Structural formula: 3 dimensional L-glutamine



- Molecular weight: 146.1445 grams / mole
- Formula weight: 146.1445 grams / mole

- Physical and chemical properties

XLogP3	-3.1
H-Bond Donor	3
H-Bond Acceptor	4
Rotatable Bond Count	4
Tautomer Count	3
Exact Mass	146.069142
MonoIsotopic Mass	146.069142
Topological Polar Surface Area	106
Heavy Atom Count	10
Formal Charge	0
Complexity	146
Isotope Atom Count	0
Defined Atom Stereocenter Count	1
Undefined Atom Stereocenter Count	0
Defined Bond Stereocenter Count	0
Undefined Bond Stereocenter Count	0
Covalently-Bonded Unit Count	1
Feature 3D Acceptor Count	3
Feature 3D Donor Count	2
Feature 3D Anion Count	1
Feature 3D Cation Count	1
Effective Rotor Count	4
Conformer Sampling RMSD	0.6
CID Conformer Count	46

- Source of L-glutamine
  The L-glutamine to be used is of natural origin, produced from fermented sugar, isolated and purified by Anjinomoto in Brazil (please see Section C.2.a and **Appendix 3**).

- Stability of L-glutamine:
  L-Glutamine is chemically stable as a dry powder and when mixed with powders. Chemical stability refers to the characteristic of a molecule or material to resist change or decomposition due to internal reaction, or due to the action of air, temperature, light, or pressure. L-Glutamine, when mixed within water-free oils, is also chemically stable when maintained under recommended storage conditions (packaging sealed from entry of ambient moisture and in a temperature-controlled environment). In the presence of water, L-glutamine is unstable at physiological pH, and breaks down to ammonium and pyroglutamate. For this reason, moisture is avoided at all steps in the production, packaging and storage of the feed supplement (please also refer to **Appendices 4, 5 and 6**).

- Biological properties:
  L-glutamine is one of twenty amino acids that are used to build proteins under the guidance of the genetic codes of animals. 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, L-glutamine is classified as an α-amino acid. L-Glutamine is also neutral, *i.e.*, it possesses no electrical charge. L-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.

  L-Glutamine has important functions within the gastrointestinal system, including the regulation of cell growth, regulation of cellular function, and cell / tissue regeneration. Under normal conditions, the body maintains circulating plasma L-glutamine concentration by dietary intake and synthesis from endogenous glutamate and branched-chain amino acids. Plasma L-glutamine concentrations decreases and tissue L-glutamine metabolism increases during catabolic states (periods of increased metabolic rate with inadequate protein intake), and thus L-glutamine is considered a "conditionally essential" amino acid.

  The pharmacokinetics of L-glutamine are based on literature data in healthy humans – there are no published pharmacokinetic studies in horses. A single oral glutamine administration at 0.1 g / kg body mass to 6 subjects raised blood L-glutamine concentration to 1.02 mM, measured 30 minutes after administration. In these subjects the terminal half-life of L-glutamine was approximately 1 hour after an i.v. bolus and the volume of distribution was approximately 200 mL / kg body mass. Pharmacokinetics following multiple oral doses have not been characterized. Metabolism is the major route of clearance of L-glutamine from the blood. The renal glomeruli filter L-glutamine, but filtered L-glutamine is nearly completely resorbed by the renal tubules such that renal elimination is very low.

C. 2. Manufacturing methods and controls

The content of this section is based on CVM Guidance for Industry 221 and the notice by

FDA on 03/08/2012: Guidance for Industry on Chemistry, Manufacturing, and Controls Information-Fermentation-Derived Intermediates, Drug Substances, and Related Drug Products for Veterinary Medicinal Use; Availability.
This guidance was found at
http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm123635.htm.

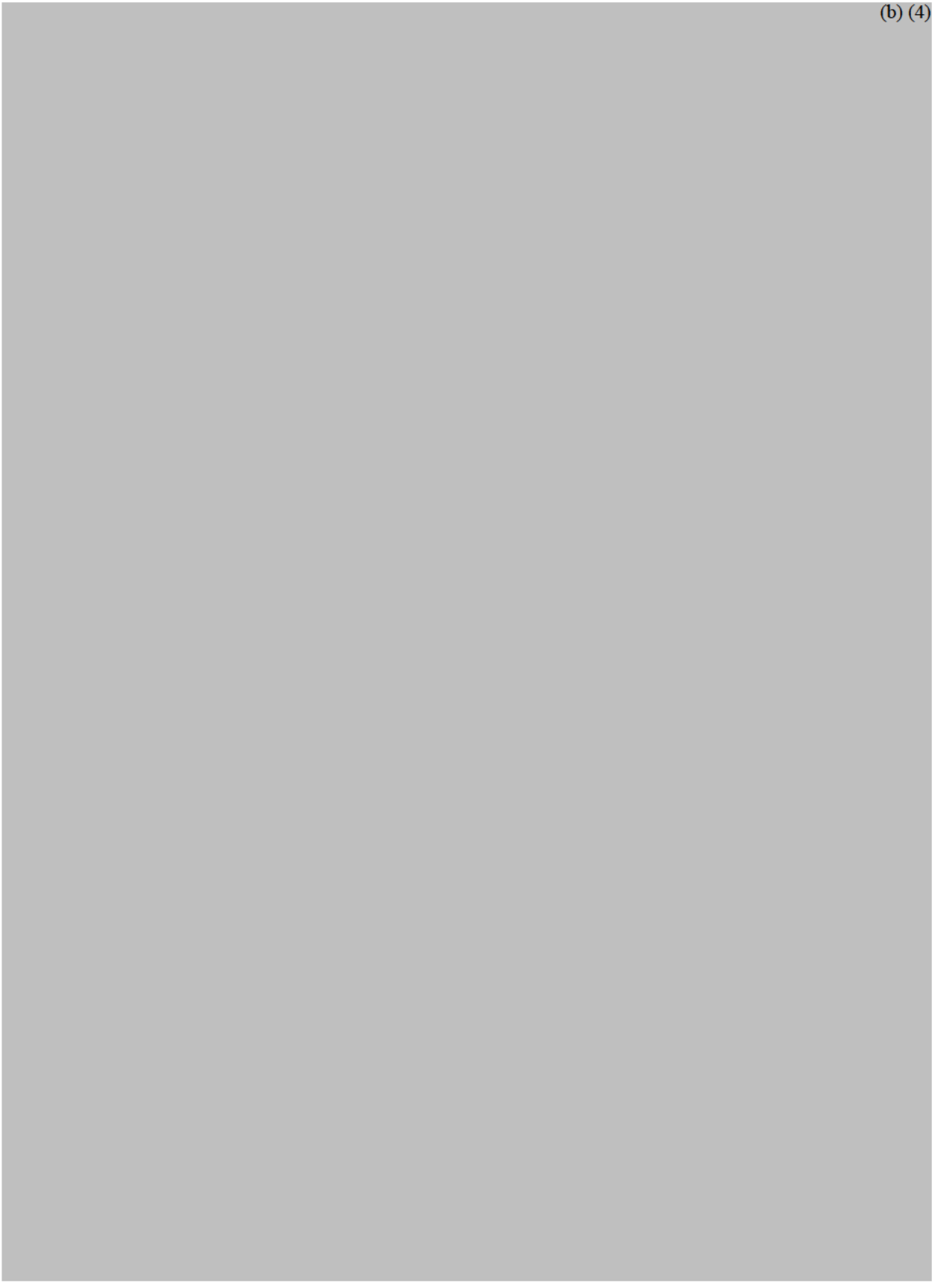
C. 2. a. Manufacturing process – L-glutamine

The country of origin for the Ajinomoto North America, Inc. L-glutamine product 20581 is Brazil, as stated in the letter dated Jan. 9, 2013 (**Appendix 3.1**). This product is purchased by the applicant (Freedom Health LLC) from Anjinomoto.
http://www.ajiaminoscience.com/products/manufactured_products/l-amino_acids/L-Glutamine.aspx

A Certificate of Good Manufacturing Practices, dated Jan. 26, 1999, was provided by the Brazilian Health Surveillance agency to Ajinomoto do Brasil Ind. E Com. De Alimentos Ltd., the company manufacturing L-glutamine by a fermentation process (**Appendix 3.2**).

An overview of the manufacturing process for L-glutamine, as provided by Ajinomoto on Jan. 28, 2011 is provided (**Appendix 3.3**) and follows the process described by Kusumoto (2001).

(b) (4)



(b) (4)

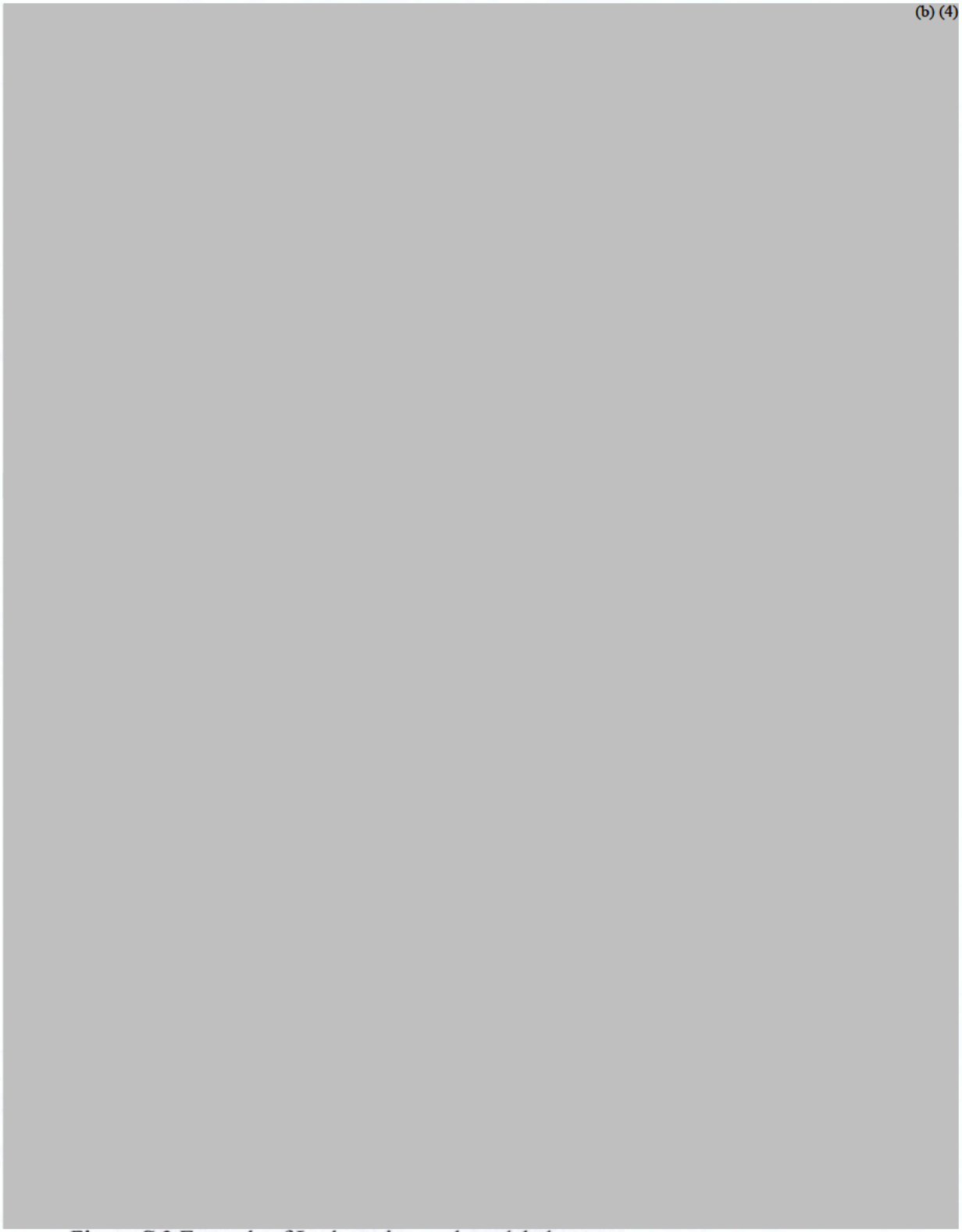


Figure C.2 Example of L-glutamine package label.

C. 2. b. Specifications for chemical identity and purity

The purity was [redacted] (b) (4) in the repeat analysis of L-glutamine Lot No 13 0081 K 03 13 (**Appendices 3.5.1 to 3.5.5**). The analytical methods are described in **Appendix 3.7**. The chemical identity of purified L-glutamine was confirmed by optical rotation (**Appendix 3.7.1**). Results for tests of purity of 5 different lots are shown in Table C.2.3.

Table C.2.3 Compositional Specifications and Analytical Data for 5 Batches of L-Glutamine

Parameter	Units	FCC (2008) specifications [1]	Feed Additive Specifications	Analytical Results				
				Lot 130081K 03B	Lot 130082K 03B	Lot 130082K 03B	Lot 130082K 03B	Lot 130082K 03B
Assay (L-glutamine)	% w/w (dry weight basis)	98.5 to 101.5	99 to 101	[redacted] (b) (4)				
Identification (FT-IR)	λ maxima	Complies	Complies					
Specific Rotation (D-Line, 20° – H_2O)	Degrees	+6.3 to +7.3	+6.3 to +7.3					
Specific Rotation (D-Line, 20° – HCl)	Degrees	-	+31.5 to +33					
Loss on drying	% w/w	Max. 0.3	Max. 0.3					
Residue on Ignition (sulphated)	% w/w	Max. 0.1	Max. 0.1					

[1] FCC, 2008 (FOOD CHEMICALS CODEX, 2008 EDITION)

C.2.1 Methods of Analysis

A number of tests were performed to check for levels of common contaminants including turbidity and color (**Appendix 3.7.2**), chloride (**Appendix 3.7.3**), ammonium (**Appendix 3.7.4**), sulfate (**Appendix 3.7.5**), iron (**Appendix 3.7.6**), heavy metals (**Appendix 3.7.7**), arsenic (**Appendix 3.7.8**), contaminant amino and nucleic acids (**Appendix 3.7.9**), water content (**Appendix 3.7.10**), ash content – inorganic impurities (**Appendix 3.7.11**), total amino acid content (**Appendix 3.7.12**), microbials (**Appendix 3.7.13**) and pH (**Appendix 3.7.14**). The analyses consistently showed negligible levels of contaminants (Table C.2.4).

Table C.2.4 Purity Specifications and Analytical Data for 5 Batches of L-Glutamine								
Parameter	**Units**	**FCC specifications**	**Feed Additive Specifications**	**Analytical Results**				
				Lot 130081K 03B	**Lot 130082K0 3B**	**Lot 130082K 03B**	**Lot 130082K 03B**	**Lot 130082K 03B**
Purity Criteria								
Chloride	% w/w	-	Max. 0.02	(b) (4)				
Ammonium	% w/w	-	Max. 0.10					
Sulphate	% w/w	-	Max. 0.02					
Iron	ppm	-	Max. 10					
Heavy metals (lead)	ppm	Max. 5	Max. 10					
Arsenic	ppm	-	Max. 1					
Microbiological Criteria								
Bacteria	CFU/g	-	Max. 500	(b) (4)				
Coliforms	CFU/g	-	Negative					
Fungi	CFU/g	-	Max. 50					

C. 2. 1. a. Stability and shelf life of L-glutamine

L-glutamine, when present as a dry powder and mixed with powders is chemically and physically stable under the recommended storage conditions of "Keep container tightly closed in a dry and well-ventilated place" (Sigma-Aldrich Material Safety Data Sheet for L-glutamine (**Appendix 4**)). In liquid form, L-glutamine is chemically and physically unstable at physiological pH, and is broken down to ammonium and pyroglutamate at rates problematic to biomanufacturing applications. L-Glutamine should also not be exposed to high temperatures and strong oxidising agents as it may decompose on oxidation to form nitrogen oxides.

Shelf life and stability data for L-glutamine supplied by Ajinomoto North America, Inc. are provided in **Appendix 5**. The L-glutamine product is shown to be stable for (b) (4) (**Appendix 5.1**) when stored within the original packaging (**Appendix 3.6** (b) (4)

Accelerated and real-time stability studies demonstrating the shelf-life of L-glutamine have been performed in accordance with International Conference on Harmonization

guidelines for industry on the testing of new substance drugs and products (ICH, 2003 – http://www.ich.org/products/guidelines/quality/quality-single/article/stability-testing-of-new-drug-substances-and-products.html). The results are summarized in Tables C.2.5 and C.2.6, and the study reports provided in **Appendix 5.2.** No degradation was reported.

Table C.2.5 Accelerated Stability Data for 3 Commercial Batches of L-glutamine (40 ± 2°C; 75% ± 5%RH)

Parameter	Units	Acceptance Criteria	Analytical Results (time in months)					
			Lot 050045K03BA		Lot 050046K03BA		Lot 050047K03BA	
			0	6	0	6	0	6
Loss On Drying	%w/w	Max 0.3	(b) (4)					
pH	pH units	4.5-6.0						
L-Glutamine content	%w/w	99.0 to 101.0						
Specific Rotation - HCl	Degrees	31.5 to 33.0						
Specific Rotation - H_2O	Degrees	6.3 to 7.3						
Ammonium	% w/w	Max 0.1						

RH = relative humidity

Table C.2.6 Long-Term Stability Data for 3 Commercial Batches of L-glutamine (25° ± 2° C; 60%RH)

Parameter	Units	Specification	Analytical Results (time in months)					
			Lot 050045K03BA		Lot 050046K03BA		Lot 050047K03BA	
			0	36	0	36	0	36
Loss On Drying	%w/w	Max 0.3	(b) (4)					
pH	pH units	4.5 to 6.0						
L-glutamine content	%w/w	99.0 to 101.0						
Specific Rotation - HCl	Degrees	31.5 to 33.0						
Specific Rotation - H_2O	Degrees	6.3 to 7.3						
Ammonium	% w/w	Max 0.1						

RH = relative humidity

C. 2. 1. b. Food Grade Assurance Letters

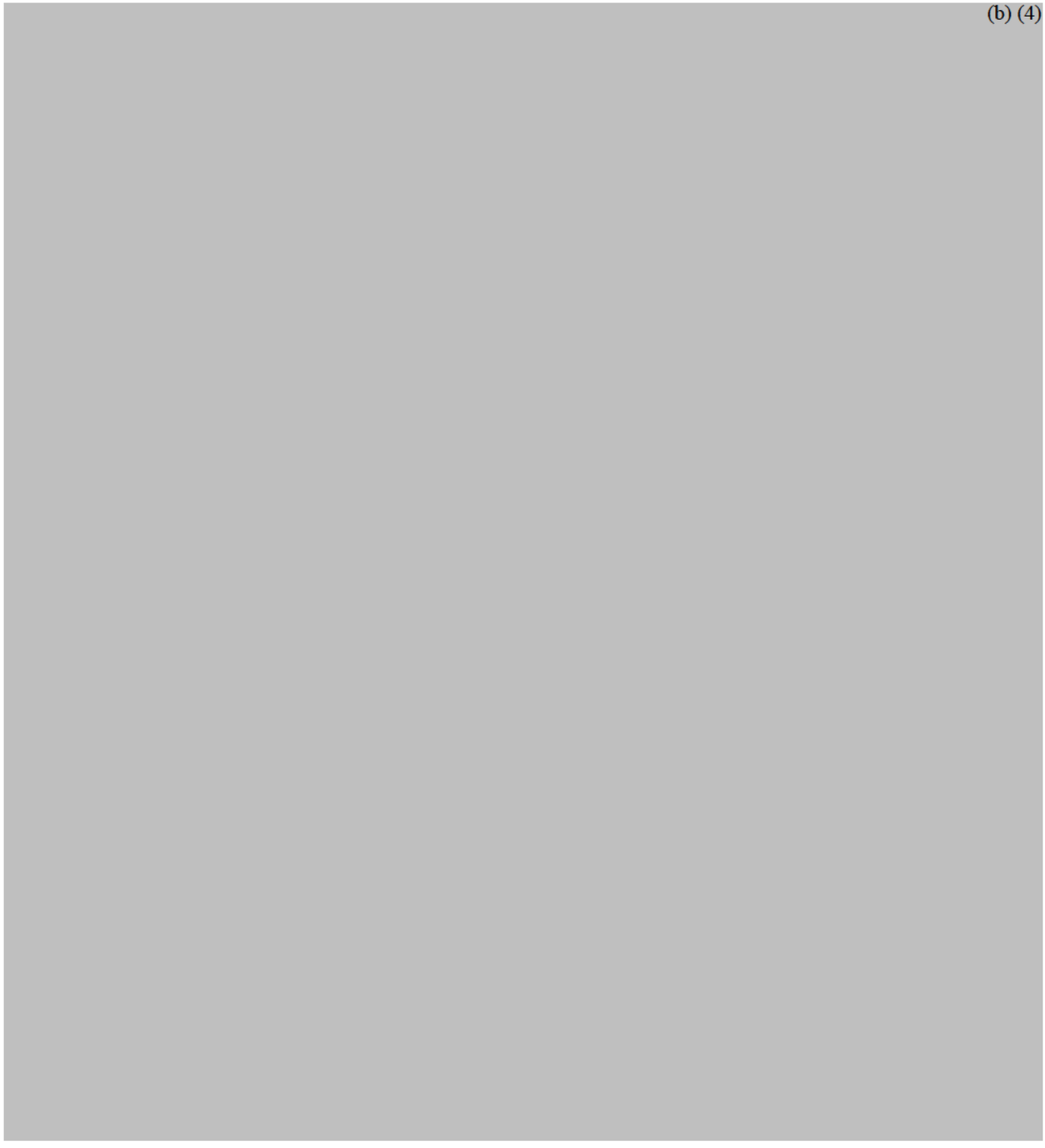
The intended use of L-glutamine is as an ingredient in a feed supplement for horses. The quality of the purified L-glutamine as suitable for food for human and animal consumption was confirmed following analysis using Fourier Transform Infrared Spectroscopy FTIR (**Appendix 5.3**). The nutritional content is provided in **Appendix 5.4.**

C. 3. L-glutamine containing feed supplement

C. 3. a. Manufacturing process – L-glutamine containing feed supplement(s)

The intended use of L-glutamine is as an ingredient in a feed supplement for horses. Two feed supplement formulations are considered, paste and granule, both of which are intended to deliver a standardized amount of L-glutamine.

(b) (4)



(b) (4)

[redacted]

C. 3. b. Mixability and stability of L-glutamine in feed supplements

The ability of L-glutamine to be mixed homogenously into typical feed supplement formulations (as per Table C.3.1) has been studied under conditions representative of commercial production.

The quantity of L-glutamine in paste and granule formulations was determined using (b) (4)

[redacted]

A typical HPLC chromatogram of an amino acid analysis of a representative sample of paste formulation (Appendix 6.1.1) and the accompanying report provided by (b) (4) (**Appendix 6.1.2**) is provided in **Appendix 6.** Lot by lot homogeneity of paste formulations was tested in 10 samples of feed supplement (**Appendix 6.1.3**) and the results summarised in Table C.3.3.

Table C.3.3 Homogeneity of a Typical Paste Feed Supplement Containing L-Glutamine (Lot L1323412)

Parameter	Analytical Results			Statistics	
	Mean (n=10)	Min.	Max.	SD	CV
L-Glutamine content (g/100 g)	3.50	3.08	3.76	0.21	5.9

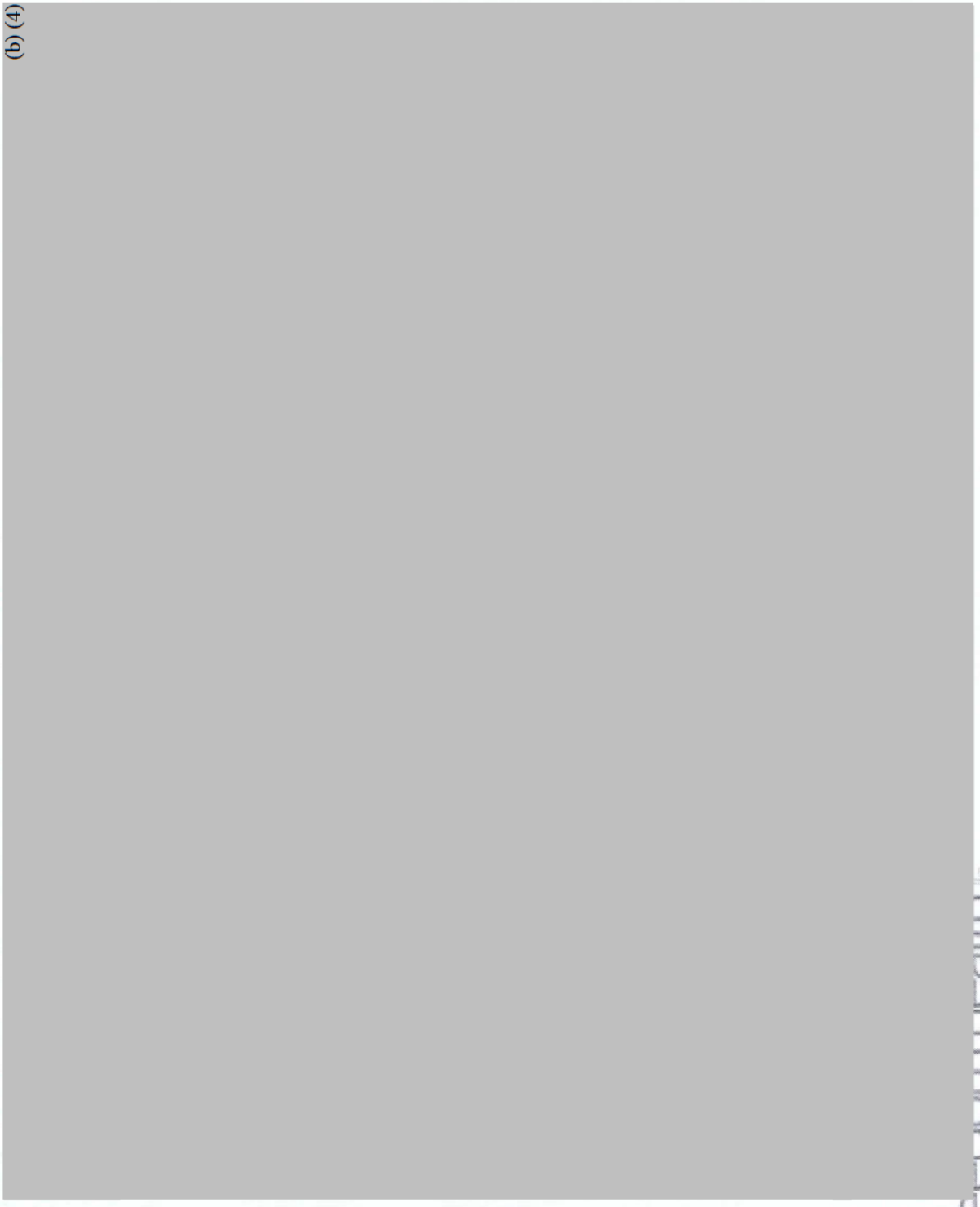
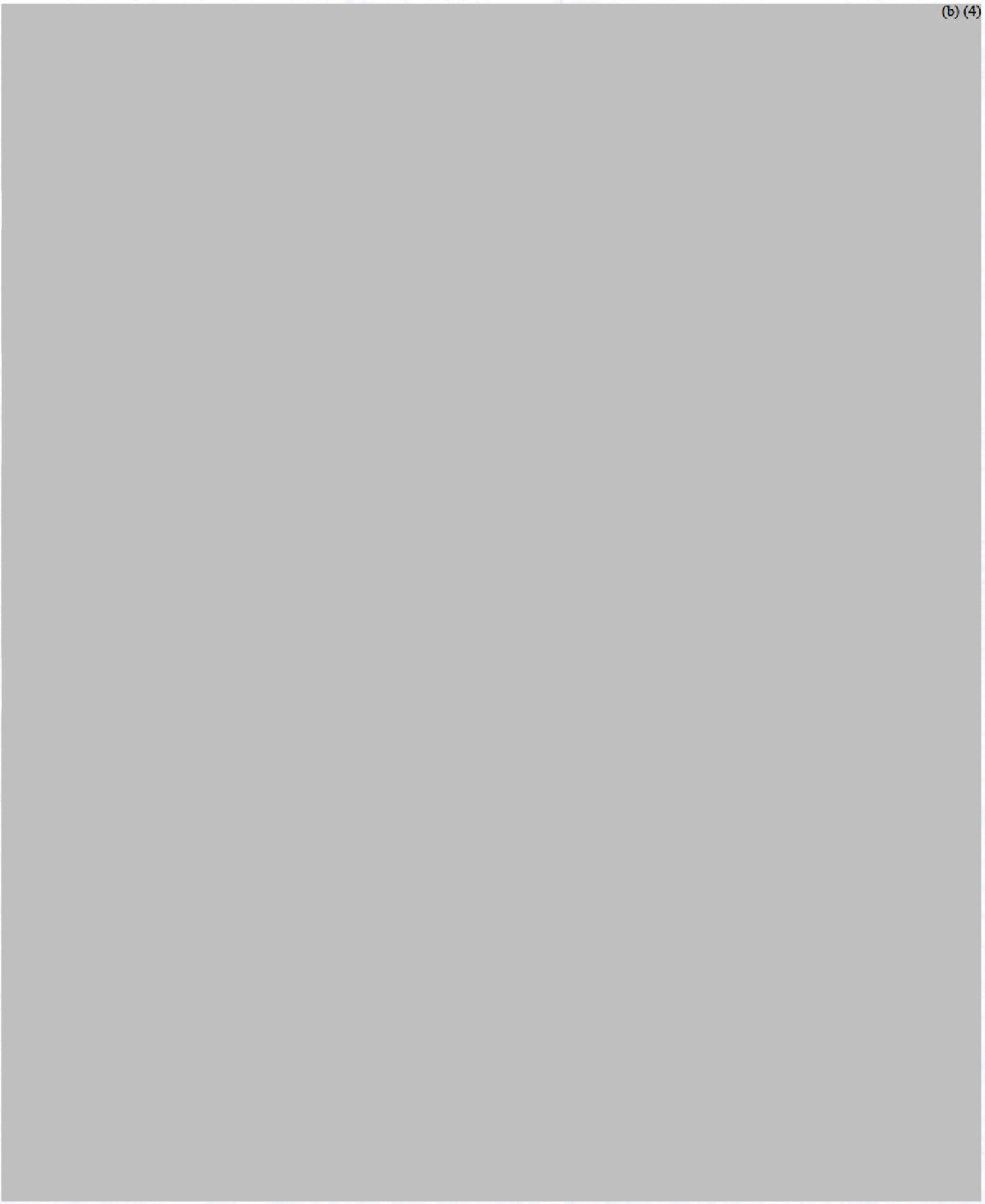


Figure C.3.1. Process for packaging syringes containing Succeed® product.

(b) (4)



A typical HPLC chromatogram of an amino acid analysis of a representative sample of granular formulation (**Appendix 6.2.1**) and the accompanying report provided by (b) (4) (**Appendix 6.2.2**) is provided in **Appendix 6.** Lot by lot homogeneity of paste formulations was tested in 10 samples of feed supplement (**Appendix 6.2.3**) and the results summarised in Table C.3.4.

Table C.3.4 Homogeneity of a Typical Granular Feed Supplement Containing L-Glutamine (Lot L1323414)

Parameter	Analytical Results			Statistics	
	Mean (n=10)	Min.	Max.	SD	CV
L-Glutamine content (g/100 g)	3.54	3.35	3.68	0.12	3.4%

These data demonstrate that both granular and paste complementary feeds can be produced homogenously with a coefficient of variation of 3 and 6%, respectively.

Stability data for Succeed® paste and granule formulations are presented in **Appendix 7**. Representative samples of the granular product of the typical composition outlined above (L-glutamine content *ca.* 4%) have been studied at various ages and the results are summarised in Table C.3.5. The samples were stored at ambient temperature in the original (commercial) packaging.

Table C.3.5 L-Glutamine Content of Aged Granular Formulations

Parameter	Analytical Results (g/100 g) and Age of Sample					
	Lot AJF10BFG	Lot AFG05ABA	Lot BFC01GED	Lot BJG10DGF	Lot BLF17BHW	Lot BNG17AEO
	16 months	40 months	54 months	63 months	99 months	103 months
L-Glutamine content; initially = ca. 4 g / 100 g	(b) (4)					

The results demonstrate that after storage for significant periods of time (up to 103 months) typical supplement formulations contained acceptable levels of L-glutamine. These data indicate that L-glutamine will be stable in typical feed supplements under the conditions of intended storage and use.

D. Intended Use and Use Level, and Labeling

D. 1. Intended use and use level

The use of purified L-glutamine as an ingredient in a feed supplement designed to support nutrition of intestinal epithelial cells is GRAS based on scientific procedures, specifically the data and information provided in the peer-reviewed scientific literature. L-Glutamine is an important nutrient in many animals and has been shown to have nutritional, immune function, performance and general health benefits in healthy mammals such as humans, dairy cattle, rabbits, sheep and swine. L-Glutamine is safe under its intended conditions of use.

The basis for GRAS determination is provided by publicly available scientific information, including the peer-reviewed scientific literature, pertaining to the functions and safety of ingested L-glutamine when provided as a supplement to typical equine forage and grain diets.

The peer-reviewed scientific literature includes:

- dietary exposure assessments in omnivores (humans, rodents) and herbivores, including hindgut fermenters such as horses and rabbits,
- biological effects within mammals including the target species, horses.

PLEASE REFER TO SECTION E FOR THE SCIENTIFIC RATIONALE

D. 1. 1. The type of animal food in which the notified substance will be used:

The intended use of L-glutamine is as an ingredient in a feed supplement for horses.

D. 1. 2. The use level of the notified substance to be used in each type of animal food:

A daily serving (up to 56 grams) of the feed supplement will provide up to 4 grams per day of L-glutamine above that already present in a horse's typical forage and grain diet. The supplement is served with the horse's normal feed up to two times daily, in accordance with manufacturer's recommendation and normal feeding practices in the equine industry. The intended use level is not higher than the amount reasonably required to accomplish the intended effect, even though safety data supports a higher tolerance.

D. 1. 3. Proposed maximum and typical use levels expressed as a concentration by weight

L-glutamine is intended to be an ingredient in a feed supplement for horses. The feed supplement will be provided to the horse once or twice daily, at a single serving of up to 28 grams. The supplement will contain L-glutamine at up to 2 g / 28 g. Therefore, a horse consuming the supplement twice a day (56 g) will ingest up to 4 grams of

supplemental L-glutamine daily. The typical use level is intended to be 2 grams / day, and the maximum use level is intended to be 4 grams / day.

A technologically self-limiting use level for oral L-glutamine has not been determined in any species, including humans and horses.

D. 1. 4. Statement of the intended effect(s) of the additive

The intended effect of the L-glutamine is as a nutrient to support the cellular metabolism of intestinal epithelial cells for horses of both sexes, post-weaning, in stages of life where metabolic requirements for L-glutamine are increased, such as occurs during growth, physical work, gestation and lactation. The intended effect is specifically to provide a nutrient that has been demonstrated scientifically to be required by cells of the intestinal system for these cells to function optimally, and so contribute to a healthy state of the whole horse. L-Glutamine provides nutritional support to the cells of the intestinal system. A healthy intestinal system supports immune function and general health. The target animal species, the horse, includes performance horses and other horses with elevated metabolic states (gestation, lactation, during and post- exercise) for which supplemented L-glutamine provides the functional benefits noted above.

D. 2. Labeling

Examples of the labels have been provided, above, as Figures C.3.2 and C.3.3. Label content and quality meet applicable federal and state requirements and guidelines.

E. Data Establishing the Intended Effect

E. 1. Summary

The Expert Panel searched the peer-reviewed, published scientific literature, the patent literature, and selected web sites in detail and reviewed studies that pertained to the biology, use, function and safety of L-glutamine in horses, humans and rodents. Across mammalian species studied, L-glutamine occurs naturally and high L-glutamine inclusion rates in supplemented diets are well tolerated. Safety studies using mice and rats did not show detrimental effects with dietary levels several-fold greater than used at the highest reported rates of oral supplementation in horses and humans. Safety studies in horses and humans reported no adverse events.

The scientific literature reflects beneficial physiological and health responses to L-glutamine supplementation in healthy humans and during periods of increased metabolism, disease and nutrient deficiencies. The patent literature also contains evidence of the benefits of L-glutamine supplementation in horses. The peer-reviewed scientific literature contains few reports regarding benefits of L-glutamine supplementation in horses specifically, although lower levels and shorter

supplementation durations have been studied than in humans.

E. 2. Terms of Reference

i. A complete literature review to identify and determine any relevant materials including relevant studies that reflect the safety (or lack thereof) of supplementary L-glutamine in horses.

ii. A complete literature review to identify and determine any beneficial effects resulting from the dietary supplementation of L-glutamine in horses and other mammals.

iii. The following search engines and databases were used to search for the relevant literature:

Agricola, Animal Health and Production Compendium, Biological Sciences, BioMed Central, BIOSIS Previews (Biological Abstracts), CAB Direct, clinicaltrials.gov, Compendium of Veterinary Products, CCRIS, DailyMed (http://dailymed.nlm.nih.gov/), DART (Developmental and Reproductive Technology), Food Science and Technology Abstracts, Google Patents, Google Scholar, Knovel, Plant Science, PubChem, PubMed, ToxNet – Hazardous Substances Database, ToxNet – LactMed (Drugs and Lactation Database), ToxNet – ToxLine, United States Patent and Trademark Office, and Web of Science.

iv. The following search terms were used either singly or in various combinations:

L-glutamine, glutamine, supplement, supplementation, requirement
Horse, equine safety, toxic, efficacy, dietary supplement, feed supplement, utility

v. The following adverse reactions databases were searched for the term 'glutamine'.

Adverse Drug Experience Reports (FDA - Veterinary)
http://www.fda.gov/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/ucm055369.htm

MedWatch: The FDA Safety Information and Adverse Event Reporting Program
http://www.fda.gov/Safety/MedWatch/default.htm

MedEffect Cananda
http://www.hc-sc.gc.ca/dhp-mps/medeff/databasdon/index-eng.php

Adverse Experience Reports Program (AERP) – Australia (APVMA)
http://www.apvma.gov.au/publications/reports/index.php

E. 3. L-Glutamine: Nutritional and Physiological Benefits in Horses and other Mammals

E. 3. 1 Introduction

Many amino acids, including L-glutamine, are ingested in proteins and peptides and as free amino acids as part of the normal diet. Some amino acids can be synthesized within the body, while others cannot. The ability to synthesize certain amino acids varies by species and developmental stage.

The main dietary sources of L-glutamine include animal and plant proteins from beef, pork and poultry, milk, yogurt, ricotta cheese, cottage cheese, raw spinach, raw parsley and cabbage (Li et al. 2011). In most mammals, L-glutamine is the most abundant, free α-amino acid in plasma (about 20% of total plasma amino acids) and skeletal muscle (Gleeson 2008; Watford 2008; Xi et al. 2011; Wu et al. 2013). The total amount of L-glutamine in the body is approximately 80 g in adult humans and approximately 400 g in adult horses. L-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 (Wu 2010; Albrecht et al. 2010; Albrecht et al. 2010a; Xi et al. 2011; Wu et al. 2013).

Amino acids have been classified as those that are nutritionally essential (or indispensable) amino acids (EAAs) – those whose carbon framework cannot be synthesized versus those that can be synthesized *de novo*. L-Glutamine can be synthesized *de novo* in many mammals and, accordingly, had originally been classified as a nonessential (or dispensable) amino acid (NEAAs). It has long been erroneously assumed that mammals could synthesize the required amounts of all NEAAs, and thus did not require dietary sources of NEAAs in order to maintain optimum nutrition, growth, development and health (Wu 2010; Wang et al. 2015).

There is now good evidence that L-glutamine, and some other NEAAs, are not synthesized in sufficient amounts during periods of increased metabolic rates 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 (Wu et al. 2013). These amino acids have therefore been re-classified as "conditionally essential" (Lacey and Wilmore 1990' Watford 2015) 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. L-Glutamine is now 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 (Watford 2015).

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 (Lobley et al. 2001; Watford 2008; Wu 2010; Albrecht et al. 2010; Xi et al. 2011). Fürst et al. (1997) characterized L-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. Therefore, supplementation of FAAs such as L-

glutamine, in amounts adequate to meet nutritional and metabolic requirements, has been proposed by the authors as a nutritional strategy to maintain or improve health, growth and development and to prevent diseases (see also **Appendix 8**, which addresses patents relating to glucosamine and horses). Xi et al. (2011) reported that adequate, high concentrations of intracellular and extracellular L-glutamine are associated with marked reductions in infection, sepsis, severe burn, cancer, and other pathologies. For example, oral L-glutamine supplementation in healthy humans performing moderate intensity exercise prevented the exercise-induced increase in intestinal permeability (Zuhl et al. 2014), thus maintaining integrity of the intestinal – immune system during periods of elevated metabolism.

The turnover of L-glutamine in the human body is approximately 80 g / day, while in horses, it is approximately 400 g / day (Watford 2008). These values are equivalent to the whole body content of L-glutamine being turned over on a daily basis. However, L-glutamine turnover is increased during periods of increased metabolism, such as exercise, recovery from exercise, periods of increased growth and in some disease states. During normal periods of increased metabolism, the cellular requirement for L-glutamine often cannot be met through *de novo* synthesis or the normal diet (Watford 2008). Because the utilization rate of L-glutamine is high, it is important to replenish L-glutamine through diet. Since the demonstration that L-glutamine supplementation stimulates protein synthesis and inhibits proteolysis in healthy rat skeletal muscle, there has been increasing research in the use of L-glutamine as an FAA to improve protein, nitrogen and energy balance under various physiological conditions in humans (for review, see Xi et al. 2011).

Key points

1. Dietary L-glutamine supplementation is associated with maintained health, growth and development compared to conditions of no supplementation.

2. L-Glutamine is the most abundant free α-amino acid in plasma and skeletal muscle of many mammals, including humans and horses.

3. L-Glutamine can be obtained from the diet as a free amino acid or in the form of peptides and proteins.

4. Under periods of inadequate dietary intake, inadequate tissue concentrations of L-glutamine are associated with impaired health, growth, development, intestinal function and immunity.

5. During periods of accelerated metabolic rate, e.g., high-intensity exercise training regimens, lactation, periods of active growth in horses, the cellular demand for L-glutamine is increased for many metabolic processes and such demand exceeds dietary intake and de novo synthesis.

E. 3. 2 L-Glutamine is an important nutrient in horses

Most of what is known about the physiology and biochemistry of amino acids in general, and

L-glutamine in particular, comes from research in humans and non-equine "production" mammals, such as cattle and swine. There is also considerable basic and mechanistic research in mouse and rat models. Although there is little difference in the fundamentals of nutritional amino acid physiology and biochemistry between horses and many other mammals, this section of the analysis will focus on equine metabolism, with an emphasis on the intestinal cells because it is these cells that nutritionally benefit directly from L-glutamine supplemented to normal diets.

E. 3. 2. 1 The small intestine of horses is similar to that of other mammals

The equine small intestine is similar to that of other mammals with respect to nutrient absorption, relationship with the immune system, cellular nutrient and energy requirements, cellular transport functions and cellular gene responses to changes in the cellular environment. On this basis, therefore, intestinal responses to dietary L-glutamine reported in other species are transferable to horses.

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-saccharides (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 (Hintz 1975; Merediz et al. 2004; Cehak et al. 2009; Dyer et al. 2002, 2009; Daly et al. 2012; Rasoamanana et al. 2012; Lindinger and Ecker 2013).

The small intestine also has immune functions and is highly integrated with other parts of the immune system, with which it works to provide for the health of the entire body (Santaolalla and Abreu 2012). Via the circulatory system, the body supports the small intestine by providing nutrients for enterocytes (the nutrient absorptive cells of the intestine) and transporting away signaling molecules and cellular byproducts.

The acidic environment of the stomach, together with hydrolytic enzymes, breaks down many peptides and proteins into their amino acid components (i.e., proteolysis). In humans, proteolysis of dietary protein and peptide sources provides about 87% of L-glutamine within the body, while the remaining 13% arises from *de novo* synthesis (Kuhn et al. 1999). Proteins and peptides that are not hydrolyzed within the stomach enter the intestinal system (the enteral environment), where enzymes and gut flora act on them. Amino acids, in particular the NEAAs, entering the small intestine from the stomach have several fates, including transport into enterocytes, from within the intestinal lumen, where they are oxidized to provide fuel (ATP) to support these cells' transport functions.

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 L-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 (Wu 2010). Enterocytes are the major site of L-glutamine extraction and oxidative ATP production, particularly the absorptive columnar epithelial cells of the small intestine (Watford 2008). While one-third of L-glutamine not extracted by epithelial cells lining the intestinal lumen enters the portal circulation, cells of the small intestine also absorb some of this L-glutamine from the arterial circulation. This route of L-glutamine entry into intestinal cells appears to be important for the maintenance of gut health and immune function. In contrast to L-glutamine, most EAAs entering the small intestine are not extensively metabolized within enterocytes.

Enterocytes along the length of the small intestine are well endowed with a variety of transporters for neutral (e.g. L-glutamine) and cationic amino acids (Woodward et al. 2010), although there are some modest differences in the amino acid transporter rates and affinities between horses and omnivores (Woodward et al. 2012). The large capacity for L-glutamine transport results in the majority of ingested L-glutamine being transported into enterocytes along the entire length of the intestinal system, with most of the uptake occurring from the small intestine (van der Schoor et al. 2010; Blachier et al. 2009).

Salloum et al. (1993) studied the transport of L-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. (1992) measured the capacity of the small intestine to extract L-glutamine from the arterial circulation. The extraction of L-glutamine by the equine jejunum *in vivo* more than doubled when the arterial concentration of L-glutamine was increased by bolus infusion, and jejunal extraction of L-glutamine was greater than that in the large intestine.

In summary, these studies demonstrate the importance of rapid, small intestinal extraction of ingested L-glutamine from the circulation, and that L-glutamine extracted by cells of the small intestine from the arterial circulation is important in equine and other mammalian enterocyte metabolism.

E. 3. 2. 2 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 (2013) and Miron and Cristea (2012). 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, and there are some equine data to support this.

- 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.
- 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 (Ruth

and Field 2013; Miron and Cristea 2012).

- The enterocytes also maintain barrier function between luminal contents (external environment) and the internal environment of the body (MacFie and McNaught 2002). This barrier function is dependent on dietary L-glutamine availability (Zuhl et al. 2014).
- The immune system's requirement for protein and amino acid support is well established.
- It has also been established that specific dietary amino acids (in particular, L-glutamine, glutamate, and 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).
- L-Glutamine, and these other intestinally important amino acids, each have unique properties essential for maintaining the intestine's integrity, growth and function, and for regulating local tissue and organ immune responses (Ruth and Field 2013; Miron and Cristea 2012).

L-Glutamine supplementation is important to maintain a normal intestinal barrier against pathogens and preserve mucosal integrity (Domeneghini et al. 2006; Larson et al. 2007; Rhoads and Wu 2009; Wang et al. 2009; dos Santos et al. 2010; Miron and Cristea 2012; Ruth and Field 2013; Sukhotnik et al. 2007; Zuhl et al. 2014; Wang et al. 2015). Using a mouse model of small intestinal obstruction (similar to an equine obstructive small intestinal "colic"), dos Santos et al. (2010) showed that L-glutamine supplementation prevented the large increases in intestinal permeability and bacterial translocation seen in non-supplemented animals.

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

During periods of increased metabolic activity, such as exercise and recovery, physical training and critical stages of development (post-natal growth, lactation), the need to provide dietary sources of L-glutamine to support growth and health is now well established. In their review, Ruth and Field (2013) identify the following metabolic functions.

L-Glutamine:

- serves as a precursor and energy substrate for immune and epithelial cells;
- 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;
- supports proliferative rates and reduces enterocyte apoptosis;
- protects against pathogenic bacterial damage to intestinal structure and barrier function;
- lowers inflammatory response and increases immunoregulatory cytokine production; and
- improves the proliferative responses and numbers of intestinal immune cells.

E. 3. 2. 3 L-Glutamine is also important elsewhere in the body

The Health Canada, Drugs and Health Products monograph, "L-Glutamine", (available at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/monoReq.do?id=126&lang=eng), in particular makes reference to the benefits of L-glutamine in normal individuals. These benefits include:

- restoration of plasma L-glutamine levels depleted after periods of physical work (e.g. prolonged exhaustive exercise) (Krzywkowski et al. 2001, Bowtell et al. 1999, Castell 2003; Castell and Newsholme 1997);
- support for immune system health after periods of physical work (Shils et al. 2006, Newsholme 2001, Griffiths 1999);
- support for digestive system health after periods of physical work (Shils et al. 2006, Newsholme et al. 2003, Food and Nutrition Board 2005); and
    - support for muscle cell repair after exercise (Newsholme et al. 2003, Food and Nutrition Board 2005).

Compared to other amino acids, the concentrations of L-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 L-glutamine within the body. With low to normal amounts of dietary L-glutamine, up to 100% of the L-glutamine ingested with protein is utilized by cells of the small intestine. In this typical situation, none of the dietary L-glutamine enters the systemic circulation. Instead, plasma concentrations of L-glutamine are maintained by *de novo* synthesis from metabolic precursors. In this sense L-glutamine is considered to be non-essential.

Skeletal muscle is the major tissue that synthesizes L-glutamine. The enzyme L-glutamine synthetase catalyzes the synthesis of L-glutamine from ammonia and glutamate. Mammalian skeletal muscle comprises approximately 40% of lean body mass, and intramuscular concentrations of L-glutamine serve as a regulator of the anabolic state of this major tissue. In nourished mammals, skeletal muscle releases L-glutamine into the circulation at a rate of 40 - 60 mmol/h (Lobley et al. 2001; Gleeson 2008). When dietary intake of L-glutamine is adequate, intestinal L-glutamine extraction maintains a high circulating L-glutamine concentration. When dietary intake of L-glutamine is low (e.g., as a result of typical horse forage) circulating concentrations of L-glutamine are lower, and have been reported to be 35-60% of that found when dietary L-glutamine is high. The circulatory system provides a means of transporting L-glutamine to those cells that require it and that are not capable of synthesizing adequate amounts to meet their demands.

The amino acids L-glutamine and glutamate make up 10–20% of dietary protein, and both are extensively metabolized in the small intestine of most mammals. Watford (2008) asserts that, with normal levels of dietary intake (5 – 10 g of L-glutamine daily for humans), there is no net small-intestinal absorption of L-glutamine or glutamate into the blood, such that body's L-glutamine pool results from *de novo* synthesis, primarily within skeletal muscle. In various mammals, including humans, about 20% of dietary L-glutamine may end up in the systemic circulation, but this is dependent on the amount of L-glutamine ingested and the metabolic

state. Therefore the high requirement for L-glutamine by intestinal enterocyte and immune cells result in less considerably less L-glutamine entering the systemic circulation than what is ingested.

L-Glutamine serves as a metabolic precursor for other important amino acids. Within the intestinal system (splanchnic bed), unoxidized L-glutamine and proline serve as important precursors for citrulline synthesis, which is then converted to arginine in the kidney (Bertolo and Burrin 2008). Arginine is a semi-indispensable amino acid in neonates and serves numerous metabolic roles in young and adult mammals.

L-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 (Lobley et al. 2001; Watford 2008). Most of the body's L-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.

The liver, like skeletal muscle, both synthesizes and consumes L-glutamine. The enzymes for each process are compartmentalized to different hepatic cell systems (Watford 2008). The liver normally produces a small amount of L-glutamine and plays a role in fine-tuning plasma L-glutamine concentrations.

In the kidneys, L-glutamine serves as the major substrate for ammoniagenesis, the process of removing nitrogen from the body, and in whole-body acid-base balance (Watford 2008). The glomeruli filter L-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 L-glutamine utilization, as well as increased hepatic extraction where L-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 L-glutamine synthesis. This is often accompanied by decreased intestinal L-glutamine utilization (Watford 2008), with consequent impairment of intestinal and immune function (Xi et al. 2011).

L-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. L-Glutamine newly synthesized from ammonia and glutamate by astrocytes within the brain is extracted by neurons. Enzymes then hydrolyze the intracellular L-glutamine back to glutamate, some of which is decarboxylated to produce GABA, or transaminated to aspartate (Albrecht et al. 2010).

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 (Brasse-Lagnel et al. 2009, 2010). In particular, L-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.

E. 4. The requirement for dietary L-glutamine in horses

Amino acid requirements for horses have not been well defined and have not been evaluated individually at different stages of life (Tanner et al. 2014). For horses, there is no L-glutamine recommended dietary allowance (RDA). The NRC (2007) 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 per day for a 450 kg horse, which provides up to 40 g of L-glutamine daily based on the typical proportions of amino acid in equine diets (NRC 2007). The recommendation increases to approximately 320 g protein / day for 450 kg horses in heavy work, which translates to 51 g of L-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 of crude protein / kg body weight / day (Tanner et al. 2014). 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 L-glutamine, and that these horses had a higher rate of whole body net protein synthesis. Tanner et al. (2014) concluded that, in the lower crude protein group, provision of at least one amino acid potentially limited the rate at which protein synthesis occurred.

Tissue L-glutamine concentrations in horses reported in most equine studies are lower than those reported in well-fed, healthy humans and rats (Rogero et al. 2004; Watford and Wu 2005; Routledge et al. 1999; Wong et al. 2011), and horses (Urschel et al. 2012).

- In adult horses, plasma L-glutamine averages about 0.33 mmol/L in a range of studies (King and Suleiman 1998; Rogers et al. 1984; Zicker et al. 1991, 1994; Zicker and Rogers 1994a, 1994b; Routledge et al. 1999; Urschel et al. 2010; van den Hoven et al. 2010; Nostell et al. 2012; Peters et al. 2013).
- Plasma L-glutamine concentrations as high as 0.6 mmol/L have been observed in healthy, adult research horses (Duckworth et al. 1992). This value is similar to the normal human value and raises the possibility of chronic hypoglutaminemia (due to inadequate dietary supply of L-glutamine combined with inadequate rates of endogenous synthesis from metabolic precursors) in the general horse population.
- In foals up to one year of age, L-glutamine is abundant in plasma (up to 0.6 mmol/L) and skeletal muscle (up to 2 mmol/L; Rogers et al. 1984; Zicker et al. 1991; Manso Filho et al. 2009).
- L-glutamine level differences between adult humans and horses likely reflect composition of the diet (Li et al. 2011).
- A recent study of well cared-for research horses reported average plasma L-glutamine concentrations ranging from 0.88 to 1.02 mmol/L (Urschel et al. 2012), further supporting that dietary glutamine was physiologically limiting in many other studies.

Conclusion: that the low tissue L-glutamine concentrations reported in most equine studies reflect typical equine diets that are low in dietary sources of L-glutamine such that dietary supplementation of L-glutamine may be appropriate.

During pregnancy, the placenta and/or fetus extract large amounts of L-glutamine from the circulation (Manso Filho et al. 2009b). The mammary glands extract large amounts of L-glutamine during lactation (Manso Filho et al. 2008b). L-Glutamine is also abundant in the milk of lactating mares (Manso Filho et al. 2008), although lactation is a very metabolically demanding period. After 3 months of lactation in mares, plasma and milk L-glutamine concentrations had decreased by more than 50% which, together with loss of lean body mass, is indicative of a mild catabolic state (Manso Filho et al. 2009b). The authors concluded that the decrease in circulating L-glutamine concentrations during lactation, when large amounts of L-glutamine are being extracted by the mammary gland, "means that L-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 L-glutamine, dietary intake is crucial to maintain adequate plasma concentrations. Plasma L-glutamine concentrations were reported to be 0.360 ± 0.055 mmol/L pre-fasting in seven lactating mares, but this level fell to 0.247 ± 0.031 mmol/L after 36 h of fasting and recovered to only 0.318 ± 0.032 mmol/L 6 h after additional feeding (Silver et al. 1994). While fetal L-glutamine concentrations were nearly double those of their mares, the fetal plasma L-glutamine concentrations similarly decreased after fasting. This, in part, reflects the high requirement of the developing fetus for L-glutamine, despite the high capacity of the placenta to synthesize L-glutamine and extract it from the circulation (Manso Filho et al. 2009).

Dietary composition also has pronounced effects on plasma L-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 L-glutamine concentrations (Rogers et al. 1984). This provides evidence that the provision of other nutrients to L-glutamine producing cells and tissues, increases the production and release of L-glutamine by these cells / tissues into the blood because the L-glutamine is in demand by other cells / tissues. It is also an indication that whole body L-glutamine demands have not been adequately met prior to provision of additional nutrients. Dietary supplementation of L-glutamine can help minimize or prevents the catabolic state and maintains steady L-glutamine concentrations essential for intestinal and immune function and health (Blikslager 2003).

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 (Johnson and Hart 1974; Russell et al. 1986; Hackl et al. 2006; Harris et al. 2006). When Miller and Lawrence (1988) fed diets containing 13% versus 18% crude protein for two weeks, there was no difference in plasma L-glutamine concentration (although the amino acid profile of the diets had not been determined). In response to the consumption of single meals, plasma L-glutamine concentrations peak three to five hours after feeding (Russell et al. 1986; Routledge et al. 1999), and again between 32 and 48 hours

after feeding if food is withheld (Russell et al. 1986). This sustained elevation reflects release of synthesized L-glutamine into the circulation and indicates the importance of maintaining elevated plasma L-glutamine concentrations.

Consistent with the equine studies cited above, Wu (2010) considers dietary L-glutamine to be "substantially inadequate" to meet the requirements for protein synthesis in extra-intestinal tissues in growing pigs. By extension, these authors infer that such is the case for mammals during periods of elevated metabolism (exercise, lactation, active growth and development). A typical diet does not provide sufficient arginine, proline, aspartate, glutamate, L-glutamine, or glycine for protein accretion in growing pigs (Wu 2010). The capacity of the intestinal system, skeletal muscle, liver and kidneys to extract L-glutamine and glutamate is high, and Bertola and Burrin (2008) concluded that diets rich in L-glutamine or glutamate have little effect on circulating concentrations and low potential for toxicity.

This view, with consideration of the numerous benefits afforded by adequate dietary intake of L-glutamine (see below), has led to the development, production and marketing of L-glutamine-containing dietary supplements for horses, cattle, sheep, humans and swine. Supplementing conventional diets with L-glutamine can optimize growth in young animals and help maintain health in animals and humans (Wu 2010; Wu et al. 2013).

E. 5. Effects of exercise and physical conditioning

In horses, humans and rodents, moderate to high intensity or duration exercise results in immune function suppression (Robson et al. 2003; Lagranha et al. 2005; Lagranha et al. 2008; Gleeson 2008; Walsh et al. 2011). Exercise typically, but not always, depresses plasma L-glutamine (Keast et al. 1995; Gleeson 2008; Parry-Billings et al. 1992; Walsh et al. 2011). L-glutamine supplementation also enhances the immune response to intense exercise, effects that appear to be mediated by intestinal/immune system interaction (Newsholme and Calder 1997; Rohde et al. 1998; Walsh et al. 1998; Newsholme 2001). L-Glutamine supplementation prevents the increase in intestinal permeability that occurs during moderate intensity exercise (Zhul et al. 2014). Neutrophils, which comprise 50-60% of the total leukocyte count, elicit some of the beneficial effects seen with L-glutamine supplementation (Lagranha et al. 2008). Supplemented, exercise-conditioned rats performed one hour of exercise at 85% of peak VO2 (oxygen consumption). In one group of rats, L-glutamine was supplemented by oral gavage one hour before exercise. Compared to the control group that did not receive L-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 (Lagranha et al. 2005).

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 (Gleeson 2008; Routledge et al. 1999). As a result the intramuscular and plasma concentrations of some amino acids and ammonia increase. Plasma L-glutamine concentration also rises in part as a result of proteolysis and in part due to an increased requirement to detoxify ammonia (Jahn et al. 1991).

E. 5. 1. Exercise responses in horses

With a constant speed, 20-minute duration, high intensity (about 80% of peak VO2) exercise test (Westermann et al. 2011), and with high-intensity maximal speed exercise (Hackl et al. 2009), plasma L-glutamine levels decreased significantly immediately after exercise and did not recover.

A very high intensity (about 115% of peak VO2) exercise test resulted in decreased plasma L-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 (Routledge et al. 1999). These authors attributed post-exercise L-glutamine increase to ammonia detoxification associated with the increased intramuscular ammonia production. Similar results were reported with horses completing high intensity field exercise testing (Nostell et al. 2012).

The decrease in plasma L-glutamine associated with relatively high intensity exercise contrasts with the increase in plasma L-glutamine seen during constant-speed moderate-intensity exercise (Miller and Lawrence 1988) and was of a magnitude equal to the osmotic loss of plasma fluid. Plasma L-glutamine returned gradually to pre-exercise values over a 30-minute period.

When Harris et al. (2006) supplemented dietary L-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 supplementation nearly doubled plasma L-glutamine concentrations. They concluded that increasing plasma L-glutamine concentrations through the diet has "benefit in the athletically worked horse with lowered plasma L-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 proteolysis (van den Hoven et al. 2011). The intended use level of L-glutamine provided in SUCCEED® represents 12- 25% of the amounts used in these studies. This provides sufficient L-glutamine to support intestinal cells without raising plasma L-glutamine.

When Matsui et al. (2006) 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 protein degradation and increased the rate of protein synthesis in the hind limb. Recently, van den Hoven et al. (2010) reported that oral administration of amino acids to horses within 1 hour after exercise increased the intramuscular amino acid concentrations. In this study using exercise trained horses, when the diet of horses was supplemented with amino acids for 6 weeks, high intensity exercise resulted in a 16% decrease in muscle L-glutamine, followed by a 30% increase in muscle L-glutamine 4 hours after completion of exercise. This was associated with a 25% increase in post-exercise plasma L-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 need for L-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. (2003) examined the effects of long-term endurance exercise (80 km endurance race) on plasma L-glutamine and immune function parameters.

- Pre-race plasma L-glutamine concentrations were very low, and much lower (0.279 ± 0.016 mmol/L) than in other equine studies. That there was no decrease immediately post-race, one hour post-race, and one-day and three-days post-race can be attributed to these very low starting values.
- In the post-race period, these 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 L-glutamine concentrations contributed to the severity of the observed immune depression. The results also indicate that endurance horses do not receive adequate dietary L-glutamine.

A 16-week, regular exercise training program for Thoroughbred horses (King and Suleiman 1998), and 4 to 16-week training periods of varying intensities using Standardbred horses (Westermann et al. 2011), had no effect on plasma L-glutamine concentrations 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 L-glutamine concentrations and tissue stores.

A viral challenge (equine influenza virus) of six horses resulted in a gradual and progressive approximately 30% decrease in plasma L-glutamine over a six-day period, and L-glutamine remained depressed for at least an additional eight days (Routledge et al. 1999). The study authors attributed this result to an increased requirement for L-glutamine by immune system cells. A sustained decrease in L-glutamine was suggested to impair the horses' ability to mount an effective immune response (Parry-Billings et al. 1992).

E. 6. Summary of benefits and functions of supplemented L-glutamine in horses

- Tissue L-glutamine concentrations are lower in horses than in other mammals, reflecting dietary composition and possible dietary inadequacy.
- During normal periods of increased metabolic activity (lactation, growth and development of young, exercise and training), L-glutamine requirements are increased.
- Tissue L-glutamine availability is not always adequate to meet tissue demands during periods of increased metabolic rate.
- Dietary provision of L-glutamine has utility in minimizing or preventing catabolic states associated with periods of increased metabolic rate (exercise, lactation).
- Diets deficient in L-glutamine do not provide sufficient L-glutamine to enterocytes and or other body systems.
- Dietary L-glutamine supplementation resulted in significant increase in horses' systemic L-glutamine concentrations.
- L-Glutamine supplementation can help athletic horses increase plasma L-glutamine concentrations. All athletic horses tested have had low plasma L-glutamine concentrations, typically half that of well-fed healthy horses and healthy humans.

- A sustained decrease in plasma L-glutamine impairs the horses' ability to mount an effective immune response.

In conclusion, intestinal enterocytes and immune cells can absorb and use the majority of orally ingested L-glutamine. It can benefit numerous intestinal enterocytes and immune cell functions. The equine small intestine is very similar to that of other mammals with respect to barrier mechanisms, nutrient absorptive and transport mechanisms, and immune system mechanisms. Supplementary dietary L-glutamine will support intestinal cell nutrition, the immune system, and the general health of horses.

F. Safety Evaluation

In this section we have not considered effects of L-glutamine supplementation in unhealthy or diseased animals. Only one peer-reviewed scientific study has examined safety of orally supplemented L-glutamine in horses and no adverse events were reported (Lindinger and Anderson 2014). Within this section, we have therefore summarized the peer-reviewed scientific literature on other species, and briefly summarized the results of three in-house equine studies. The reader is reminded of the similarities between horses and other mammals with respect to small-intestinal and immune-system functions. These similarities apply as well to the following section, insofar as L-glutamine provided as part of a nutritional supplement at up to 4 g / day for a 450 kg body mass (0.009 g / kg body mass) mammal has been demonstrated to be safe and has not been associated with any adverse events in any species. This level of supplementation would provide L-glutamine at about 10% above that provided as part of the normal equine diet. The capacity of the intestinal system, skeletal muscle, liver and kidneys to extract L-glutamine and glutamate is high, and Bertola and Burrin (2008) found that diets rich in L-glutamine or glutamate have little effect on circulating concentrations and low potential for toxicity.

F.1 Safety 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 (Food and Nutrition Board 2005). Within this context, and based on the absence of adverse effects, the Observed Safety Limit of L-glutamine supplementation (i.e., the highest amount one can consume that will not cause side effects) was identified to be 14 g / d in supplemental form above normal food intake in normal healthy adults (Shao and Hathcock 2008). Higher dietary intake levels have been tested and shown to be well tolerated (Novak et al. 2002; Wischmeyer 2007; Tjader et al. 2007; Bongers et al. 2007; Garlick 2001, 2004; Biolo et al. 2005; Watford 2008).

There is inadequate scientific evidence at this time that higher intake levels (50 – 60 g / day for up to 3 weeks) do not cause observable adverse events over a lifetime of use (Roth 2008). While L-glutamine supplementation is generally safe in animal models, infants, and critically ill children, it is not yet recommended for use in premature neonates or infants with late-onset sepsis (Briassouli and Briassoulis 2012; Moe-Byrne et al. 2016).

- In humans and other animals, ingestion of approximately 0.75 g L-glutamine / kg body mass (in the range of 40 – 60 grams per day) may increase plasma ammonia concentrations above the tolerated safety limit (Ward et al. 2003).
- Intake levels as high as 2.0 g / kg body mass in rats caused only an approximate 30% increase in striatal L-glutamine concentrations and only a 13% increase in striatal fluid GABA concentrations (Wang et al. 2007).
- 50 subjects aged 17 – 65 years old ingested a carbohydrate / L-glutamine (50 grams of L-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 L-glutamine supplementation was safe during pre-operative "fasting" and subsequent surgery (Borges Dock-Nascimento et al. 2011).
- Elderly men and women (69 $\pm$ 8.8 years) ingesting 0.5 g/kg supplemental L-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 (Galera et al. 2010).
- In critically ill children, several studies have shown that L-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. 2007).
- The DailyMed web site for the L-glutamine oral supplement for humans says that 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.

(http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=cd3fb572-c5b1-43da-aea2-31208985f544#section-8.3).

F.1.1 L-Glutamine side effects in humans

Supplemental L-glutamine is well tolerated in humans and did not cause severe adverse side effects when orally supplemented below 40 gram per day in humans (~0.5 g / kg body mass) (Ziegler et al. 1990; Holecek 2013). Holecek (2013) has found that when intake levels of 40 grams or more are consumed per day, L-glutamine:

- may impair amino acid transport and distribution among tissues because it competes with other amino acids for transport systems (Salloum et al. 1993), such that individuals with reduced kidney function should carefully consider L-glutamine requirements;
- may impair synthesis of endogenous L-glutamine and enhance glutamate and ammonia production;
- may impair ammonia detoxification; and
- may result in an abnormal balance of amino acids in the body.

Long term effects on the immune system have not yet been assessed. Holecek (2013) also noted that the effects of withdrawal of high-intake L-glutamine supplementation are unknown.

F.2 Safety in cultured cells

Wong et al. (2011) assessed the safety of L-glutamine using bacterial reverse mutation assays with *Salmonella typhimurium* test strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* mutant Wp2 uvrA. L-Glutamine in the range of 156 to 5000 µg / plate did not inhibit growth and had no mutagenic activity in either of these five strains. Wong also used an *in vitro* chromosomal aberration assay comprising Chinese hamster lung fibroblast cells. L-Glutamine in the range of 153 to 5000 µg / ml did not induce chromosomal aberrations in the *in vitro* chromosomal aberration assay. 5000 µg/mL L-glutamine for 24 and 48 h was associated with cytotoxicity where cell growth was reduced to 64.5 and 50.5% of the negative control, respectively.

F.3 Safety in rats

Wong et al. (2011) assessed the safety and toxicity of L-glutamine using Sprague-Dawley rats (10 / sex / group) orally supplemented for 13-weeks. The L-glutamine contents of the diet were 0, 0.5, 2.5 or 5.0% (the highest intake level was equal to 4.5 grams / kg body mass daily) of the total diet. The results showed no morbidity or mortality; no significant differences in body mass, body mass gains, ophthalmological and urinalysis parameters, or organ weights compared to controls; and no toxicological effects on hematological or blood biochemical parameters. The study concluded that the no-observed-adverse-effect-level (NOAEL) was 3.8 to 4.5 g / kg body mass / day in male and female rats, respectively.

Tsubuku et al. (2004) conducted a 13-week toxicity study using groups of 12 male and 12 female Sprague-Dawley rats. Rats were fed a standard diet supplemented with 0, 1.25, 2.5, or 5.0% of L-glutamine (Ajinomoto Co., Inc.).

- The authors chose as the NOAEL lowest dietary level tested, 1.25% (corresponding to 833 or 964 mg / kg body weight / day for male and female rats, respectively).
- Changes in some urinalysis and clinical chemistry parameters occurred in the 2.5 and 5.0%, supplement groups.
- The study also determined that the changes observed in these higher dose groups were not toxicologically relevant for a number of reasons (i.e., values were within physiological normal ranges; changes were observed in only one sex; accompanying toxicity findings were not observed; and/or effects were noted at the end of the recovery phase only).
- Therefore, no physiologically adverse findings due to L-glutamine supplementation at 5% of the diet (corresponding to 3.5 and 4.0 g / kg body weight / day for male and female rats, respectively) occurred in the 13-week study.
- Additionally, the 5% dietary amount should be taken as the NOAEL and this provided a value consistent with the findings reported by Wong et al. (2011).

Rogero et al. (2004) administered acute oral supplementation of 1 g / kg body mass L-glutamine or 1.5 g / kg alanyl-glutamine by gavage daily for 21 days. Plasma L-glutamine concentration increased from 0.94 mmol/L to 2.0 and 2.6 mmol/L, respectively, at 30 minutes and decreased to pre-gavage concentrations by 120 minutes. Compared to controls, there

were no differences in food uptake and plasma concentrations of L-glutamine, glutamate and ammonia, and there were statistically (but not likely physiologically) significant increases in skeletal muscle and liver L-glutamine contents.

Similar findings were reported in similarly-supplemented rats that underwent greater than one hour of exhaustive exercise (Rogero et al. 2006), although the inflammatory response to exercise was attenuated in both supplemented groups compared to controls (Cruzat et al. 2010).

The literature shows no evidence for neurotoxicity with dietary L-glutamine supplementation. L-glutamine is directly involved in the body's defense against excessive ammonia accumulation. Ammonia reacts with glutamate, catalyzed by L-glutamine synthase, throughout the body. Because ammonia toxicity is highly detrimental to central nervous system (CNS) function, astrocytes have a high capacity to detoxify ammonia using L-glutamine synthase. The resultant production of L-glutamine within CNS tissues has been proposed to "mediate key aspects of ammonia neurotoxicity" (Albrecht et al. 2010). To date, this has only been experimentally demonstrated in rat tissues *in vitro*, and has not been associated with high levels of dietary L-glutamine supplementation in any species.

F.4 Safety in rabbits

Chamorro et al (2010) conducted an extensive study designed to examine the effects of L-glutamine supplementation on post-weaning mortality. They measured feed intake, body mass gain, feed efficiency at day 14 after weaning and at the end of the experiment, blood, mid-jejunum and mid-ileum tissue samples for assay of intestinal enzymes, PPARγ mRNA, and histological analysis, jejunal morphology (villus height and associated crypts depth), and ileal and cecal microbiota. Supplementation with 1% L-glutamine significantly ($p < 0.05$) reduced fattening mortality during both the first two weeks and during the entire fattening period. Dietary treatment did not affect measured blood parameters, intestinal enzyme activities, gut histology or gut immune response.

With respect to jejunal morphology, L-glutamine supplementation tended ($p = 0.061$) to decrease villus height by 11.4% and crypt depth by 7.7%, but did not affect the villus height / crypt depth ratio, which averaged 4.95. In rabbits supplemented with L-glutamine, the frequency of *Helicobacter* spp. was decreased in the ileum (from 86.7 to 33.3%, $P = 0.003$) and cecum (from 86.7 to 46.7%, $P = 0.020$) and the frequency of *Clostridium* spp. was reduced in the ileum (from 86.7 to 33.3%, $P = 0.003$). Other microbial species were minimally affected.

The authors concluded that 1% L-glutamine supplementation to postweaned rabbit diets decreased fattening mortality and modified the intestinal microbiota, although no beneficial effects were observed on mucosal integrity or inflammatory and systemic immune response.

F.5 Safety in horses

A safety study of an equine feed supplement, SUCCEED®, at a daily serving of 54 grams per horse) was performed by Scott (2005) using seven horses per treatment group for eight weeks (see Lindinger and Anderson 2014). Dr. David Dzanis (2005) performed a scientific assessment of this study and reported no shortcomings with respect to State of Texas. The data from this study have been statistically analyzed and the study recently published (Lindinger and Anderson 2014). Initially, 16 horses were divided randomly between the test group and the control group. One horse was removed from each group during the course of the study, for non-nutritional reasons (lameness). On a weekly basis, outcome measures included complete blood chemistry and hematology panels, a complete urinalysis, as well as body mass, heart rate, respiratory rate and body temperature measurements. There were no statistical differences between treatment horses and control horses throughout the treatment period, and no clinical abnormalities associated with supplementation. The study concluded that SUCCEED®, when ingested by horses at 54 g / horse per day (i.e. twice the recommended daily maintenance level), was safe and does not pose a health risk when used in accordance with good feeding practice.

Pellegrini (2012) studied blood biochemistry and hematology profiles of two groups of 17 performance horses in training over a 4-month period. One group was a control group, while the test group received a daily serving of 26.5 grams of an L-glutamine-containing supplement. The single daily serving provided 0.418 grams of L-glutamine, equal to 0.84 mg / kg body mass for a 500 kg horse. In both groups, all blood parameters remained within normal reference ranges during the entire 4 months of the study. After the first 5 weeks of supplementation in the supplement group, the RBC count and hemoglobin content were increased compared to the control group. It was concluded that 4 months of daily L-glutamine supplementation of 0.418 g was not detrimental to tested blood parameters and some beneficial effects were noted at this relatively low serving level.

Pellegrini and Franco (2014) studied blood biochemistry and hematology profiles of 38 horses, of which 20 were randomly assigned to a test group and 18 served as controls. Over a 4-month period horses in the supplement group received 26.5 grams of an L-glutamine containing supplement daily. This provided a daily serving of 0.785 grams of glutamine, equal to 1.6 mg / kg body mass for a 500 kg horse. There were no significant changes over time and no detrimental effects on any tested blood parameters.

F.6 Summary of safety studies

Table F.1 summarizes key studies from the literature that have examined safety and functional effects of L-glutamine in humans, rats, mice, rabbits and horses. It is noteworthy that the equine study performed by Harris et al. (2006) used relatively low intake levels (0.03 and 0.06 g / kg BM per day, which is equivalent to 14.7 and 29.4 g per horse per day), and the control level of daily L-glutamine intake was not provided. The SUCCEED® products intend to provide about 7% of the lowest intake level used in the Harris et al. (2006) study.

Table F.1. Literature summary of dietary L-glutamine supplementation

Reference	Species	Intake level	Main effects	
Shao and Hathcock 2008, and Watford 2008 and Dioguardi 2011	Humans; review of all clinical data;	up to 45 g / day for 6 weeks; = 0.9 g / kg lean body mass /day 0.57 to 0.75 g / kg / day well tolerated	No adverse effects; though studies using more than 14 g/day did not test for safety parameters; On this basis, and on safety data for 14 g/day (0.2 g/kg / day), this is the OSL (observed safe level)	
Shao and Hathcock 2008	Reviewed animal data	rats, up to 50 g / kg of daily diet for 13 weeks	Minor increases in certain hematological and urinary parameters in the 2.5% and 5% groups, all changes were within the physiologic range. • concluded NOAEL of 1.25% (0.9 g / kg bm / day	
Rohde et al. 1998	Humans; plasma L-glutamine lowish at start (0.51 mmol/L)	0.1 g / kg body mass	Prevent post-exercise decrease in L-glutamine Did not prevent fall in lymphocyte proliferation; (similar to at least four other studies – Gleeson 2008)	
Fasina et al. 2010	Poultry, newly hatched	10 g / kg of diet for 14 days	Improved body mass gain	No effect on cecal Salmonella
Rogero et al. 2004	Rats	1g / kg body mass acute, by gavage; chronic for 21 days	Acute feeding increased plasma [glutamine] from 1 to 2 mmol/L; Chronic: no increase in fasting L-glutamine; maintained acute L-glutamine feeding response; increased tissue L-glutamine contents	
Wang et al. 2007	Rats	0.5 g / kg diet by gavage; acute only	Increased ECF and striatal GABA	Glutamine supplementation has utility in treating anxiety
Lagranha et al. 2005	Rats	1 g/ kg body mass by gavage	Increased neutrophil phagocytic activity and ROS, and decreased nitric oxide induced by exercise	
Rogero et al. 2011	Mice – early weaned	40 g / kg diet / day for 7 and 14 days	Increased serum IGF-1, albumin and muscle protein content	
Chamorro et	Rabbits, 473 g	Supplemented	Basal diet of	Decreased

al. 2010	intestinal health study	10 g / kg diet with L-glutamine; for 14 days, followed by normal diet	31 g / kg L-glutamine	fattening mortality post weaning & modified intestinal biota
Lindinger and Anderson 2014	Horses	1.72 g of L-glutamine daily supplemented to normal diet for 8 weeks	No adverse effects or events	
Harris et al. 2006	Horses	0.03 and 0.06 g / kg body mass acute; 0.06 g/kg body mass for 10 days	Single servings of oral L-glutamine supplementation increased plasma [glutamine]; 10 days of L-glutamine supplementation did not alter this response – benefit	
Miller and Lawrence 1988	Horses	increased dietary protein from 12.9% to 18.5% for 2 weeks	Plasma [glutamine] increased from 0.493 to 0.625 mmol/L in controls, and from 393 to 492 in high protein group.	

G. L-Glutamine as a nutritional supplement for non-human mammals

It is noteworthy that L-glutamine is not found in 21 C.F.R. Parts 573, 582 or 584.

Table G.1 presents daily L-glutamine intake levels that resulted in a safe upper limit in humans and rats. An estimate of an L-glutamine requirement for horses is based on the NRC (2007) recommendation for daily protein intake for non-working horses. By comparison, SUCCEED® products would deliver 3.4 g per day of L-glutamine when horses are provided two servings per day. We believe that up to 4 g per day of L-glutamine is GRAS, and our GRAS opinion covers up to 4 g per day.

Table G.1. Reference table for upper limit of L-glutamine supplementation compared to L-glutamine proposed for SUCCEED® products.

	Species	Intake level (g / kg body mass per day)	Equivalent level for 70 kg human	Equivalent level for 450 kg horse
NOAEL	rat	4.5	31.5	2025
Observed Safety Limit	human	0.2	14	90
Potential for increased ammonia	human	0.75	52.5	338
[1]NRC - Horses	horse	0.089	6.23	40
[2]SUCCEED®	horse	0.007 (up to 6 % of 28 g up to 2 times / day)	0.49	3.36

Notes: These amounts are in addition to L-glutamine normally found in a typical diet.
[1] Includes dietary L-glutamine intake (normal forage and feed).
[2] The maximum L-glutamine content of SUCCEED® is intended to be 6%.
For humans, 21 C.F.R. § 172.32.0(c)(4) allows for L-glutamine to not exceed 12.4% of total protein in the finished food.

The Food and Nutrition Board recommends protein at up to 35% of total energy intake; with 12.4% of this as L-glutamine, this equals 4.34% of total daily energy intake in the form of L-glutamine.

In horses, as in other animals, it is not possible or practical to accurately determine an optimal intake level of L-glutamine supplementation experimentally because of widely varied metabolic demands, health / disease states, multiple fates and effects within the body. What is evident from the literature in healthy mammals, however, is that there is an important nutritional requirement for L-glutamine during normal periods of increased metabolic rate, such as exercise, lactation and growth. Also, L-glutamine supplemented at intake levels of 30 g per day for humans is considered safe and is without significant negative effects (Roth 2008).

In humans, benefits of supplemental dietary L-glutamine have been seen with as little as 5 g per day, and intake levels of up to 25 g / day have been repeatedly well tolerated although an upper limit of 14 g / day is recommended as the Observed Safety Limit (Shao and Hathcock 2008). Lenders et al. (2009) suggested that L-glutamine as an oral supplement should not exceed 0.75g / kg body mass (above normal food intake) per day due to the potential to excessively increase ammonia concentrations and an absence of evidence for benefit at higher intake levels.

L-Glutamine is a commonly used supplement among human athletes, encompassing all

disciplines from endurance to strength and body building (Gleeson 2008). Exercise resulting in decreased intramuscular and plasma concentrations of L-glutamine has been hypothesized to impair immune function, contributing to the immunodepression apparent in many endurance athletes, including horses (see above). There is also some evidence that L-glutamine supplementation reduces muscle proteolysis during exercise and enhances muscle glycogen resynthesis post-exercise (Gleeson 2008).

For human and equine athletes, manufacturers and suppliers of L-glutamine supplements claim that L-glutamine provides nutritional support for the immune system, prevention of infection, improved gut barrier function, reduced risk of endotoxemia, improved cellular and whole body hydration, enhanced muscle glycogen synthesis, enhanced muscle protein synthesis, muscle hypertrophy, reduced muscle soreness, improved muscle tissue repair, and enhanced strength and endurance exercise performance. For humans, many manufacturers recommend a 1 g / day L-glutamine supplement.

Examples of two L-glutamine-containing supplements for horses are Platinum Performance (http://www.platinumperformance.com/equine-l-glutamine/productinfo/elglp1/) and SUCCEED® (http://www.SUCCEED-equine.com).

For Platinum Performance L-glutamine, it is recommended that 10 – 40 grams be given twice daily with the feed.

For the SUCCEED® Digestive Conditioning Program, the standard serving is 27 grams, with L-glutamine at 1.6%, to be administered up to two times daily for an initial 7 to 10 day period, although once daily feeding is recommended beyond this initial period. A proposed SUCCEED® formulation is intended to provide L-glutamine at 6% of a 28 gram serving, also to be given twice daily. This supplement would therefore provide 3.36 grams of L-glutamine daily. These amounts of supplementary L-glutamine will provide additional L-glutamine primarily to the enterocytes of the upper intestinal tract, as it is these cells that are heavily involved in nutrient transport into the body, as well as being integral to immune health. These oral intake levels are relevant for horses because plasma L-glutamine concentrations reported in most studies range from 0.26 to 0.5 mmol/L, and are much lower than the recommended minimum of 0.6 mmol/L for optimum health (see above).

Sustamine™ (http://www.sustamine.com) is a dipeptide (L-alanyl-L-glutamine) form of L-glutamine that, following upper intestinal system digestion, provides L-glutamine and L-alanine. The dipeptide is proposed to be of benefit because its small molecular weight allows for rapid absorption, while the dipeptide form increases stability in aqueous solutions compared to L-glutamine alone. Its touted benefits include enhanced intestinal electrolyte and water absorption, enhanced muscle glycogen and protein synthesis, attenuated muscle proteolysis, improved gastrointestinal tract protection and function, and immune system stimulation. The company responsible for the product (Kyowa Hakko America Inc., Princeton, NJ, USA) announced that it had completed GRAS self-affirmation for the product in Sept. 2008.

Ingestion of a Sustamine™ rehydration beverage by 10 active, healthy men following moderate dehydration resulted in increased exercise time to fatigue when cycling at 75% of peak VO2 (Hoffman et al. 2010). The authors concluded that this benefit was due to enhanced fluid and electrolyte absorption with the supplement compared to controls. This conclusion is consistent with a recent study showing that Sustamine™ supplementation resulted in increased intestinal absorption of L-glutamine, compared to a pure L-glutamine supplement (Harris et al. 2012). Improvements in performance due to Sustamine™ supplementation have also recently been reported for women playing competitive (NCAA Division 1) basketball (Hoffman et al. 2012).

H. Summary of evidence supporting the intended effects and use:

The prior sections describe numerous studies and cases that demonstrate beneficial effects of L-glutamine supplementation in horses and other mammals, including:

- Tissue L-glutamine concentrations in horses are lower than those reported in well-fed, healthy humans and rats (Rogero et al. 2004; Watford and Wu 2005; Routledge et al. 1999; Wong et al. 2011).
- the 'typical intake' of protein, and therefore L-glutamine, in performance horses is inadequate to achieve optimal health and performance. No published studies have examined 2 or 4 grams dietary L-glutamine / day in horses, however Pelligrini (2012, 2014) used lesser amounts (the single daily serving provided 0.418 grams of L-glutamine, equal to 0.84 mg / kg body mass for a 500 kg horse), albeit with other putative nutraceuticals, and showed a beneficial effect on red cell mass.
- With low to normal amounts of dietary L-glutamine, up to 100% of the L-glutamine ingested with protein is utilized by cells of the small intestine.
- When dietary intake of L-glutamine is low (e.g. as a result of typical horse forage) circulating concentrations of L-glutamine are lower, and have been reported to be 35-60% of that found when dietary L-glutamine is high.
- The amino acids L-glutamine and glutamate make up 10–20% of dietary protein, and both are extensively metabolized in the small intestine of most mammals. Watford (2008) asserts that, with normal levels of dietary intake (5 – 10 g of L-glutamine daily for humans), there is no net small intestinal absorption of L-glutamine or glutamate into the blood.
- Amino acid requirements for horses have not been well defined and have not been evaluated individually at different stages of life (Tanner et al. 2014). For horses, there is no L-glutamine recommended dietary allowance (RDA). The NRC (2007) 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 per day for a 450 kg horse, which provides up to 40 g of L-glutamine daily. The recommendation increases to approximately 320 g protein / day for 450 kg horses in heavy work, which translates to 51 g of L-glutamine daily.
- When Harris et al. (2006) supplemented dietary L-glutamine (single feeding and 10 days of supplementation at 30 and 60 mg / kg body mass; approximately 15 and 30 grams per day respectively) in athletically-worked horses, they found that supplementation nearly doubled plasma L-glutamine concentrations. They concluded

that increasing plasma L-glutamine concentrations through the diet has "benefit in the athletically worked horse with lowered plasma L-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 proteolysis (van den Hoven et al. 2011).

I. Environmental Assessment

We hereby provide that the action requested qualifies for a categorical exclusion. There are two bases for this. To the applicant's knowledge, no extraordinary circumstances exist.

First, 21 C.F.R. § 25.32 (f) sates: "Affirmation of a food substance as GRAS for humans or animals on FDA's initiative or in response to a petition, under parts 182, 184, 186, or 582 of this chapter, and establishment or amendment of a regulation for a prior-sanctioned food ingredient, as defined in 170.3(l) and 181.5(a) of this chapter, if the substance or food ingredient is already marketed in the United States for the proposed use." 21 C.F.R. § 172.320 provides that L-glutamine, as a food additive, may be safely used as a nutrient added to foods for human consumption.

Second, there is no environmental residue resulting from ingestion of L-glutamine supplemented to the diet of mammals because all of the ingested L-glutamine is metabolized, with a half-life of about 20 minutes, with up to 2/3 of ingested L-glutamine being metabolized by cells of the intestinal system (Windmueller and Spaeth 1974; Heitmann and Bergman 1980; Mittendorfer et al. 2001; Meijer et al. 1997).

J. Conclusion

Controlled studies indicate mammals supplemented with L-glutamine in the diet have better health, particularly during periods of increased metabolism such as occurs during growth, lactation, exercise training and exercise recovery. One review in the published scientific literature suggested that L-glutamine supplementation may contribute to neurotoxicity due to excessive increase in ammonia or glutamate. However there are no reports of L-glutamine being given as a food in such excessive amounts to cause neurotoxicity in mammals. After reviewing the literature regarding the safety and efficacy of L-glutamine in mammalian nutrition, the Panel unanimously agrees that: 1) there are times when L-glutamine is conditionally essential such that L-glutamine provided as part of the normal diet is not adequate to maintain optimal function and health; 2) that inadequacy of dietary L-glutamine is reflected in a decreased blood concentrations of L-glutamine; and 3) that L-glutamine supplemented to the normal diet provides important nutrition to cells of the intestinal tract that support cellular function and whole body health.

The manufacturing processes for both L-glutamine and the L-glutamine-containing feed supplements were reviewed and considered by the Panel to ensure provision of a high quality feed supplement for horses. Under the conditions of manufacture and use, L-glutamine is chemically and physically stable and remains stable in pure form for at least (b) (4) and in the feed supplement for up to (b) (4)

Many safety studies have been conducted using L-glutamine including acute, subchronic, developmental and reproductive toxicology studies in rodents and in vitro mutagenicity assays. None of the studies indicated that the L-glutamine was toxic at ingestion rates greater than 10-fold higher / kg body mass of that intended to be used ingested daily. Studies conducted in several animal species, have provided a large base of experience and toxicological data (summarized in **Appendix 9**). None of the reports have suggested any toxicological or safety issues associated the use of L-glutamine at the intended ingestion rate.

In addition, L-glutamine is presently found in a large range of commercially available supplements for animal and human used, in numerous countries around the world, at levels up to 10-fold higher than intended to be used in the products described herein, with no reports of adverse findings that can be attributed to L-glutamine. Taken together, these data provide support for the establishment of GRAS status for L-glutamine at use levels proposed to be supplemented to normal diets.

K. Proposed Statement

We have concluded, based on the foregoing information, that L-glutamine for use in a feed supplement for horses is Generally Recognized among experts qualified by scientific training and experience to evaluate its safety as Safe (GRAS) for consumption by horses post-weaning at up to 4 grams per day, or approximately 9 mg / kg body wt / day.

The determination of GRAS status is on the basis of scientific procedures, in accordance with 21 C.F.R. § 570.30(b) and conforms to the guidance provided by FDA in 62 Fed. Reg. 18938 (April 17, 1997) and FDA's Notice of Pilot Program: Substances Generally Regarded as Safe Added to Food for Animals, 75 Fed. Reg. 31806 (June 4, 2010).

Michael L. Lindinger, Ph.D.	Jan. 9, 2016 Date
E. Murl Bailey, Jr., DVM, Ph.D., DABVT	Date
David A. Dzanis, DVM, Ph.D., DACVN	Date

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Michael L. Lindinger, Ph.D. — Date

E. Murl Bailey, Jr., DVM, Ph.D., DABVT — 14 Feb 2016

David A. Dzanis, DVM, Ph.D., DACVN — Date

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Michael L. Lindinger, Ph.D. — Date

E. Murl Bailey, Jr., DVM, Ph.D., DABVT — Date

David A. Dzanis, DVM, Ph.D., DACVN — FEB 14, 2016 — Date

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APPENDICES

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APPENDIX 1
CURRICULA VITAE OF EXPERT PANEL MEMBERS

APPENDIX 1. CURRICULA VITAE OF EXPERT PANEL MEMBERS

A.1.1 DR. MICHAEL LINDINGER

CURRICULUM VITAE

11 Page(s) Withheld in Full Pursuant to FOIA Exemption (b)(6) immediately following this page

A.1.2 DR. MURL BAILEY

CURRICULUM VITAE

20 Page(s) Withheld in Full Pursuant to FOIA Exemption (b)(6) immediately following this page

A.1.3 DR. DAVID DZANIS

CURRICULUM VITAE

12 Page(s) Withheld in Full Pursuant to FOIA Exemption (b)(6) immediately following this page

APPENDIX 2
AGENT APPOINTMENT LETTER

APPENDIX 2. AGENT APPOINTMENT LETTER

65 Aurora Industrial Parkway
Aurora, Ohio 44202-8088
330.562.0888
330.562.1445

February 10, 2016

U.S. Food and Drug Administration
Center for Veterinary Medicine
Division of Animal Feeds (HFV-224)
7519 Standish Place
Rockville, MD 20855

Re: Authorization to Act as Agent for Freedom Health, LLC

Dear Sir or Madam:

This letter is to advise that The Nutraceutical Alliance, its employees, associates and agents, specifically including but not limited to Michael I. Lindinger, are authorized to act as agents on behalf of Freedom health, LLC with regard to its Generally Regarded as Safe (GRAS) notification for L-Glutamine, submitted to the U.S. Food and Drug Administration, Center for Veterinary Medicine.

This letter is our authorization to you to permit The Nutraceutical Alliance to undertake appropriate communications relevant to making submissions or inquiries as to the status of the above referenced GRAS Notification filed by or on behalf of Freedom Health, LLC, including examination of all relevant information including confidential business, proprietary, and trade secret information submitted or develop under the Federal Food, Drug and Cosmetic Act.

Sincerely,

Patrick Warczak, Jr.
VP, Marketing
FREEDOM HEALTH LLC

freedom health llc

Manufactured with SUCCEED

APPENDIX 3
L-GLUTAMINE MANUFACTURING

APPENDIX 3. L-GLUTAMINE MANUFACTURING

A.3.1 COUNTRY OF ORIGIN OF L-GLUTAMINE

AJINOMOTO.

AJINOMOTO NORTH AMERICA, INC.
Technical Services
4020 Ajinomoto Drive, Raleigh, NC 27610
Tel: 919-723-2089 Fax: 919-233-6612

Date: January 9, 2013

To Whom It May Concern:

Re: Country of Origin

We, Ajinomoto North America, Inc. are pleased to respond to your inquiry in regards to the country of origin for the product listed below.

SAP #	Product	Size	Country of Origin	HTS #
20581	L-Glutamine	25kg	Brazil	2924.19.11.50

If you have any additional questions, please do not hesitate in contacting the Ajinomoto technical support staff by e-mail at nctechnicalservices@ajiusa.com

Technical Services
Ajinomoto North America, Inc.

A.3.2 CERTIFICATE OF MANUFACTURE - GMP

SELENE CUBEROS PEREZ
TRADUTOR PÚBLICO
INGLÊS - PORTUGUÊS
TRADUÇÃO OFICIAL
Alphaville: Alameda Araguaia, 1293 - 7º andar - sala 706 - Barueri - SP - 06455-000
TEL. 55 11 4191-6868 – Fax: 55 11 4191-2888 – E-mail: alphaville@fidelity.com.br
São Paulo: Rua Libero Badaró, 377 - 29º andar - São Paulo - SP - 01009-906
TEL. 55 11 2166-4444 – Fax: 55 11 2166-4466 – E-mail: sp@fidelity.com.br
Campinas: TEL. 55 19 3295-4000 – E-mail: campinas@fidelity.com.br
Rio de Janeiro: TEL. 55 21 2507-1958 – E-mail: rj@fidelity.com.br
Belo Horizonte: TEL. 55 31 3274-4343 – E-mail: mg@fidelity.com.br
Curitiba: TEL. 55 41 3322-0677 – E-mail: pr@fidelity.com.br
Porto Alegre: TEL. 55 51 3346-1111 – E-mail: rs@fidelity.com.br
Brasília: TEL. 55 61 3223-7000 – E-mail: df@fidelity.com.br
Website: www.fidelity.com.br

Matr. JUCESP Nº 1695
C.C.M. 9.382.440-0

C.P.F. Nº 701.395.718-68
R.G. 5.266.238

TRADUÇÃO Nº I-103295/11 LIVRO Nº 952 FOLHAS Nº 1

I, SELENE CUBEROS PEREZ, a Sworn Translator and Commercial Interpreter for the English language, duly sworn by the Board of Trade of the State of São Paulo - Federative Republic of Brazil, DO HEREBY CERTIFY that a document issued in the PORTUGUESE language was submitted to me, which I faithfully translated into ENGLISH, as follows: -.-

[Coat of Arms]

MINISTRY OF HEALTH
BRAZILIAN HEALTH SURVEILLANCE AGENCY
GENERAL MANAGEMENT OF INSPECTION AND CONTROL OF INGREDIENTS, DRUGS AND PRODUCTS
CERTIFICATE OF GOOD MANUFACTURING PRACTICES
PHARMACEUTICAL INGREDIENT FIELD

Number/Year: 04/2011.

According to Law no. 9.782, dated January 26th, 1999, Decree no. 3.029, dated April 16th, 1999 and Resolution – RE no. 3.445, dated August 8th, 2011, published in the Federal Official Gazette on August 8th, 2011, I do hereby certify that the company described below satisfies the current health legislation relative to the Good Manufacturing Practices concerning Intermediate Products and Active Pharmaceutical Ingredients, as required by the Brazilian health authority, which are consonant to the recommendations of the World Health Organization. We further certify that the company manufacturing sites are subjected to regular inspections.

Company: Ajinomoto do Brasil Ind. e Com. de Alimentos Ltda.
CNPJ [Corporate Taxpayer Registration]: 46.344.354/0001-54.
Address: Via Anhangüera – KM 131, S/N.
District: Jaguari. **CEP:** 13480-970.
City: Limeira. **State:** São Paulo.
Operation Permit No.: 1.08.465-7.

Certificate of Good Manufacturing Practices for Intermediate Products and Active Pharmaceutical Ingredients

Active Pharmaceutical Ingredients obtained by fermentation: L-glutamine and L-isoleucine.

Valid until: August 7th, 2013.
Brasília – DF, August 24th, 2011.
Signed: [illegible signature]
Name: Luiz Roberto da Silva Klassmann.

SELENE CUBEROS PEREZ

TRADUTOR PÚBLICO

INGLÊS - PORTUGUÊS

TRADUÇÃO OFICIAL

Alphaville: *Alameda Araguaia, 1293 - 7º andar - sala 706 - Barueri - SP - 06455-000*
TEL.: 55 11 4191-6868 – Fax: 55 11 4191-2888 – E-mail: alphaville@fidelity.com.br
São Paulo: *Rua Libero Badaró, 377 - 29º andar - São Paulo - SP - 01009-906*
TEL.: 55 11 2166-4444 – Fax: 55 11 2166-4466 – E-mail: sp@fidelity.com.br
Campinas: *TEL.: 55 19 3295-4000 – E-mail: campinas@fidelity.com.br*
Rio de Janeiro: *TEL.: 55 21 2507-1988 – E-mail: rj@fidelity.com.br*
Belo Horizonte: *TEL.: 55 31 3274-4343 – E-mail: mg@fidelity.com.br*
Curitiba: *TEL.: 55 41 3322-0077 – E-mail: pr@fidelity.com.br*
Porto Alegre: *TEL.: 55 51 3346-1111 – E-mail: rs@fidelity.com.br*
Brasília: *TEL.: 55 61 3223-7000 – E-mail: df@fidelity.com.br*
Website: www.fidelity.com.br

Matr. JUCESP Nº 1695
C.C.M. 9.382.440-0

C.P.F. Nº 701.395.718-68
R.G. 5.266.238

TRADUÇÃO Nº I-103295/11 LIVRO Nº 952 FOLHAS Nº 2

Title: General Manager of Inspection and Control of Ingredients, Drugs and Products.
This Certificate is only valid if bearing the raised seal of the Health Surveillance Authority of the Ministry of Health.

NOTHING FURTHER WAS CONTAINED IN THE DOCUMENT SUBMITTED.
I verified it and certify to it.
The Sworn Public Translator.
Barueri, September 09, 2011.

SELENE CUBEROS PEREZ
Tradutor Público

8.º Cartório de Notas da Capital - SP - Tabelião Bel. Douglas Eduardo Dualibi

Reconheço por SEMELHANÇA a(s) firma(s) de SELENE CUBEROS PEREZ
que conferem com os padrões depositados neste cartório.
São Paulo, 09 de setembro de 2011. ... semelhança sem valor econômico
Válido somente com o selo de autenticidade

FIRMA 1
1026AA943078

A.3.3 MANUFACTURING FLOW

AJINOMOTO.

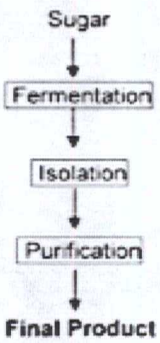
Limeira, January 28, 2011

AJINOMOTO DO BRASIL INDÚSTRIA E COMERCIO DE ALIMENTOS LTDA
Via Anhanguera, Km 131 - B. Jaguari - 13480-970 - Limeira - SP - Brasil

To: Whom it may concern

Re: Manufacturing Flow

We, AJINOMOTO DO BRASIL INDÚSTRIA E COMÉRCIO DE ALIMENTOS LTDA, are pleased to respond to your inquire in regards to the manufacturing flow for L-Glutamine.



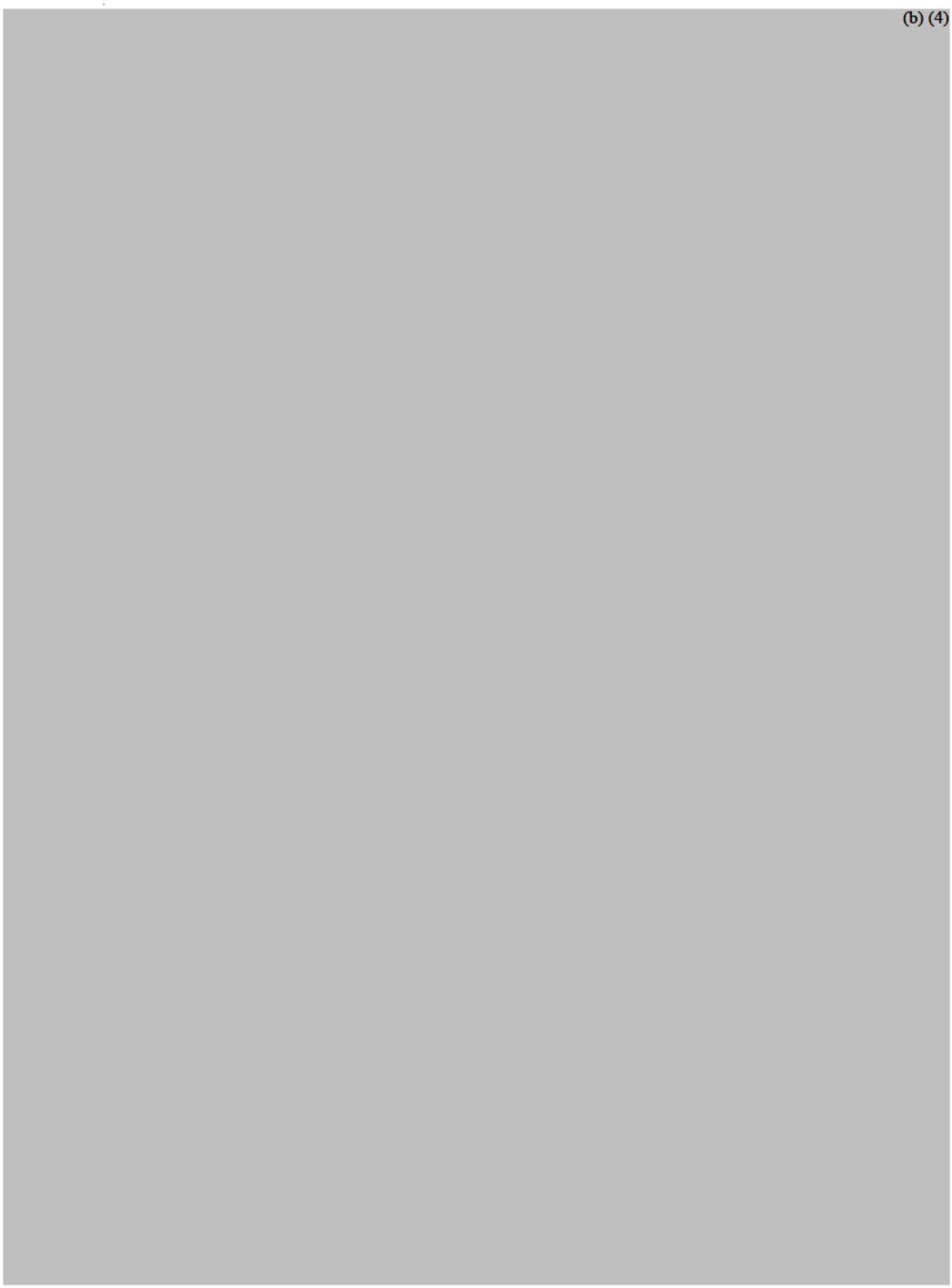
Sincerely,

(Signature)
Olivier Zanella Filho
Quality Assurance and Control Manager

(Signature)
Carlos Tetsuya Takata
Plant Director

A.3.4 L-GLUTAMINE PARTICLE SIZE DISTRIBUTION

(b) (4)



(b) (4)

(b) (4)

A.3.5 CERTIFICATES OF ANALYSIS FOR L-GLUTAMINE

(3.5.1)

AJINOMOTO.

CERTIFICATE OF ANALYSIS

Ajinomoto do Brasil Ind. e Com. de Alimentos Ltda.
Rodovia Anhanguera, Km 131
Bairro Jaguari - Limeira - SP - Brasil

ANALYTICAL RESULTS OF: L-GLUTAMINE

LOT N°: 13 0081 K 03 B

ITEM	LIMIT	RESULT
Description		(b) (4)
Identification (FT-IR)		
Specific Rotation (D-Line,20°) – H_2O		
Specific Rotation (D-Line,20°) – HCl		
State of solution (Transmittance)		
State of solution		
Chloride (Cl)		
Ammonium (NH_4)		
Sulfate (SO_4)		
Iron (Fe)		
Heavy metals (Pb)		
Arsenic (As_2O_3)		
Related substances		
Loss on drying		
Residue on ignition (sulfated)		
Assay		
pH		
Bacteria		
Coliform Bacteria		
Fungi		

We certify that quality of this product conforms to USP residual solvents requirement.
We certify the quality of this product conforms to DAB and USP

Manufacturing Date: (b) (4)
Retest Date:

Manufacturer:

Manufacturing Site:

(3.5.2)

AJINOMOTO.

CERTIFICATE OF ANALYSIS

Ajinomoto do Brasil Ind e Com. de Alimentos Ltda.
Rodovia Attilio Gianetti, Km 131
Bairro Jaguari - Limeira - SP - Brasil

ANALYTICAL RESULTS OF: L-GLUTAMINE

LOT N°: 13 0082 K 03 B

ITEM	LIMIT	RESULT
Description		(b) (4)
Identification (FT-IR)		
Specific Rotation (D-Line,20°) – H_2O		
Specific Rotation (D-Line,20°) – HCl		
State of solution (Transmittance)		
State of solution		
Chloride (Cl)		
Ammonium (NH_4)		
Sulfate (SO_4)		
Iron (Fe)		
Heavy metals (Pb)		
Arsenic (As_2O_3)		
Related substances		
Loss on drying		
Residue on ignition (sulfated)		
Assay		
pH		
Bacteria		
Coliform Bacteria		
Fungi		

We certify that quality of this product conforms to USP residual solvents requirement.
We certify the quality of this product conforms to DAB and USP

Manufacturing Date: (b) (4)
Retest Date:

Manufacturer:

Manufacturing Site:

(3.5.3)

AJINOMOTO.

CERTIFICATE OF ANALYSIS

Ajinomoto do Brasil Ind. e Com. de Alimentos Ltda.
Rodovia Anhanguera, Km 131
Bairro Jaguari - Limeira - SP - Brasil

ANALYTICAL RESULTS OF: L-GLUTAMINE

LOT N°: 13 0083 K 03 B

ITEM	LIMIT	RESULT
Description		(b) (4)
Identification (FT-IR)		
Specific Rotation (D-Line,20°) – H_2O		
Specific Rotation (D-Line,20°) – HCl		
State of solution (Transmittance)		
State of solution		
Chloride (Cl)		
Ammonium (NH_4)		
Sulfate (SO_4)		
Iron (Fe)		
Heavy metals (Pb)		
Arsenic (As_2O_3)		
Related substances		
Loss on drying		
Residue on ignition (sulfated)		
Assay		
pH		
Bacteria		
Coliform Bacteria		
Fungi		

We certify that quality of this product conforms to USP residual solvents requirement.
We certify the quality of this product conforms to DAB and USP

Manufacturing Date: (b) (4)
Retest Date:

Manufacturer:

Manufacturing Site:

(3.5.4)

AJINOMOTO.

CERTIFICATE OF ANALYSIS

Ajinomoto do Brasil Ind. e Com. de Alimentos Ltda
Rodovia Anhanguera, Km 121
Bairro Jaguari - Limeira - SP - Brasil

ANALYTICAL RESULTS OF: L-GLUTAMINE

LOT N°: 13 0084 K 03 B

ITEM	LIMIT	RESULT
Description		(b) (4)
Identification (FT-IR)		
Specific Rotation (D-Line,20°) – H_2O		
Specific Rotation (D-Line,20°) – HCl		
State of solution (Transmittance)		
State of solution		
Chloride (Cl)		
Ammonium (NH_4)		
Sulfate (SO_4)		
Iron (Fe)		
Heavy metals (Pb)		
Arsenic (As_2O_3)		
Related substances		
Loss on drying		
Residue on ignition (sulfated)		
Assay		
pH		
Bacteria		
Coliform Bacteria		
Fungi		

We certify that quality of this product conforms to USP residual solvents requirement.
We certify the quality of this product conforms to DAB and USP

Manufacturing Date: (b) (4)
Retest Date:

Manufacturer:

Manufacturing Site:

(3.5.5)

AJINOMOTO.

CERTIFICATE OF ANALYSIS

Ajinomoto do Brasil Ind. e Com. de Alimentos Ltda.
Rodovia Anhanguera, Km 131
Bairro Jaguari - Limeira - SP - Brasil

ANALYTICAL RESULTS OF: L-GLUTAMINE

LOT N°: 13 0085 K 03 B

ITEM	LIMIT	RESULT
Description		(b) (4)
Identification (FT-IR)		
Specific Rotation (D-Line,20°) – H_2O		
Specific Rotation (D-Line,20°) – HCl		
State of solution (Transmittance)		
State of solution		
Chloride (Cl)		
Ammonium (NH_4)		
Sulfate (SO_4)		
Iron (Fe)		
Heavy metals (Pb)		
Arsenic (As_2O_3)		
Related substances		
Loss on drying		
Residue on ignition (sulfated)		
Assay		
pH		
Bacteria		
Coliform Bacteria		
Fungi		

We certify that quality of this product conforms to USP residual solvents requirement.
We certify the quality of this product conforms to DAB and USP

(b) (4)

Manufacturing Date:
Retest Date:

Manufacturer:

Manufacturing Site:

A.3.6 PACKAGING INFORMATION

AJINOMOTO.

AJINOMOTO NORTH AMERICA, INC.
Technical Services
4020 Ajinomoto Drive, Raleigh, NC 27610
Tel: 919-723-2089 Fax: 919-233-6612

Date: May 20, 2013

Subject: Packaging Information for L- Glutamine Oral Grade

Dear Valued Customer:

Ajinomoto North America, Inc. is providing the following packaging information for the product L-Glutamine and product identifier (SAP number):

SAP#	Package size	Outer Container	Outer Liner	Inner Liner (Product Contact)	Silica Gel Number
20581	(b) (4)				
32826					

(b) (4)

If you have additional questions concerning this product or its storage conditions, please contact the Ajinomoto technical support staff by email at nctechnicalservices@ajiusa.com.

Technical Support Staff
Ajinomoto North America, Inc.

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APPENDIX 4
SAFETY DATA SHEET, L-GLUTAMINE

APPENDIX 4. SAFETY DATA SHEET, L-GLUTAMINE

SAFETY DATA SHEET

Version 4.9
Revision Date 07/11/2014
Print Date 11/12/2015

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : L-Glutamine

Product Number : G5792
Brand : Sigma
Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada Co.
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Telephone : +1 9058299500
Fax : +1 9058299292
Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300
Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

Manufacturer : Sigma-Aldrich Corporation
3050 Spruce St.
St. Louis, Missouri 63103
USA

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

Not WHMIS controlled.

Not a dangerous substance according to GHS.

HMIS Classification

Health hazard: 0
Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.
Skin May be harmful if absorbed through skin. May cause skin irritation.
Eyes May cause eye irritation.
Ingestion May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : (*S*)-2,5-Diamino-5-oxopentanoic acid
L-Glutamic acid 5-amide

Formula : $C_5H_{10}N_2O_3$
Molecular Weight : 146.14 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
L-Glutamine			
56-85-9	200-292-1	-	<=100%

4. FIRST AID MEASURES

APPENDIX 5
L-GLUTAMINE STABILITY

APPENDIX 5. L-GLUTAMINE STABILITY

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APPENDIX 6
SUCCEED® ANALYSES (CONFIDENTIAL)

APPENDIX 6. SUCCEED® ANALYSES (CONFIDENTIAL)

11 Page(s) Withheld in Full Pursuant to FOIA Exemption (b)(4) immediately following this page

APPENDIX 7
RESULTS OF AGING STUDIES – SUCCEED®
(CONFIDENTIAL)

APPENDIX 7. RESULTS OF AGING STUDIES – SUCCEED®
(CONFIDENTIAL)

(b) (4)

(b) (4)

APPENDIX 8
PATENTS PERTAINING TO "GLUTAMINE" AND "HORSE"

APPENDIX 8. PATENTS PERTAINING TO "GLUTAMINE" AND "HORSE"

8.1 US2003 / 0162723 A1, Aug. 28, 2003, and EP1267635 B1, Sep. 29, 2011. Supplementation of equine feedstuffs. RC Harris and PA Harris (Waltham Nutrition; Mars Inc.)

> The "invention provides equine feedstuffs and methods by which plasma L-glutamine concentrations in equine animals can be increased or maintained" by use of a L-glutamine supplement with or without high levels of fats or oils. It is stated that "glutamine supplementation provides a pharmacological benefit to the equine animal, for example in respect of one or more of over-training, infection, other stressful conditions or catabolic stress."
>
> "Preferably", the L-glutamine is provided in the range of 30 to 60 mg / kg body mass (up to 200 mg / kg in EP1267635) per day. The L-glutamine is provided as free L-glutamine or a L-glutamine-rich extract of peptides and proteins, admixed with dry feeds or given in a drinkable solution.
>
> Ingestion of a meal containing 60 mg / kg body mass free L-glutamine increased plasma L-glutamine concentration from 0.27 to 0.66 mmol/L by 1 hour, and L-glutamine returned to normal at 3 h post-feeding. This single serving response was identical after 10 days of adaptation to the L-glutamine supplement, indicating that intestinal uptake is maintained high, and suggests an ongoing requirement for this amino acid. When L-glutamine was added within a feed mix, the increase in plasma L-glutamine was blunted but of longer duration.

8.2 US 7,824,706 B2, Nov. 2, 2010 and US 2011 / 0045107 A1, Feb. 24, 2011. Dietary supplement and method for the treatment of digestive tract ulcers in equines. PMJ Bedding and FL Pellegrini (Freedom Health, LLC).

> The dietary supplement is effective in treating and / or preventing gastric ulcers and in treating colonic ulcers. The principle ingredient of the dietary supplement is oat oil, which "strengthens the mucous gut membrane lining the inside of the stomach wall. This, in combination with the other ingredients, provides a synergistic, beneficial effect. Among the other ingredients, L-glutamine and L-threonine are naturally produced amino acids that act to "increase the integrity of the mucous gut membrane lining the inside wall of the stomach".
>
> Results are described where stomach ulcers of up to grade 3 were completely resolved by 16 weeks of supplementation, and this was associated with improved health and physical (exercise) performance. Consistent with reduced ulceration, there was an increase in red blood cell number and mass, and an improvement in white cell count was consistent with improved immune response.

8.3 EP1374693 A2, Jan. 2, 2004. Method for preventing decrease of breast milk amount in mammals. H. Kobayashi and 3 others. (Ajinomoto Co., Inc.)

This patent provides for a method for preventing a decrease in breast milk in mammals as a result of L-glutamine supplementation, administered orally or by injection. The primary mammals identified are cows, goats, sheep and water buffalo, and also pigs and horses. The orally administered L-glutamine ranges from 0.1 g / kg body mass to 5 g / kg per day. A single oral serving can prevent the decrease in milk amount for up to 2 weeks. The L-glutamine can be provided in purified form or as low molecular weight protein hydrolysates.

8.4 EP1868586 A1, Dec. 26, 2007, and EP1868586 B1, Nov. 21, 2012. Anti-inflammatory formulation. JN Larkins (AKL Inflammatory Ltd.)

The patents describes formulation for an anti-inflammatory or analgesic containing apocynin and paenol as the active ingredients, and for which L-glutamine is the main ingredient by mass of the formulations. The formulation is administered as a capsule, tablet or liquid form. The range of daily supplementation for L-glutamine is 0.001 to 0.2 g / kg body mass. Five (5) horse examples. One horse is described as having degenerative joint disease (osteoarthritis) which, after 10 days administration, returned to full soundness and successfully competed in endurance races. Another horse with osteoarthritis was administered a formulation for 5 days and became clinically sound. A race horse with joint swelling was administered a formulation, with full rest, and improvement was seen within 1 week. An elderly horses that had numerous leg injuries was administered a formulation and experienced improvement. Other examples are described using dogs and humans.

8.5 EP1887881 B1, Apr. 25, 2012. Composition and method for providing L-glutamine. LK Gross and C Khoo. (Hill's Pet Nutrition, Inc.)

The patent describes a process for preparing a L-glutamine-supplemented food product that is "useful for feeding an animal to increase L-glutamine absorption or to strengthen immune function". The process uses heat to prepare the product, and free L-glutamine is heat labile, therefore the process does not use free L-glutamine but rather L-glutamine is present in protein hydrolysates. Similar patents include EP672352 describes various solutions containing a L-glutamine rich peptide preparation, and US5849335 which provides for a composition and method for providing L-glutamine to a human or animal using carob germ protein hydrolysate. The product is deemed "suitable" for non-human mammals including horses, even though horse meat can be used as a source of protein.

APPENDIX 9
SUMMARY OF STUDIES REPORTING BENEFITS OF L-GLUTAMINE SUPPLEMENTED TO CONVENTIONAL DIETS

APPENDIX 9. SUMMARY OF STUDIES REPORTING BENEFITS OF L-GLUTAMINE SUPPLEMENTED TO CONVENTIONAL DIETS

Experimental studies reported that L-glutamine may protect cells, tissues, and whole organisms from physical work and injury through the following mechanisms: attenuation of NF (nuclear factor)-κB activation, a balance between pro- and anti-inflammatory cytokines, reduction in neutrophil accumulation, improvement in intestinal integrity and immune cell function, and enhanced of heat shock protein expression. A sample of the peer-reviewed, scientific literature is provided, showing the range and depth of research supporting claims of benefit of supplemented L-glutamine in humans. Such literature is absent for horses.

Table A. 9. 1. Benefits of dietary L-glutamine supplementation

Benefits	Type of Study	Reference
Maximize growth potential in young animals	Review of experimental research and clinical trials	Wu 2010
Prevent diseases (obesity, diabetes, necrotizing enterocolitis, intrauterine growth retardation)	Review of experimental research and clinical trials	Wu 2010
Optimal fetal, neonatal and post-weaning growth in pigs	Review of experimental research	Wu et al. 2013
Decreased fattening mortality and modified the intestinal microbiota	Experimental research; post-weaning rabbits	Chamorro et al. 2010
Enhanced intestinal structure, growth performance and immune response in poultry	Experimental research	Bartell and Batal 2007
Stimulates protein-synthesis and inhibits protein degradation in skeletal muscle of diabetic rats	Experimental research	Lambertucci et al. 2012
• decreases oxidative stress-related gene expression • increases the antioxidant potential • may attenuate renal oxidative damage in rats with STZ-induced diabetes	Experimental research	Tsai et al. 2012
Improves the prognosis of critically ill patients, presumably by: • maintaining the physiologic intestinal barrier • reducing the frequency of infections	Review of clinical trials and experimental research	De-Souza and Greene 2005
• improved structure of intestinal mucosa • trophic agent for mucosal repair, improvement of barrier function and gut adaptation	Experimental research; swine as an animal model	Domeneghini et al. 2006
10 days of oral L-glutamine supplementation inhibits whole-body protein degradation in in children with Duchenne muscular	Experimental research	Mok et al. 2006

Benefits	Type of Study	Reference
dystrophy		
Clinical trials conducted in patients indicate that L-glutamine improves nitrogen balance, increases cellular proliferation, decreases the incidence of infection, and shortens hospital stay in some catabolic patients	Reviews of clinical trials and experimental research	Newsholme and Calder 1997 Sacks 1999
Enhanced gut glutathione production	Experimental research using rats	Cao et al. 1998
Trophic and cytoprotective effects in small bowel and colonic mucosal cells	Review of experimental research and clinical trials	Ziegler et al. 2000
Reduced complications of chemotherapy in cancer treatment	Review of experimental research and clinical trials	Guarav et al. 2012
Neuroprotective effects in Alzheimer's disease	Experimental research using in vitro cell systems	Chen and Herrup 2012
Diminished risks of high-dose chemotherapy and radiation during clinical oncology treatments	Review of experimental and clinical data	Kuhn et al. 2010
Reduced peripheral neuropathy associated with chemotherapy	Review of clinical trials	Amara 2008
Enhanced protective immunity against viral infection	Experimental research using mice	Uyangaa et al. 2012
Reduced myocardial injury and clinical complications in patients undergoing cardiac surgery	Clinical research	Sufit et al. 2012
• benefits patient with severe inflammation • beneficial in burn or trauma patients	Review of clinical research	Soeters and Grecu 2012
Anti-infarct cardioprotection through up-regulation of COX-2	Experimental research; rabbit model	McGuinness et al. 2009
• enhanced milk production • support maximum growth, development, and production performance of swine	Review	Wu et al. 2011
Improved body mass (BM) and BM gain in poultry challenged with Salmonella	Experimental research	Fasina et al. 2010
Probable improvement of cellular re-dox state when taken before long-term exercise	Experimental research; rats as a model	Cruzat and Tirapegui 2009
Attenuated inflammation biomarkers and inflammatory response induced by prolonged exercise	Experimental research; rats as a model	Cruzat et al. 2010
Alleviated fall in intramuscular L-glutamine content during lactation, and may alleviate some of the catabolic effects of lactation	Experimental research; swine	Manso Filho et al. 2012
Improved insulin signaling in liver and muscle of rats with diet-induced obesity	Experimental research	Prada et al. 2007

Benefits	Type of Study	Reference
Can become "conditionally essential" because of elevated needs during pathological conditions	Review of clinical research	Dioguardi 2011
Bone-marrow transplantation there appears to be some benefit from oral L-glutamine in reducing mucositis and graft v. host disease	Review of clinical research	Crowther 2009

In addition, a number of studies noted a lack of effect of supplemented L-glutamine and stated the need for additional research.

Lack of effect	Type of Study	Reference
Scientific evidence is not available to support the use of these supplements for performance enhancement in human fitness and bodybuilding athletes.	Review of clinical trials and human experimental research	Mason and Lavallee 2012
No benefit of 4 months oral L-glutamine on muscle mass or function in ambulatory boys with Duchenne muscular dystrophy	Experimental research	Mok et al. 2009
Justification and safety of long-term L-glutamine supplementation is yet to be established in severe burn injury patients	Review	Windle 2006
Available data from randomised controlled trials are insufficient to determine whether L-glutamine supplementation has any important benefits for young infants with severe gastrointestinal disease	Meta-analysis and review	Wagner et al. 2012
Available data from 11 randomised, controlled trials do not provide evidence that L-glutamine supplementation confers important benefits for preterm infants	Meta-analysis and review	Moe-Byrne et al. 2012

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Responses to CVM review of Notification

1. CVM noted that most of the information in the notice which supported supplementation of L-glutamine having some utility was in disease models using rats and mice.

Response:

- Some literature used in support of the notification reflects that supplementation of L-glutamine has some utility in disease models and bona fide diseases in mammals, including humans. However, these are review papers that summarize key topics and the occasional "clinical" conclusion is provided in support of the evidence that ingestion of adequate and / or supplemental L-glutamine is important in maintaining a healthy, growing, functioning and performing mammal. None of these "clinical" references are primary sources, so when such conclusions are noted, we deem them to be a relevant and appropriate by-product of the body of scientific evidence that supports the nutritional utility of glutamine.
- Indeed, L-glutamine supplementation given to healthy animals was shown to be able to maintain a healthy / healthier status in a stressful environment, compared to in the absence of supplemented glutamine. This effect cannot be equated as providing the supplement to an animal already in a disease state.
- Maintaining a healthy state, or improving the health status by providing important nutrients such as L-glutamine has been an area of progressive research for more than two centuries. Maintaining health by providing supplementary L-glutamine to healthy animals while simultaneously imposing an unhealthy condition experimentally demonstrates nutritional utility and both a health and an economic benefit.
- For example:

Page 27

"Xi et al. (2011) reported that adequate, high concentrations of intracellular and extracellular L-glutamine are associated with marked reductions in infection, sepsis, severe burn, cancer, and other pathologies."

- This review summarizes the peer-reviewed scientific literature reporting that healthy mammals having high concentrations of L-glutamine from dietary supplementation maintain normal health even in the face of stressful conditions. This leads to the inference that high (within the range now considered normal) concentrations of L-glutamine are beneficial to maintain a healthy state in healthy animals.

Page 27:

"For example, oral L-glutamine supplementation in healthy humans performing moderate intensity exercise prevented the exercise-induced increase in intestinal permeability (Zuhl et al. 2014), thus maintaining integrity of the intestinal – immune system during periods of elevated metabolism."

Page 27:

> "During normal periods of increased metabolism, the cellular requirement for L-glutamine often cannot be met through de novo synthesis or the normal diet (Watford 2008)." This means that supplementary L-glutamine is required to maintain health. Similar statements are made by other scientists, some of which have been cited throughout the notification.

Recent research in support of health maintenance effects of supplemented L-glutamine in healthy animals NOT cited in the Notification:

Br J Nutr. 2016 Jul;116(2):211-22. doi: 10.1017/S0007114516001860. Epub 2016 May 18.

Whole-body and splanchnic amino acid metabolism in sheep during an acute endotoxin challenge.

McNeil CJ[1], Hoskin SO[1], Bremner DM[1], Holtrop G[2], Lobley GE[1].

The study investigated whole-body and splanchnic tissue metabolism in response to a lipopolysaccharide (LPS) challenge with or without supplementation of six amino acids, including glutamine. Sheep were infused with either saline (Control), LPS, or LPS plus six amino acids (including glutamine). LPS reduced absorption glutamine, but not the other amino acids, and increased its hepatic removal so that the levels of glutamine decreased at greater than the level at which glutamine was supplemented. Hence, glutamine supplementation is particularly important for the maintenance of a healthy state in healthy animals.

Nutr Rev. 2016 Apr;74(4):225-36. doi: 10.1093/nutrit/nuv052. Epub 2016 Mar 2.

Glutamine metabolism in advanced age.

Meynial-Denis D[1].

Glutamine, reviewed extensively in the last century, is a key substrate for the splanchnic bed in the whole body and is a nutrient of particular interest in gastrointestinal research.
Oral glutamine supplementation initiated before rats reach an advanced age increases gut mass and maintained the villus height of mucosa, thereby decreasing normal, age-related gut changes.

Curr Opin Clin Nutr Metab Care. 2016 Jan;19(1):62-6. doi: 10.1097/MCO.0000000000000233.

Interrelationships between glutamine and citrulline metabolism.

Marini JC[1].

The beneficial effects of glutamine supplementation may be partially mediated by the effects of glutamine on citrulline synthesis by the gut and the de-novo synthesis of arginine by the kidney and other tissues. Although there is no strong evidence to support that glutamine is a major precursor for citrulline synthesis in humans, glutamine has the potential to increase overall gut function and in this way increase citrulline production.

Amino Acids. 2014 Oct;46(10):2403-13. doi: 10.1007/s00726-014-1793-0. Epub 2014 Jul 15.

Dietary L-glutamine supplementation modulates microbial community and activates innate immunity in the mouse intestine.

Ren W[1], Duan J, Yin J, Liu G, Cao Z, Xiong X, Chen S, Li T, Yin Y, Hou Y, Wu G.

This study was conducted to determine effects of dietary supplementation with 1% L-glutamine for 14 days on the abundance of intestinal bacteria and the activation of intestinal innate immunity in mice. In the ileum, glutamine supplementation induced a shift in the Firmicutes:Bacteroidetes ratio in favor of Bacteroidetes, and enhanced mRNA levels for Tlr4, pro-inflammatory cytokines, and antibacterial substances participating in NF-κB and JNK signaling pathways. These results indicate that the effects of glutamine on the intestine vary with its segments and compartments, but collectively, dietary glutamine supplementation of mice beneficially alters intestinal bacterial community and supports the innate immunity in the small intestine through NF-κB, MAPK and PI3K-Akt signaling pathways.

2. CVM indicated that the notice provided some information that L-glutamine may have utility in early weaned piglets and lactating sows. In the studies with piglets and sows, benefits were only seen at 1 to 1.5% glutamine supplementation levels; lower levels of supplementation show no effect.

Response:

The CVM statement is not accurate. In fact, table 2 of Wu et al. (2013) provides that the "recommended" "dietary requirement" for glutamine ranges from 1.2 to 2.7 % of the total diet; also, referring to the original research studies (cited by Wu et al. 2013) shows ability to maintain health status and growth at supplementation levels that are at and above the NRC (2012) recommended daily requirement.

- This is only one group of studies that show utility. It is intended to complement that of other studies that also show benefit – see examples from the notification copied below.
- We also acknowledge that one may not be able to translate directly serving amounts provided to pigs in various stages of growth and development to maintenance of health in horses. It has, however, been demonstrated scientifically that supplemental L-glutamine is beneficial to maintaining health, growth and development. Horses, as described in detail below, are similar to other mammals in this and other key aspects.
- Please refer to these excerpts from the notification:

Page 28:

"In humans, proteolysis of dietary protein and peptide sources provides about 87% of L-glutamine within the body,..."

and

"Amino acids, in particular the NEAAs, entering the small intestine from the stomach have several fates, including transport into enterocytes, from within the intestinal lumen, where they are oxidized to provide fuel (ATP) to support these cells' transport functions."

Kuhn et al. 1999 is referenced.

Page 29:

"Enterocytes are the major site of L-glutamine extraction and oxidative ATP production, particularly the absorptive columnar epithelial cells of the small intestine (Watford 2008)."

"Salloum et al. (1993) studied the transport of L-glutamine into equine luminal enterocytes isolated from the jejunum."

"The extraction of L-glutamine by the equine jejunum in vivo more than doubled when the arterial concentration of L-glutamine was increased by bolus infusion, and jejunal extraction of L-glutamine was greater than that in the large intestine."

Page 30:

"L-Glutamine, and these other intestinally important amino acids, each have unique properties essential for maintaining the intestine's integrity, growth and function, and for regulating local tissue and organ immune responses (Ruth and Field 2013; Miron and Cristea 2012)."

"L-Glutamine supplementation is important to maintain a normal intestinal barrier against pathogens and preserve mucosal integrity (Domeneghini et al. 2006; Larson et al. 2007; Marc Rhoads and Wu 2009; Wang et al. 2009; dos Santos et al. 2010; Miron and Cristea 2012; Ruth and Field 2013; Sukhotnik et al. 2007; Zuhl et al. 2014; Wang et al. 2015)."

"Using a mouse model of small intestinal obstruction (similar to an equine obstructive small intestinal 'colic'), dos Santos et al. (2010) showed that L-glutamine supplementation maintained normal intestinal permeability and bacterial translocation compared to the large increase in intestinal permeability seen in non-supplemented animals." – note that these are healthy animals maintained their healthy state in the face of stressful conditions.

"In their review, Ruth and Field (2013) identified the following metabolic functions:

L-Glutamine:

- serves as a precursor and energy substrate for immune and epithelial cells;
- 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;
- supports proliferative rates and reduces enterocyte apoptosis;
- protects against pathogenic bacterial damage to intestinal structure and barrier function;
- lowers inflammatory response and increases immunoregulatory cytokine production; and
- improves the proliferative responses and numbers of intestinal immune cells."

Page 31:

"With low to normal amounts of dietary L-glutamine, up to 100% of the L-glutamine ingested with protein is utilized by cells of the small intestine. In this typical situation, none of the dietary L-glutamine enters the systemic circulation."

"Watford (2008) asserts that, with normal levels of dietary intake (5 – 10 g of L-glutamine daily for humans), there is no net small-intestinal absorption of L-glutamine or glutamate into the blood,"

The conclusion arrived at, from our review of the peer-reviewed, scientific literature is stated as such on page 29:

"In summary, these studies demonstrate the importance of rapid, small intestinal extraction of ingested L-glutamine from the circulation, and that L-glutamine extracted by cells of the small intestine from the arterial circulation is important in equine and other mammalian enterocyte metabolism."

Additional supporting excerpts from the literature that is cited in the notification, and some recent ones not cited:

From Wu et al. (2013):

Also, results of our microarray studies involving early-weaned pigs supplemented with or without glutamine indicated that early weaning resulted in increased (52–346 %) expression of genes related to oxidative stress and immune activation, but decreased (35–77 %) expression of genes related to macronutrient metabolism and proliferation of cells in the gut (Wang et al. 2008). Dietary glutamine supplementation increased intestinal expression (120–124 %) of genes that are necessary for cell growth and removal of oxidants, while reducing (34–75 %) expression of genes that promote oxidative stress and immune activation (Wang et al. 2008). In addition, glutamine enhances the MTOR signaling and protein synthesis in both skeletal muscle (Xi et al. 2011) and small intestine (Xi et al. 2012).

[G]lutamine reduces net utilization of asparagine, lysine, leucine, valine, ornithine and serine by jejunal or ileal mixed bacteria (Dai et al. 2012b, c). These results have important implications for developing new means to formulate diets for animals.

Dietary requirements of NEAA should be based on the metabolic needs of all AA for the maintenance, tissue protein synthesis, generation of physiologically important non-protein metabolites, and their regulatory functions (Fig. 2). Thus, dietary NEAA requirements likely vary with nutritional, physiological, pathological, and environmental factors (Wu 2010).

[R]esults of recent studies indicate that (1) diets must contain sufficient amounts of arginine and glutamine to support optimal fetal, neonatal and post-weaning growth in pigs (Kim and Wu 2004, 2009; Wu et al. 2004, 2010, 2011b)

From Wu 2014:

[C]areful analysis of the scientific literature reveals that over the past century there has not been compelling experimental evidence to support this assumption [2]. Indeed, in the 1960s and 1970s, A.E. Harper and other investigators found that the absence of NEAA from chicken and rat diets could not support maximal growth of these animals [11-15]. Growing evidence shows that nearly all of these synthesizable AA are inadequately present in typical plant protein (e.g., corn- and soybean meal)-based diets for growing swine relative to optimal whole-body protein synthesis [16].

At present, little is known about dietary requirements for NEAA by mammals, birds, or fishes.

Conclusion and perspectives

Amino acids have versatile and important physiological functions beyond their roles as the building blocks of protein [101]. Thus, dietary NEAA and EAA are necessary for the survival, growth, development, reproduction and health of animals. Growing evidence shows that pigs and poultry cannot synthesize sufficient amounts of all NEAA to achieve their maximum genetic potential [95-100].

NEAA (e.g., glutamine, glutamate, proline, glycine and arginine) play important roles in regulating gene expression, cell signaling, antioxidative responses, neurotransmission, and immunity. Additionally, glutamate, glutamine and aspartate are major metabolic fuels for the small intestine to maintain its digestive function and to protect its mucosal integrity.

From Hou et al. 2015:

It had long been assumed that NEAA are synthesized sufficiently in animals and humans to meet the needs for maximal growth and optimal health.10–13 However, no experimental data substantiate this assumption.2,14–17

Animal studies to determine dietary requirements of NEAA

Much of our current knowledge about dietary requirements of NEAA has been built from studies with economically important animals and laboratory animals. They include:(1) swine, an excellent animal model for human nutrition research78; (2) rats and mice; (3) chickens and other poultry species; and (4) fish.2 In published studies, animals were fed typical or conventional diets that were supplemented with or without an NEAA.17,79

Recent advances in the analysis of glutamate, glutamine, aspartate, and asparagine in food and animal-tissue proteins have made it possible to quantify dietary intakes of these four AA by animals and humans.80,81

Large amounts of emerging evidence indicate that this century-old concept has major limitations in protein nutrition, such that efficiencies of nutrient utilization in farm animals and humans remain relatively low despite much effort on establishing dietary requirements of EAA. While all organisms are known to have metabolic needs for all proteinogenic and other physiologically important AA, the needs of dietary NEAA for animals and humans have largely been ignored in animal production and human health. Based on new developments of AA biochemistry and nutrition, we propose that mammals, birds, and fish have dietary needs of all NEAA for optimal growth, development, lactation, reproduction, and health. This new paradigm shift in nutrition has now led to the recognition of dietary essentiality of "nutritionally non-essential AA" for animals and humans.

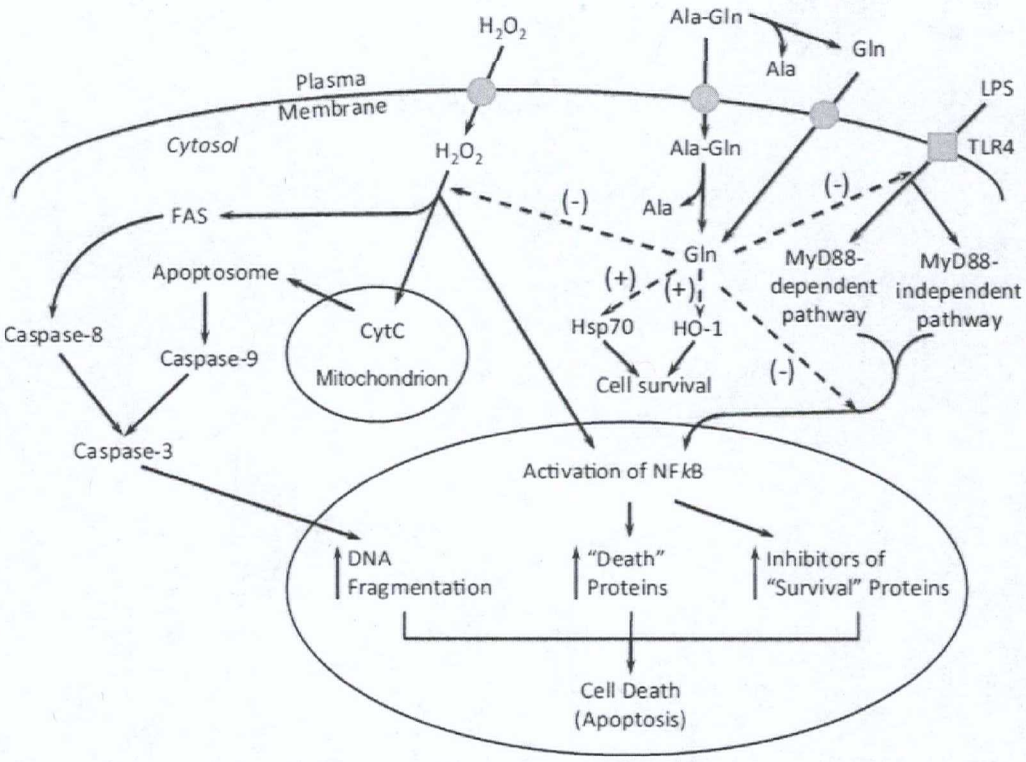

Figure 2 Proposed mechanisms for glutamine to prevent intestinal cells from oxidant- or lipopolysaccharide (LPS)-induced apoptosis. Exposure of intestinal epithelial cells to oxidants (e.g. H_2O_2) or LPS results in DNA damage and apoptosis, which are rescued by supplementation with either glutamine or its dipetide alanyl-glutamine. CytC: cytochrome C; HO-1: heme oxygenase; hsp: heat shock protein. Adapted from Haynes *et al.*[41]

Exp Biol Med (Maywood). 2015 Aug;240(8):997-1007. doi: 10.1177/1535370215587913. Epub 2015 Jun 2.

Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans.

Hou Y[1], Yin Y[2], Wu G[3].

Nutritionally non-essential amino acids (NEAA) are those amino acids that can be synthesized de novo in adequate amounts by the animal to meet requirements for maintenance, growth, development, and health and, therefore, need not be provided in the diet. There are no compelling data to substantiate that NEAA are synthesized sufficiently in animals and humans to meet the needs for maximal growth and optimal health. NEAA play important roles in regulating gene expression, cell signaling pathways, digestion and absorption of dietary nutrients, DNA and protein synthesis, proteolysis, metabolism of glucose and lipids, endocrine status, men and women fertility, acid-base balance, antioxidative responses, detoxification of xenobiotics and endogenous metabolites, neurotransmission, and immunity. ***Emerging evidence indicates dietary essentiality of "nutritionally non-essential amino acids" for animals and humans to achieve their full genetic potential*** for growth, development, reproduction, lactation, and resistance to metabolic and infectious diseases.

J Anim Sci Biotechnol. 2014 Jun 14;5(1):34. doi: 10.1186/2049-1891-5-34. eCollection 2014.

Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition.

Wu G[1].

It was assumed that all the "nutritionally nonessential amino acids (NEAA)" were synthesized sufficiently in the body to meet the needs for maximal growth and optimal health, but there has not been compelling experimental evidence to support this assumption. NEAA, including glutamine, play important roles in regulating gene expression, cell signaling, antioxidative responses, fertility, neurotransmission, and immunity and as major metabolic fuels for the small intestine to maintain its digestive function and to protect the integrity of the intestinal mucosa. Thus, animal diets must contain glutamine and other NEAAs to optimize their survival, growth, development, reproduction, and health. Furthermore, adequate provision of all amino acids (including NEAA) in diets enhances the efficiency of animal production.

Curr Opin Clin Nutr Metab Care. 2016 Oct 5. [Epub ahead of print]

Glutamine and the regulation of intestinal permeability: from bench to bedside.

Achamrah N[1], Déchelotte P, Coëffier M.

Glutamine is the most abundant amino acid in plasma and plays a key role in maintaining the integrity of intestinal barrier by modulating intestinal permeability and tight junction protein expression.

3. CVM noted that the notice did not include any reference to reporting the utility of L-glutamine supplementation in horses.

Response:

- The notification provides summary information from numerous studies performed on horses, as detailed below. The horse is not so unique amongst mammals and bears relevant and important similarities to many other mammals as provided for throughout the notification.
- The notification details on pages 27 – 33 similarities amongst mammals and between some mammals and horses, with an emphasis on gastrointestinal cell function and health. The small intestine of horses, humans, rats, pigs and many other mammals are remarkably similar with respect to their cellular nutrition, cellular metabolism, metabolic and transport functions. Equine studies are cited in this regard.
- There is no scientific basis to argue that the equine small intestine differs in a significant way with regards to these functions, **and with respect to requirement for glutamine**, than in those species where direct benefit has been established.

What we know about horses specifically:

Page 33:

- Amino acid requirements for horses have not been well defined and have not been evaluated individually at different stages of life (Tanner et al. 2014).
- Tissue L-glutamine concentrations in horses reported in most equine studies are lower than those reported in well-fed, healthy humans and rats (Rogero et al. 2004; Watford

and Wu 2005; Routledge et al. 1999; Wong et al. 2011), as well as horses (Urschel et al. 2012).

- In adult horses, plasma L-glutamine averages about 0.33 mmol/L in a range of studies (King and Suleiman 1998; Rogers et al. 1984; Zicker et al. 1991, 1994; Zicker and Rogers 1994a, 1994b; Routledge et al. 1999; Urschel et al. 2010; van den Hoven et al. 2010; Nostell et al. 2012; Peters et al. 2013).

- Plasma L-glutamine concentrations as high as 0.6 mmol/L have been observed in healthy, adult research horses (Duckworth et al. 1992). ***Note: this is two-fold higher than that typically reported.***

- L-glutamine level differences between adult humans and horses likely reflect composition of the diet (Li et al. 2011). From this paper, the authors state: "*The objective of the present study was to determine complete composition of NEAA (including glutamate, glutamine, aspartate, and asparagine) in feed ingredients of animal diets for comparison with EAA content.*"

- A recent study of well cared-for research horses reported average plasma L-glutamine concentrations ranging from 0.88 to 1.02 mmol/L (Urschel et al. 2012), further supporting that dietary glutamine was physiologically limiting in many other studies. ***Note: this is three to four-fold greater than reported in most studies.***

Page 34:

- (Manso Filho et al. 2009b). The authors concluded that the decrease in circulating L-glutamine concentrations during lactation, when large amounts of L-glutamine are being extracted by the mammary gland, "means that L-glutamine availability for maternal organs, such as the small intestine and immune cells, may be limiting as lactation proceeds". ***Note: this means that additional L-glutamine is required.***

- [T]he dietary provision of even non-glutamine-containing supplements added to forage more than doubled plasma L-glutamine concentrations (Rogers et al. 1984). This provides evidence that the provision of other nutrients to L-glutamine-producing cells and tissues increases the production and release of L-glutamine by these cells / tissues into the blood **because the L-glutamine is in demand by other cells / tissues.**

- Dietary supplementation of L-glutamine can help minimize or prevent the catabolic state and maintains steady L-glutamine concentrations essential for intestinal and immune function and health (Blikslager 2003). ***Note: L-glutamine appears to be beneficial for maintenance of health status.***

Page 36:

- When Harris et al. (2006) supplemented dietary L-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 supplementation nearly doubled plasma L-glutamine concentrations. They concluded that increasing plasma L-glutamine concentrations through the diet has "benefit in the athletically worked horse with lowered plasma L-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 proteolysis (van den Hoven et al. 2011). ***Implication: Proteolysis, the breakdown of muscle protein (which is not desirable), occurs to provide amino acids, like glutamine, that are in demand at high rates during and following periods of increased metabolism that occurs normally in athletic horses.***

- When Matsui et al. (2006) 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 protein degradation and increased the rate of protein synthesis in the hind limb. Recently, van den Hoven et al. (2010) reported that oral administration of amino acids to horses within 1 hour after exercise increased the intramuscular amino acid concentrations. In this study using exercise-trained horses, when the diet was supplemented with amino acids for 6 weeks, high intensity exercise resulted in a 16% decrease in muscle L-glutamine, followed by a 30% increase in muscle L-glutamine 4 hours after completion of exercise. This was associated with a 25% increase in post-exercise plasma L-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 need for L-glutamine, as well as some other amino acids, as a result of exercise, even in horses receiving daily supplements of amino acids.***

Page 37:

- In these athletic horses, it appears that low L-glutamine concentrations contributed to the severity of the observed immune depression. The results also indicate that endurance horses do not receive adequate dietary L-glutamine. ***Implication: that supplemental L-glutamine is required to maintain health status.***

- A viral challenge (equine influenza virus) of six horses resulted in a gradual and progressive approximately 30% decrease in plasma L-glutamine over a six-day period, and L-glutamine remained depressed for at least an additional eight days (Routledge et al. 1999). The study authors attributed this result to an increased requirement for L-glutamine by immune system cells. A sustained decrease in L-glutamine was suggested to impair the horses' ability to mount an effective immune response (Parry-Billings et al. 1992). ***Implication: that supplemental L-glutamine is required to maintain health status.***

Implications for utility in horses:

Page 35:

- Consistent with the equine studies cited above, Wu (2010) considers dietary L-glutamine to be "substantially inadequate" to meet the requirements for protein synthesis in extra-intestinal tissues in growing pigs. By extension, these authors infer that such is the case for mammals during periods of elevated metabolism (exercise, lactation, active growth and development).

- The capacity of the intestinal system, skeletal muscle, liver and kidneys to extract L-glutamine and glutamate is high, and Bertola and Burrin (2008) concluded that diets rich

in L-glutamine or glutamate have little effect on circulating concentrations and low potential for toxicity. ***Explanatory note: increasing dietary glutamine has little effect on circulating glutamine concentrations BECAUSE the demand and capacity for extraction of the intestinal system, etc., is high.***

This leads to the statement on page 36:

The intended use level of L-glutamine provided in SUCCEED® represents 12- 25% of the amounts used in these studies. This provides sufficient L-glutamine to support intestinal cells without raising plasma L-glutamine.

4. CVM indicated that there was only one report showing that supplementing L-glutamine (60 mg / kg of body weight) transiently increases plasma levels of glutamine in horses. This report is not sufficient to demonstrate utility.

Response:

As provide in the response to point 3 (above) there are numerous equine studies that have been relied on and that are supportive when taken together. The notification relies on all of the information provided therein, with emphasis on the equine-specific research highlighted in the response to point number 3.

The abstract of the "one report" is provided here to show the authors' tone and conclusions:

Equine Vet J Suppl. 2006 Aug;(36):637-42.

Plasma glutamine concentrations in the horse following feeding and oral glutamine supplementation.

Harris RC[1], Harris PA, Routledge NB, Naylor JR, Wilson AM.

Author information

Abstract

REASONS FOR PERFORMING STUDY:

Pharmacological benefits of glutamine supplementation have been shown in athletically and clinically stressed human subjects. In the horse, infection and intense exercise have also been shown to significantly decrease plasma glutamine concentrations, but little is known on how best to supplement.

OBJECTIVE:

To evaluate whether ingestion of different foodstuffs, with or without L-glutamine (G) or a peptide (Pep) containing 31.5% w/w G in a water-stable form, could affect plasma glutamine concentrations (P-GC).

MATERIALS AND METHODS:

Nine feeds (molassed sugar beet-pulp (mSB); naked oats (nO); commercial mix (CM); mSB with 30 or 60 mg/kg bwt G or the G-molar equivalent of Pep; and CM with 60 mg/kg bwt G or

equivalent Pep) were offered to 6 healthy mature horses on different days following overnight food restriction. The changes in P-GC were monitored for 8 h post feeding.

RESULTS:

After 1.5 h mean +/- s.d. AP-GC were -0.9 +/- 10.2% (mSB), +12.5 +/- 7.1% (nO) and +44.7 +/- 15.9% (CM; P<0.05). deltaP-GC with mSB supplemented with G was +60.9 +/- 30.0% (30 mg; P<0.05) and +156.8 +/- 34.6% (60 mg; P<0.05) at 1 h; deltaP-GC with Pep was 51.0 +/- 31.0% (30 mg equivalent, P<0.05) and +91.1 +/- 9.5% (60 mg equivalent, P<0.05) at 1 h. After 10 days of supplementation with 60 mg/kg bwt G, AP-GC following a further 60 mg/kg bwt G challenge showed a similar increase at 1 h of +154.3 +/- 37.9%; prevalues were unchanged. G and Pep added to CM, increased P-GC by 246.3 +/- 55.3 (+99.2%) and 252.3 +/- 94.2 micromol/l (96.7%) at 1.5 h with concentrations still above prevalues at 8 h (P<0.05). Apart from the CM (with or without supplement), pre P-GC was always regained by 4 h. Plasma NH3 and plasma protein concentrations were unaffected by supplementation with G or Pep.

CONCLUSION:

P-GC may be modified by appropriate supplementation with no apparent adverse effects.

POTENTIAL RELEVANCE:

Increasing P-GC through appropriate supplementation may be of benefit in the athletically or clinically stressed horse with lowered plasma glutamine concentrations.

5. CVM requested that the notifier define with some type of measurement what the notifier means "when they say the horse does better" with L-glutamine supplementation.

Response:

Page 27:

Key points

1. Dietary L-glutamine supplementation is associated with sustained health, growth and development compared to no supplementation.
2. L-Glutamine is the most abundant free α-amino acid in plasma and skeletal muscle of many mammals, including humans and horses.
3. L-Glutamine can be absorbed from the diet as a free amino acid or in the form of peptides and proteins.
4. Under periods of inadequate dietary intake, low tissue concentrations of L-glutamine are associated with impaired health, growth, development, intestinal function and immunity.
5. During periods of accelerated metabolic rate, e.g., high-intensity exercise training regimens, lactation, and periods of active growth in horses, the cellular demand for L-glutamine is increased for many metabolic processes and exceeds dietary intake and de novo synthesis.

Pages 29-30:

- 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 (Ruth and Field 2013; Miron and Cristea 2012).
- The enterocytes also maintain barrier function between luminal contents (external environment) and the internal environment of the body (MacFie and McNaught 2002). This barrier function is dependent on dietary L-glutamine availability (Zuhl et al. 2014).
- The immune system's requirement for protein and amino acid support is well established.
- It has also been established that specific dietary amino acids (in particular, L-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).

From pages 37-38:

E. 6. Summary of benefits and functions of supplemented L-glutamine in horses

- Tissue L-glutamine concentrations are lower in horses than in other mammals, reflecting dietary composition and possible dietary inadequacy.
- During normal periods of increased metabolic activity (lactation, growth and development of young, exercise and training), L-glutamine requirements are increased.
- Tissue L-glutamine availability is not always adequate to meet tissue demands during periods of increased metabolic rate.
- Dietary provision of L-glutamine has utility in minimizing or preventing catabolic states associated with periods of increased metabolic rate (exercise, lactation).
- Diets deficient in L-glutamine do not provide sufficient L-glutamine to enterocytes and or other body systems.
- Dietary L-glutamine supplementation resulted in significant increase in horses' systemic L-glutamine concentrations.
- L-Glutamine supplementation can help athletic horses increase plasma L-glutamine concentrations. All athletic horses tested have had low plasma L-glutamine concentrations, typically half that of well-fed healthy horses and healthy humans.
- A sustained decrease in plasma L-glutamine impairs the horses' ability to mount an effective immune response.

In conclusion, intestinal enterocytes and immune cells can absorb and use the majority of orally ingested L-glutamine. It can benefit numerous intestinal enterocytes and immune cell functions. The equine small intestine is very similar to that of other mammals with respect to barrier mechanisms, nutrient absorptive and transport mechanisms, and immune system mechanisms. Supplementary dietary L-glutamine will support intestinal cell nutrition, the immune system, and the general health of horses.

6. CVM requested that the notifier provide their amendment within 30 days from the date of the meeting.

Response:

The delay in responding was associated with the need to get a copy of the minutes of the meeting.

7. CVM requested that the notifier provide information demonstrating that silicon anti-foam (b) (4) is acceptable for use in animal food in the US.

Response:

(b) (4)

8. CVM requested a copy of the label for the L-glutamine that Freedom Health uses.

Response:

The label appears as Figure C.2 on page 14 of the notification.

This is the notifier’s 3rd submission

Trull, Chelsea G-6

Subject: FW: AGRN 019
Attachments: Extractive fermentation with non-ionic surfactants.pdf

Importance: High

From: Perron, Jeannie [mailto:jperron@cov.com]
Sent: Monday, July 03, 2017 3:24 PM
To: Conway, Charlotte
Cc: Michael Lindinger; Patrick Warczak (pwarczak@freedomhealthllc.com)
Subject: AGRN 019
Importance: High

Confidential

Dear Charlotte:

I am writing with respect to AGRN 019. As you know, Freedom Health, LLC is the sponsor of AGRN 019, and I am Freedom Health's counsel. Dr. Michael Lindinger, copied here, is the Notifier.

The February 6, 2017 letter from Dr. Edwards issued with respect to that Notice contains the following comment:

"The notifier should provide written documentation demonstrating that Silicon Anti-foam (b) (4) is acceptable for use in animal food."

Freedom Health's glutamine supplier, Ajinomoto, has told us that the anti-foaming agent it uses, (b) (4) is polyoxyethylene polyoxypropylene glyceryl ether, CAS No. 9082-00-2. I understand that the polymer has a number of synonyms, which include:

Polyoxyethylene (24) polyoxypropylene (24) glyceryl ether
Polyoxyethylene (30) polyoxypropylene (20) glyceryl ether
Polyoxypropylene (20) polyoxyethylene (30) glyceryl ether
Polyoxypropylene (24) polyoxyethylene (24) glyceryl ether
Polyoxypropylene (66) polyoxyethylene (12) glyceryl ether
PPG-20-Glycereth-30
PPG-24-Glycereth-24
PPG-66-Glycereth-12
Glycerol propoxylate-block-ethoxylate
Glycerol poly(oxyethylene, oxypropylene) ether
Glycerol, ethylene oxide, propylene oxide polymer
Glycerol, propylene oxide, ethylene oxide polymer
Propylene oxide ethylene oxide polymer, ether with glycerol (3:1)
Propylene oxide, ethylene oxide, glycerol adduct
Oxirane, 2-methyl-, polymer with oxirane, ether with 1,2,3-propanetriol (3:1)
Oxirane, methyl-, polymer with oxirane, ether with 1,2,3-propanetriol (3:1)
Polyglycol 15-200
Methyl oxirane polymer with oxirane, ether with 1,2,3-propanetriol
VORANOL 4701 Polyol DA

(b) (4) was used in the production of the polyglutamic acid for human food, which was the subject of GRN 339 filed by Ajinomoto Corporate Services, LLC, Freedom Health's supplier. *See* p. 00020 of that GRAS Notice, available here. FDA had no questions about GRN 339. Under the heading "Safety Assessment of DISFOAM GD Anti-foaming Agent" in Section IX H, GRN 339 said:

"A safety assessment and toxicological evaluation of the anti-forming agent DISFOAM GD was performed to support its use in the fermentation process used to PGA. Dr. Timothy Adams reviewed all of the available safety data on the substance and evaluated a worst case potential exposure from the proposed use of PGA and determined the experimental no effect level was 10,000,000 times greater than any likely intake (Appendices 111-1 and 111-2).

The acute oral LD_{50} of DISFOAM GD in male and female Sprague-Dawley CD rats was greater than 2000 mg/kg bw (OECD Guidelines No. 401). In a 28-day repeated dosing study (OECD Guidelines No. 407) in male and female Sprague Dawley rats, the No Observed Adverse Effect Level was 94 mg/kg bw/day, the highest dose tested. The concentrations tested were 0, 100, 300, 1000 ppm, equivalent to dose of 0, 9.9, 28 or 94 mg/kg bw/day. DISFOAM GD showed no evidence of mutagenicity in a bacterial reverse mutation assay in *Salmonella typhimurium* strains TA 1535; TA 1537, TA 1538, TA 100: TA 98 and TA 102 and *Escherichia coli* strain WP2 uvr A with and without metabolic activation (OECD Nos. 471, 472)."

See also, 56 Fed Reg. 15275, 15276 (April 16, 1991), in which FDA assessed the safety of these compounds for use in food contact substances when the agency was amending 21 C.F.R. § 177.1680 (which incorporates these polymers for use in food contact substances). We have attempted to determine whether (b) (4) is covered by 21 C.F.R. § 172.808, but were unable to reach a definitive conclusion on that point.

In addition, I understand that in the manufacturing process, at least some of the antifoaming agent is recycled, so expect that little, if any, remains with the fermented product. *See, e.g.*, Dhamole, Pradip B., Zhilong Wang, Yuanqin Liu, Bin Wang, and Hao Feng. "Extractive Fermentation with Non-Ionic Surfactants to Enhance Butanol Production." *Biomass and Bioenergy* 40 (May 2012): 112–19. doi:10.1016/j.biombioe.2012.02.007 (in which the surfactant L62 from the extractive fermentation process used in butanol production was recaptured for reuse), attached.

Taking into account the foregoing, Freedom Health has analyzed Silicon Anti-foam (b) (4) in terms of its appropriateness for use in animal feed. Freedom Health additionally understands that in the glutamine production process, Silicon Anti-foam (b) (4) is used at a rate of less than 1 part in 1,000 and is then recovered for re-use. Given the safety of the surfactant and its lack of detectability in Freedom Health's product, Freedom Health has assessed Silicon Anti-foam (b) (4) to be safe and appropriate for use within the production of substances intended for use as ingredients in animal feed.

Please let me know if you have any additional questions or need anything else.

Respectfully submitted,

Jeannie Perron

Jeannie Perron, JD, DVM

Covington & Burling LLP
One CityCenter, 850 Tenth Street, NW
Washington, DC 20001-4956
T +1 202 662 5687 | jperron@cov.com
www.cov.com