PARABEL

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OFFICE OF
FOOD ADDITIVE SAFETY

Division of Biotechnology and GRAS Notice review
Attn. Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

November 6, 2017

Re: GRAS notice for LENTEIN™ Complete and Degreened LENTEIN™ Complete as a nutritive ingredient in human food

Dear Dr. Gaynor,

In accordance with regulation 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Parabel Ltd. hereby informs the United States Food and Drug Administration that Parabel's LENTEIN™ Complete and Degreened LENTEIN™ Complete – which are produced in accordance with FDA Good Manufacturing Practices requirements and which meet the product specifications as set forth in Section II.E.2 of the provided safety evaluation and as required by FDA regulation, 21 CFR 182.1 (b)(1)- are considered to be Generally Recognized As Safe when consumed as a nutritional ingredient in commercial food products at a maximum level of 24 grams per serving.

Pursuant to the regulatory and scientific procedures established by the regulation at 21 C.F.R.§ 170.225 (c)(5), the intended use of Parabel's LENTEIN Complete and Degreened LENTEIN™ Complete are exempt from premarket approval requirements of the United States Federal Food, Drug and Cosmetic Act, because we determine that such use is GRAS.

Along with this cover letter, I am submitting one digital version of Parabel' s Generally Recognized As Safe ("GRAS") notice for its LENTEIN™ Complete and Degreened LENTEIN™ Complete. The digital notification is provided in a DVD-R (CD). I hereby certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Kaspersky Anti-virus Version 10.2.5.3201.

Should you have any questions regarding this GRAS Notice or require additional information to aid in the review, please do not hesitate to contact us via email atiarks@parabel.com or at vcarpio@parabel.com, so that we may provide response in a timely manner.

Yours sincerely,

(b) (6)
[redacted]

Anthony Tiarks
CEO
Parabel Ltd.

COMPREHENSIVE GRAS ASSESMENT

of

LENTEIN™ Complete (LC) and Degreened LENTEIN™ Complete (DGLC)

Food Usage Conditions for General Recognition of Safety

For

Parabel Ltd.

7898 Headwaters Commerce St.
Fellsmere, FL 32948, USA

Prepared by Valentina Carpio Téllez

November 6, 2017

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List of Acronyms and Abbreviations

LC	LENTEIN Complete
DGLC	Degreened LENTEIN Complete
LPC	Lemna Protein Concentrate
WHO	World Health Organization
GMP	Good Manufacturing Practices
FAO	Food and Agriculture Organization
DM	Dry Matter, also noted as dmb
SCFA	Short Chain Fatty Acid
PPM	Parts Per Million
ND	Not Detected
HACCP	Hazards Analysis and Critical Control Points
ISO	International Organization for Standardization
FDA	United States Food and Drug Administration
LOD	Lethal Overdose
TOTOX	Total Oxidation Value
GRAS	Generally Recognized As Safe
JECFA	Joint FAO/WHO Expert Committee on Food Additives
BW	Body Weight
AID	Acceptable Daily Intake
EFSA	European Food Safety Authority Panel
GRN	GRAS Notification
CP	Crude Protein
ME	Metabolizable Energy
STTD	Standardized Total Tract Digestibility
SID	Standardized Ileal Digestibility
AA	Amino Acid
SBM	Soybean Meal
Sp.	Specie
w/w	Weight to weight
N	Nitrogen
LTM	Lemna Trisculaca Meal
ADF	Acid Detergent Fiber
NDF	Neutral Detergent Fiber
NPU	Net Protein Utilization
TD	True Digestibility
BV	Biological Value
PER	Protein Efficiency Ratio
RDA	Recommended Dietary Allowance
AI	Adequate Intake
UL	Tolerable Upper Intake Levels
ORP	Oxidation-Reduction Potential
NHANES	National Health and Nutrition Examination Survey
USDA	United States Department of Agriculture

CFR	Code of Federal Regulations
IOM	Institute of Medicine
CSFII	Continuing Survey of Food Intakes by Individuals
UPMC	University of Pittsburgh Medical Center
WK	Week
COA	Certificate of Analysis

I. SIGNED STATEMENTS AND CERTIFICATION

A. Compliance with 21 CFR 170.255 Part 1

Parabel Ltd. ("Parabel") is hereby submitting a GRAS Notice in accordance with 21 CFR 170.255 Part 1.

B. Name and Address of the Notifier

Parabel Ltd.
7898 Headwaters Commerce St.
Fellsmere, FL 32948, USA
+1 321 405 2130

Persons responsible for the dossier:

Valentina Carpio Téllez
Regulatory Affairs Manager and Products Development Specialist

Parabel Ltd.
7898 Headwaters Commerce St.
Fellsmere, FL 32948, USA
+1 321 405 2130

As the notifier, Parabel accepts responsibility for the GRAS determination that has been made for Parabel's LENTEIN Complete and Parabel's Degreened LENTEIN Complete, as described in the subject notification. Consequently, the LC and DGLC preparations, meeting the conditions described herein, are exempt from premarket approval requirements for food ingredients.

C. Common or Usual Name of The Notified Substance

The common name or usual name of the two notified substances is water lentil (whole *Lemnaceae*) protein powder. The green-powder version of the product is to be marketed as LENTEIN™ Complete (LC). Parabel's degreened-powder version is identified in this notice as Parabel's degreened LENTEIN Complete (DGLC). Both are manufactured by Parabel Ltd. (Parabel's LC or Parabel's DGLC). The proposed brand name for DGLC is LENTEIN Lean. Refer to Part II.

D. Conditions of Intended Use in Food

Parabel's LENTEIN Complete and Parabel's Degreened LENTEIN Complete are a water lentil (whole *Lemnaceae*) protein powders intended to be used as a food ingredient in human food products and component of the human diet as a protein source.

Parabel's LENTEIN Complete and Parabel's Degreened LENTEIN Complete are both intended for use as a food ingredient at the level of 1.0-24g/serving in the food categories listed in Section III, Table 19. The foods in which the substance will be used include beverages and beverage bases, breakfast cereals, fresh fruits and fruit juices, frozen dairy desserts and mixes, grain products and pastas, milk products, plant protein products, processed fruits and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, and soups and soup mixes. By considering the food categories and use levels depicted in Section III, Table 20, the estimated upper daily intake for Parabel's LENTEIN Complete and Parabel's Degreened LENTEIN Complete would be nearly 201 g/person/day. The subpopulation expected to consume food products containing the notified substance, Parabel's LENTEIN Complete and Parabel's Degreened LENTEIN Complete, are individuals over 10 years of age.

E. Basis for GRAS determination

Pursuant to 21CFR 170.30 (a), the intended use of LC and DGLC have been determined to be GRAS based on scientific procedures as discussed in the detailed description provided below. A comprehensive literature search conducted through May 2017 was used for this safety evaluation. There is sufficient qualitative and quantitative scientific evidence, including compositional analysis and animal data, to determine safety-in-use for LC and DGLC. The safety determination of Parabel's LC and DGLC is based on the totality of available evidence, including composition analysis, history of use, feeding studies in a variety of animals, EDI, and toxicity studies.

Currently, Parabel's LC and DGLC are not included in the list of approved food additives or GRAS substances in the U.S. However, another plant-based protein ingredient, such as soy protein (GRN 134), already is listed as a GRAS ingredient. Also, the FDA had no question on GRAS notices of *Spirulina platensis*, whose macronutrient composition (53-65% protein; 4% nucleic acids; 17-25% carbohydrates, 4-6% lipids, 8-13% minerals; 3-6% moisture) is comparable to Parabel's LC and DGLC (GRNs 127, 394, and 417).

F. Exemption from Premarket Approval

Parabel Ltd. believes that the notified substances, LENTEIN Complete and Degreened LENTEIN Complete, are not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that the notified substances are GRAS under the conditions of their intended use.

G. Availability of information

The data and information that serve as the basis for this GRAS evaluation are available and will be sent to the USA Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of Parabel Ltd. located at 7898 Headwaters Commerce St. Fellsmere, FL 32948, USA.

Parabel Ltd. will provide FDA with a complete copy of the data and information used as a basis for the GRAS conclusion either in an electronic format that is accessible for FDA's evaluation or on paper.

H. Disclosure of Confidential Information

Parabel Ltd. states that any of the safety-related data and information in Parts 2 through 7 of this GRAS notice are not exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

I. GRAS Notice Certification

Parabel Ltd. certifies that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to Parabel Ltd. and pertinent to the evaluation of the safety and GRAS status of the use of the substances Parabel's LENTEIN Complete and Parabel's Degreened LENTEIN Complete.

(b) (6)

Signed: [redacted]

Anthony Tiarks
CEO
Parabel Ltd.
7898 Headwaters Commerce St.
Fellsmere, FL 32948, USA

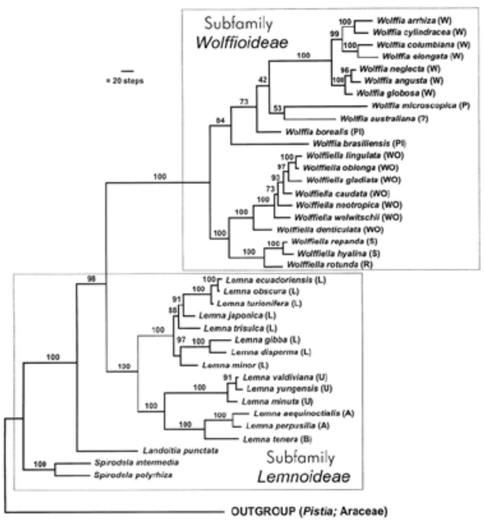
Date: November 6, 2017

II. IDENTITY, METHOD OF MANUFACTURE, SPECIFIATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Background

The family *Lemnaceae,* member of Araceae, consists of several species of small, free floating aquatic plants that are capable of rapid reproduction. The plant is commonly called duckweed, lemna, water lentils, and mud midget. The *Lemnaceae* family includes the subfamilies *Wolffioideae* and *Lemnoideae.* Phylogenetic analysis has shown that the *Wolffioideae* subfamily is comprised of two genera, *Wolffia* and *Wolffiella,* whereas the *Lemnoideae* subfamily is comprised of three genera, *Lemna, Landoltia*, and *Spirodela* (Les et al., 2002). A phylogenetic cladogram of the *Lemnaceae* family is shown in Figure 1.

Figure 1. *Lemnaceae* Family Cladogram[a]



[a] Adapted from Les et al. (2002)

Water lentils (*Lemnaceae* family) are tiny free-floating vascular plants with worldwide distribution. *Lemnaceae* grow best in tropical and temperate zones, but are found in all but arctic zones (Goopy and Murray, 2003). Water lentils are monocotyledonous aquatic plants, which are represented by 37 species (Appenroth et al., 2013). Figure 2 summarizes the species categorization into five genera: Spirodela (2 species), Landoltia (1), Lemna (13), Wolffiella (10) and Wolffia (11) (Appenroth et al., 2013). Parabel's water lentil crop includes a mono or poly-culture of the Lemnaceae family, generally consisting of plants in the Lemna genus (20%-100%), Wolffia genus (0 - 80%), Wolfiella (0 – 30% of the crop) and Landoltia (0 - 30% of the crop). Figure 2 represents 3 of these water lentil genera. A selected list of Lemnaceae that Parabel actively grows, as well as identifying features is shown in Table 1.

Figure 2. Size Comparison of Different Water lentils [a]

Scale is 1mm

[a] This figure portrays three distinct water lentil genera: The largest water lentils shown is Spirodela. The medium size water lentils are Lemna and the smallest are Wolffia (Photograph by Gerald Carr, University of Hawaii).

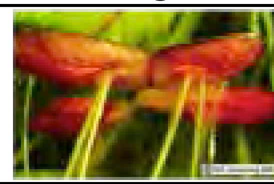
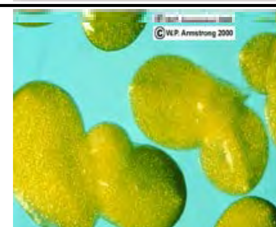
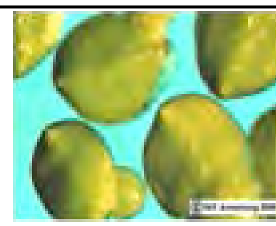
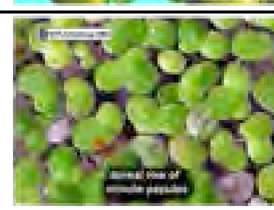
Figure 3. *Lemnaceae* specie categorization (Sree et al., 2016)

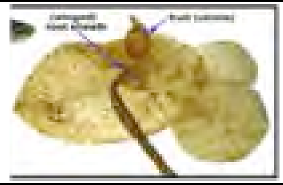
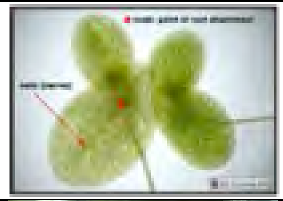
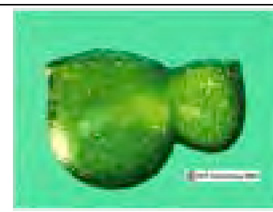
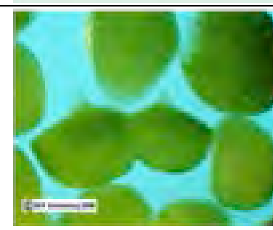
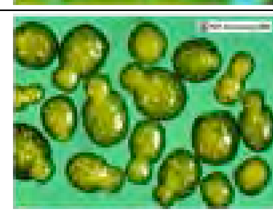
Table 1: Presently accepted genera and species of the plant family Lemnaceae Dumort

Subfamily	Genera	Species
Lemnoideae Engl.	*Spirodela* Schleid.	*S. polyrhiza* (L.) Schleid.
		S. intermedia W.Koch
	Landoltia Les & Crawford	*L. punctata* (G.Mey.) Les & D.J.Crawford
	Lemna L.	*L. disperma* Hegelm.
		L. gibba L.
		L. japonica Landolt
		L. minor L.
		L. obscura (Austin) Daubs
		L. turionifera Landolt
		L. trisulca L.
		L. aequinoctialis Welw.
		L. perpusilla Torr.
		L. tenera Kurz
		L. minuta Kunth
		L. valdiviana Phil.
		L. yungensis Landolt

Wolffioideae Engl.	*Wolffiella* Hegelm.	*W. caudata* Landolt
		W. denticulata (Hegelm.) Hegelm.
		W. gladiata (Hegelm.) Hegelm.
		W. lingulata (Hegelm.) Hegelm.
		W. neotropica Landolt
		W. oblonga (Phil.) Hegelm.
		W. welwitschii (Hegelm.) Monod
		W. rotunda Landolt
		W. hyalina (Delile) Monod
		W. repanda (Hegelm.) Monod
	Wolffia Horkel ex Schleid.	*W. angusta* Landolt
		W. arrhiza (L.) Horkel ex Wimm.
		W. columbiana H.Karst.
		W. cylindracea Hegelm.
		W. elongata Landolt
		W. globosa (Roxb.) Hartog & Plas
		W. neglecta Landolt
		W. australiana (Benth.) Hartog & Plas
		W. borealis (Engelm. ex Hegelm.) Landolt
		W. brasiliensis Wedd.
		W. microscopica (Griff.) Kurz

Table 1. Selected species grown by Parabel

Strain	Defining features	Images
Landoltia punctata (synonym to Spirodela punctata)	2-4 roots per frond. Red/Purple ventral common	
Lemna gibba	1 root, gibbous on bottom	
Lemna minor	1 root, not reddish on lower surface, can be red on upper. 3 veins.	
Lemna japonica	Hybrid of L minor and L turionifera. 1 root, row of dorsal papillae	
Lemna obscura	Prominent apical papule on smooth dorsal surface	
Lemna turionifera	Shiny, red blotches on ventral side, and occasionally dorsal. Typically has dorsal papule row. Turions often present (seasonal)	
Lemna valdiviana	Plant bodies connected in 4-8 colonies, thin transparent with single vein extending more than 3/4 from root node to apex	

Lemna aequinoctialis	2-3.5 mm, no red color, rounded at base, very distinct papillae near apex and root node, base of root has sheath with 2 wing-like appendages	
Lemna minuta	Frond 1-2mm, one vein extending less than 2/3 from node to apex	
Wolfiella Gladiata	Fronds are usually connected to form star like colonies. The fronds are 5-10 mm long; the flowers are extremely small and difficult to see. Wolfiella floats just beneath the surface of the water	
Wolffia globosa	Plant body 0.4-0.8 mm, longer than wide (ovoid-cylindrical), upper surface barely rounded (flattened along top)	
Wolffia arrhiza	Plant body 0.8-1.3 mm, ovoid to nearly spherical. Distinctly flattened dark green dorsal. No brown pigment cells.	
Wolffia brasilensis	Plant body 0.7-1.2 mm, ovoid-ellipsoid, upper surface flattened with a minute-prominent papule in the center; dead plants dotted with brown pigment cells	
Wolffia columbiana	Plant body 0.8-1.2 mm, almost spherical, most of upper surface clearly rounded, the uppermost top area flat	

Water lentils are monocotyledons and of relatively simple morphology with no stems or true leaves. The plant usually consists of a single or a few flat, oval shaped, leaf-like fronds, with a single thread-like root attached, seldom exceeding 5 mm long (Hillman, 1961; van der Speigel et al., 2013; FAO, Date Unknown; Les et al., 2002; Goopy and

Murray, 2003). According to Wang et al. (2010), water lentils "exhibit tiny, closely-related and often morphologically similar features".

Members of the Lemnaceae family are found worldwide, except in Polar Regions. However, the distribution of the species is far from uniform, with the Americas having over 60% of recorded species, and Australia and Europe each having less than 30% of the total (Goopy and Murray, 2003).

In the environment, water lentils grow in still or slow-moving fresh water, flourishing ponds or in areas rich in organic matter, where they can double every 1-2 days if the conditions are right. Some species grow in brackish water. In general, the plants thrive within a pH range of 4.5-7.5, with slight variation in range depending on the species. Water lentils are tolerant of both full sun and low light conditions, and can grow in layers up to 1 cm thick. Optimal growth temperatures range from 20 – 30 ºC, although they will grow in water temperatures ranging from 6 – 33 ºC (Hillman, 1961; Leng, 1999). In addition, water lentils are well suited for hydroponic cultivation.

Water lentils reproduce by budding new “daughter” fronds on alternative side pockets from the mature “mother” frond. These “daughter” fronds may or may not remain attached to the “mother” frond before they undergo the reproductive process. The mother frond tends to die after six reproductive cycles (Leng, 1999).

Most the water lentils frond is composed of chlorenchymatous (chloroplast-containing) cells, surrounded by air pockets for buoyancy. Some Lemnaceae are also known to contain anthocyanin pigments (Leng, 1999).

Water lentils are consumed by many species including poultry, fish, and herbivorous animals, as well as by humans in many parts of the world (Rusoff et al., 1980). Water lentils are a dietary staple of a variety of animals, including ducks and other waterfowl, fish, and muskrats (Hillman, 1961; Leng, 1999). For over 35 years, water lentils have been investigated as a dietary source of protein for fish (Fasakin et al., 1999; Gaigher et al., 1984; Bairagi et al., 2002; Hassan and Edwards, 1992; van Dyke and Sutton, 1977; El-Shafai et al., 2004 a, b), poultry, ruminants, and swine.

In southern Asia, water lentils are a traditional part of the human diet (van der Spiegel et al., 2013). Dried water lentils have high protein (30-45%, dry matter [DM] basis), calories (3,500-3,800 kcal/kg), and micronutrients (Men et al., 1995; Porath et al., 1979). Burmese, Laotians, and the people of northern Thailand have used water lentils as a nutritious vegetable for generations (Bhanthumnavin and McGarry, 1971).

Therefore, given its rapid reproduction and small size, water lentils have become of economic interest, and thus the subject of a multitude of studies beginning in the late 1800s. In underdeveloped or developing countries, at least 900 million people suffer from hunger and malnutrition (World Hunger Education Service, 2012). Protein deficiency is common among large segments of the world population due to a shortage of protein and foods.

Therefore, various protein concentrates have been developed and recommended for alleviating protein malnutrition. Parabel's LC and DGLC are a potential solution to help solve this nutritional crisis.

B. Chemical Identity of LENTEIN Complete and Degreened LENTEIN Complete

1. Common or Usual Name

The specific substances that are the subject of this safety evaluation are identified as Parabel's LENTEIN Complete and Parabel's degreened version of LENTEIN Complete (LENTEIN™ Lean) from Lemnaceae poly-genus, including Wolffia, Lemna, Landoltia, and Spirodella, as grown, harvested and produced and sold by Parabel. The common or usual names of the product that may be used to describe the product are: lemna protein, water lentil protein, LC or DGLC.

2. Chemical Composition

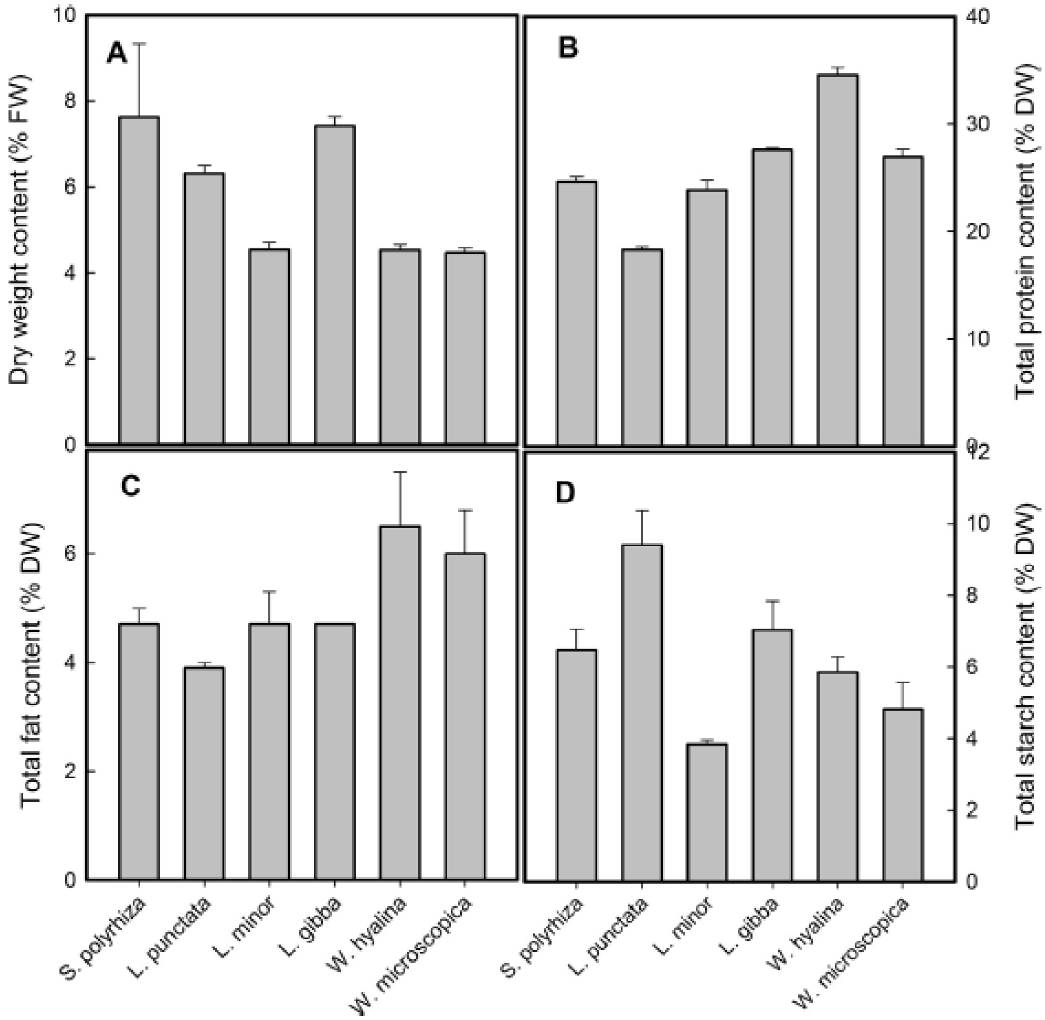
a. Chemical Composition of *Lemnaceae* as Reported in the Scientific Literature

Over the years, Parabel Ltd. has investigated the nutritional composition of water lentils and the products derived from the aqua-plant. A recent report by Appenroth et al. (2016), analyzed the chemical composition of six species of water lentils "representing all five genera Spirodela polyrhiza, Landoltia punctata, Lemna gibba, Lemna minor, Wolffiella hyalina, and the recently rediscovered species Wolffia microscopica (Sree, Maheshwari et al., 2015)." As represented in Figure 4, the results of the study indicate that the six-species dry weight content ranges from 4 to 8%, the protein ranged from 20 to 35% (dmb), the fat content 4 to 7% (dmb), and the starch 4 to 10% (dmb). In addition, water lentils contain micronutrients including minerals and phytonutrients.

Another report by Damry et al. (2001) indicated that water lentils contain a variable amount of crude protein, ranging from 15-45%, depending on growth conditions, and highlighted that the protein is generally composed of a remarkable amino acid profile. The micro-aquatic plant is also composed of 40-45% carbohydrates where the clear majority is composed of dietary fiber, and less than 10% fat highly composed of essential fatty acids.

As a result, this section will emphasize the compositional similarities across species according to numerous literature sources. Table 2 lists the proximate composition of several water lentil species, cultivated at different nutrient water media.

Figure 4. Main components in six species of water lentils (Appenroth et al., 2016).



(A) Dry weight, (B) Total protein content, (C) Fatty acid content, (D) Starch content. Data (B–D) are related to dry weight. Means ± standard deviations are given.

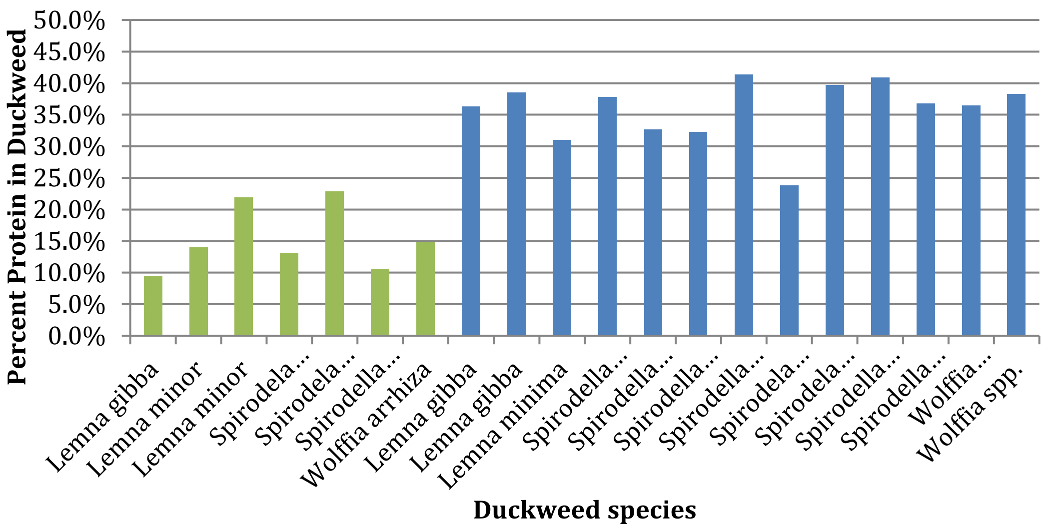
Table 2. Water lentil protein Levels are Driven by Nutrient Levels in Water

										Adjusted to % solids				
Species	**Species Geographic Source**	**Nutrient Source**	**Nutrients (Low = 1, Hi = 3)**	**Moisture %**	**Solids %**	**Protein %**	**Fiber %**	**Ash %**	**Fat %**	**Protein %**	**Fiber %**	**Ash %**	**Fat %**	**Data Source**
Lemna gibba	USA	Low Nutrient Lagoon	1	0.0%	100.0%	9.4%	17.0%	16.8%	1.8%	9.4%	17.0%	16.8%	1.8%	Culley et al., 1981
Lemna minor	*Bangladesh*	Pond	1	0.0%	100.0%	14.0%	11.1%	12.0%	1.9%	14.0%	11.1%	12.0%	1.9%	Zaher et al., 1995
Lemna minor	*Bangladesh*	Ditch	1	0.0%	100.0%	21.9%	N/A	N/A	N/A	21.9%	0.0%	0.0%	0.0%	Majid et al., 1992 (mean protein)
Spirodela polyrhiza	*USA*	Low Nutrient Lagoon	1	0.0%	100.0%	13.1%	N/A	N/A	N/A	13.1%	0.0%	0.0%	0.0%	Culley et al., 1981
Spirodela polyrhiza	*Bangladesh*	Ditch	1	0.0%	100.0%	22.9%	N/A	N/A	N/A	22.9%	0.0%	0.0%	0.0%	Majid et al., 1992 (average protein)
Landoltia punctata	*USA*	Low Nutrient Lagoon	1	0.0%	100.0%	10.6%	11.3%	14.1%	2.3%	10.6%	11.3%	14.1%	2.3%	Culley et al., 1981
Wolffia arrhiza	*Bangladesh*	Ditch	1	0.0%	100.0%	14.9%	N/A	N/A	N/A	14.9%	0.0%	0.0%	0.0%	Majid et al., 1992 (average protein)
Lemna gibba	*USA*	High Nutrient Lagoon	3	0.0%	100.0%	36.3%	10.1%	15.5%	6.3%	36.3%	10.1%	15.5%	6.3%	Culley et al., 1981
Lemna gibba	*USA*	Dairy waste Lagoon	3	0.0%	100.0%	38.5%	9.4%	16.4%	3.0%	38.5%	9.4%	16.4%	3.0%	Hillman and Culley et al., 1978
Lemna minima	*USA*	unknown		0.0%	100.0%	31.0%	10.0%	14.0%	2.0%	31.0%	10.0%	14.0%	2.0%	Shireman, Colle and

														Rottmann (1977)
Landoltia punctata	*USA*	Dairy waste Lagoon	3	0.0%	100.0%	37.8%	7.3%	12.0%	3.8%	37.8%	7.3%	12.0%	3.8%	Hillman and Culley et al., 1978
Landoltia punctata	*USA*	Treated wastewater effluent	3	0.0%	100.0%	32.7%	13.5%	20.3%	6.3%	32.7%	13.5%	20.3%	6.3%	Culley and Epps 1973
Landoltia punctata	*USA*	Septic Tank Effluent	3	0.0%	100.0%	32.3%	N/A	N/A	N/A	32.3%	0.0%	0.0%	0.0%	Culley and Epps 1973
Landoltia punctata	*USA*	Swine waste Lagoon	3	0.0%	100.0%	41.4%	8.3%	12.9%	5.1%	41.4%	8.3%	12.9%	5.1%	Culley and Epps 1973
Spirodela polyrhiza1	*Thailand*	Septage-fed pond	3	0.0%	100.0%	23.8%	N/A	18.3%	3.8%	23.8%	N/A	18.3%	3.8%	Hassan and Edwards (1992)
Spirodela polyrhiza	*USA*	High Nutrient Lagoon	3	0.0%	100.0%	39.7%	N/A	12.8%	5.3%	39.7%	N/A	12.8%	5.3%	Culley et al., (1981)
Spirodella polyrhiza	*USA*	Dairy waste Lagoon	3	0.0%	100.0%	40.9%	N/A	12.9%	6.7%	40.9%	N/A	12.9%	6.7%	Hillman and Culley (1978)
Landoltia punctata	*USA*	High Nutrient Lagoon	3	0.0%	100.0%	36.8%	N/A	15.2%	4.8%	36.8%	N/A	15.2%	4.8%	Culley et al., (1981)
Wolffia columbiana	Louisiana, USA	dairy wastewater	3	0.0%	100.0%	36.5%	N/A	N/A	N/A	36.5%	N/A	N/A	N/A	Rusoff et al., 1980
Wolffia sp.	Fellsmere	SR01	3	12.2%	87.8%	38.3%	14.4%	17.0%	6.4%	43.7%	16.4%	19.4%	7.3%	Parabel Data; S. Reed

A study conducted on Wolffia arrhiza 1971 found that dry plants contained 19.8 % protein by weight (Bhanthumnavin and McGarry, 1971). In a comparative study by Rusoff et al. (1980), authors reported crude protein levels for four water lentil species, as follows: 25.2% Lemna gibba; 28.7% Spirodela punctata; 29.1% Spirodela polyrhiza; and 36.5% Wolffia columbiana. Mbagwu and Adeniji (1988) compared the crude protein levels of Lemna paucicostata obtained from three different locations in Nigeria. They reported crude protein levels ranging from 26.3-45.5% (dry weight basis). In a study of aquatic plants collected near Calcutta, India, Banerjee and Matai (1990) determined a crude protein content of 20.4% (dry weight) for Lemna minor. Figure 5 represents protein data from many studies indicating that the variability of the protein content and composition of water lentils is more related to the nutrient content of the growth media than the species pertaining to the Lemnaceae family. This is also confirmed by a list of studies summarized by Appenroth et al. (2016): according to Xu, Cui, Cheng, and Stomp (2011), "water lentils biomass transferred from artificial swine medium to clean source water or salty water showed a decrease in the protein content but at the same time an increase of the starch content." Similarly, Zhao et al. (2015) examined the balance between protein and starch under the influence of nitrate and phosphate deficiency. Finally, Landolt & Kandeler (1987) emphasized the high impact of cultivation conditions followed by high light intensity and high nitrate concentrations on the protein content (ranging from 6.8% to 45% of dry weight) in different water lentil species. As a result, authors concluded that the protein content of water lentils can be easily controlled by optimizing the cultivation conditions of species, leading to a protein content close to 40% (dmb).

Figure 5. Percent Protein in Water lentils and Relative Nutrient Concentration in Growth Media

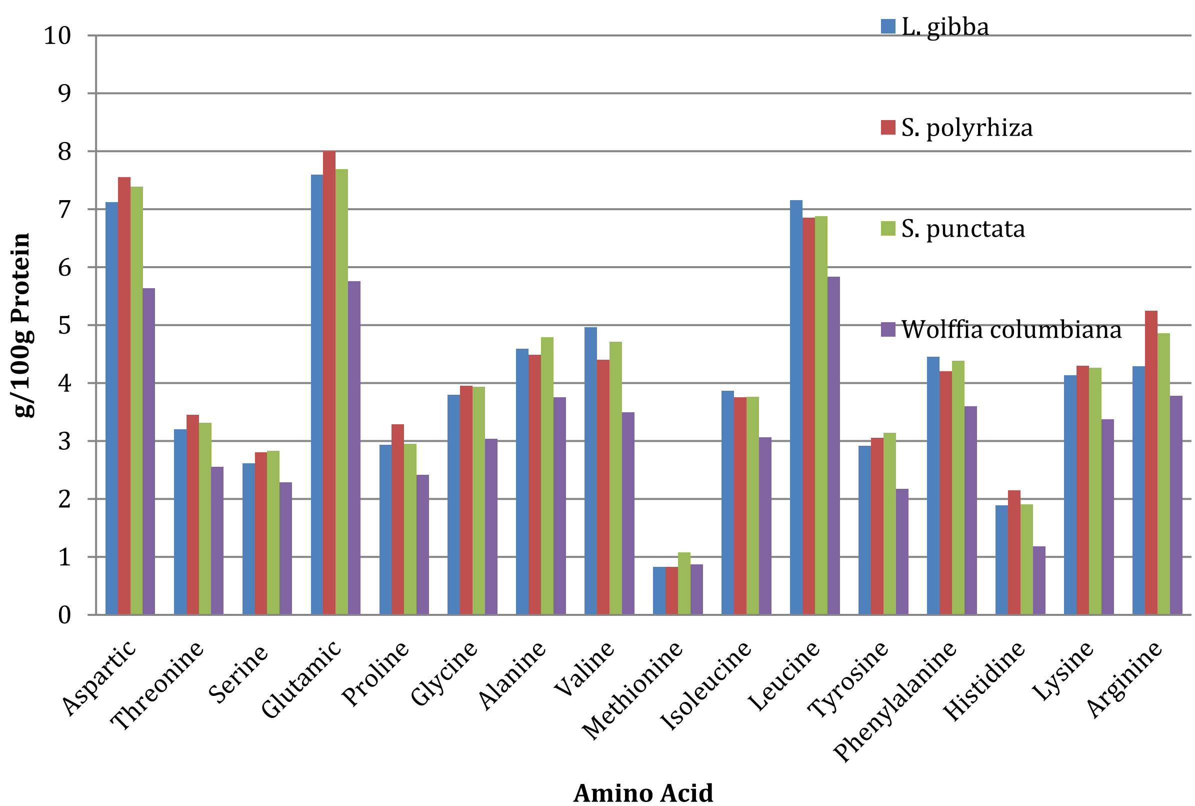


Green bars indicate percent protein of water lentils grown in low nutrient media, blue bars indicate percent protein of water lentils grown in high nutrient media (Culley et al., 1981; Zaher et al., 1995; Majid et al., 1992; Hillman and Culley et al., 1978; Shireman, Colle and Rottmann (1977); Culley and Epps 1973; Hassan and Edwards (1992); Rusoff et al., 1980; Parabel Data; S. Reed.).

Similarly, the taxonomic species mix of Parabel's water lentil crop may differ between farm locations, but the macro composition is generally influenced more by growth conditions and water chemistry than taxonomic species (Culley et al., 1981; Zaher et al., 1995; Majid et al., 1992 (averaged protein); Hillman and Culley et al., 1978; Shireman, Colle and Rottmann (1977); Culley and Epps 1973; Hassan and Edwards (1992); Rusoff et al., 1980; Parabel Data; S. Reed.).

The amino acid profile is similar for all members of the Lemnaceae (refer to Figure 6 and Table 3). This means that regardless of the species blend (and therefore geographic location) that Parabel uses to produce LENTEIN Complete and its degreened version, the amino acid composition is nearly the same. According to Rusoff et al. (1980), the amino acid composition of four species "showed very little variation". A similar finding was echoed in Porath, Hepher and Koton (1979) stating that the "variation [of four species of water lentils] in amino acid composition was only negligible". The amino acid composition of total protein in six water lentil species are also represented in Table 4 (Appenroth et al., 2016).

Figure 6. Amino Acid Profiles of Water Lentil Species [a]



[a] Data from Rusoff et al. 1980.

Table 3. Typical Amino Acid Content of LENTEIN Complete and Degreened LC Compared to Other Water Lentils

Amino Acid	Percent of total dry L. Gibba [a]	Percent of total dry S. Polyrhiza [a]	Percent of total dry S. Punctata [a]	Percent of total dry W. Columbiana [a]	% Amino Acid in LC (per 100g protein as-is) [b]	% Amino Acid in DGLC (per 100g protein as-is) [c]
Alanine	4.59	4.48	4.79	3.75	4.62	4.87
Arginine	4.29	5.25	4.86	3.78	5.41	5.62
Aspartic acid	7.12	7.55	7.38	5.63	7.46	7.64
Cysteine	NR	NR	NR	NR	0.99	2.90
Glutamic acid	7.60	8.00	7.69	5.76	8.93	9.27
Glycine	3.79	3.95	3.93	3.04	3.84	4.04
Histidine	1.89	2.15	1.90	1.18	1.86	1.93
Isoleucine	3.87	3.75	3.76	3.06	4.00	4.22
Leucine	7.15	6.85	6.88	5.83	7.29	7.72
Lysine	4.13	4.30	4.26	3.37	5.58	5.93
Methionine	0.83	0.83	1.07	0.87	1.78	See Cysteine
Phenylalanine	4.45	4.20	4.38	3.60	4.81	See Tyrosine
Proline	2.93	3.28	2.95	2.41	3.46	3.71
Serine	2.61	2.80	2.83	2.28	3.43	3.73
Threonine	3.20	3.45	3.31	2.55	3.63	3.81
Tryptophan	NR	NR	NR	NR	2.34	2.42
Tyrosine	2.91	3.05	3.14	2.17	3.34	8.54
Valine	4.96	4.40	4.71	3.49	4.88	5.12

[a] From Goopy and Murray, 2003
[b] Mean of 4 commercial representative batches (Refer to MedFinalReport_AA_G3.2, EUNF-b1, b2, b5.pdf in CoA folder or Appendix A2)
[c] Batch DGLC 170116 or 2017-MED-0986-02 (See Appendix A7)
S = Spirodela; W = Wolffia; L = Lemna
NR Not reported

Table 4. Amino acid composition of proteins from different water lentil species [g/ 100 g protein].

Amino acid	*Spirodela polyrhiza*	*Landoltia punctata*	*Lemna minor*	*Lemna gibba*	*Wolffiella hyalina*	*Wolffia microscopica*
CYS	0.8	1.1	0.9	0.9	1.0	1.2
MET	1.6	1.6	1.6	1.6	2.0	1.6
ASP	7.8	8.1	8.2	10.6	7.3	10.4
THR	4.2	4.1	4.0	4.0	4.2	4.7
SER	4.1	4.0	4.1	4.2	4.3	4.7
GLU	9.6	9.5	9.8	10.3	10.5	10.9
GLY	4.3	4.5	4.6	4.6	5.0	4.7
ALA	5.4	5.3	5.1	6.0	6.0	7.8
VAL	4.4	4.6	4.6	4.5	4.8	4.9
ILEU	3.3	3.5	3.7	3.4	3.9	3.7
LEU	6.8	7.3	7.3	7.2	8.0	7.7
TYR	3.1	3.1	3.1	3.2	3.8	3.3
PHE	3.97	4.5	4.4	4.3	5.1	4.2
LYS	4.2	4.1	5.0	4.2	5.8	5.7
HIS	1.6	1.6	1.5	1.6	1.7	1.7
ARG	4.7	4.7	4.8	4.9	4.7	5.2
PRO	3.5	4.1	3.8	3.9	3.7	3.6

Moreover, it has been reported in the literature that water lentils are a source of dietary fiber. Bhanthumnavin and McGarry studied the composition of dried Wolffia arrhiza, and found that carbohydrates made up 43.6% of the dry matter (Bhanthumnavin and McGarry, 1971). Kalita et al. (2007) reported 42% total carbohydrates (on dry basis) for Lemna minor collected in northeast India. A summary of studies determining the fiber content of various water lentil species is presented in Table 5.

Table 5. Percent Crude Fiber in Water lentil Species

Fiber Source	Crude Fiber (% dmb)	Reference
Lemna perpusilla	7.6	Hassan and Edwards, 1992
Water lentils	8.3	Hang, 1998
Lemna paucicostata	8.4-10.0	Mbagwu and Adeniji, 1988
Lemna minor	8.7	Men et al., 2001
Wolffia globosa L. Winn.	8.76	Chantiratikul et al., 2010
Spirodela polyrhiza	8.8	Rusoff et al., 1980
Lemna minor	8.9	Men et al., 2002
Lemna minor L.	9.15	Hanczakowski et al., 1995
Spirodela punctata	9.2	Rusoff et al., 1980
Lemna gibba	9.4	Rusoff et al., 1980
Lemna minor	10.0	Kalita et al., 2007
Lemna perpusilla	10.48	Khandaker et al., 2007
Wolffia columbiana	11.0	Rusoff et al., 1980
Lemna sp.	11.0	Becerra et al., 1995
Spirodela polyrrhiza	11.7	Hassan and Edwards, 1992
Water lentils	12.07	Islam et al., 1997
Wolffia arrhiza	13.3	Bhanthumnavin and McGarry, 1971
Lemna minor	15.7	Banerjee and Matai, 1990
Lemna gibba	16.1	Haustein et al., 1994
Lemna sp.	18.7	Men et al., 1995

The fiber in water lentils is largely composed of cellulose, hemicellulose, and lignin. A summary of studies determining the fiber composition in water lentils is provided in Table 6.

Table 6. Fiber Composition of Water lentils (on dry matter basis)

Source	Neutral Detergent Fiber	Acid Detergent Fiber	Hemicellulose	Cellulose	Lignin	Reference
Spirodela	471 g/kg	215 g/kg	256 g/kg	NR	32.6 g/kg	Huque et al., 1996
Lemna	574 g/kg	203 g/kg	371 g/kg	NR	NR	Huque et al., 1996
Wolffia	476 g/kg	227 g/kg	249 g/kg	NR	12.6 g/kg	Huque et al., 1996
Lemna sp. & *Spirodela* sp.	47.50-48.15%	21.50-22.20%	NR	NR	NR	Zetina-Cordoba et al., 2013

Regarding fat, a quick review of the literature indicates that water lentils (the whole plant biomass) tend to have low-levels of fat (<10% of the dry weight) (Table 7). Bhanthumnavin and McGarry (1971) reported a 5.0% (dry weight) fat content for *Wolffia arrhiza*. In a comparative study by Rusoff et al. (1980), they reported fat levels for four water lentil species, as follows: 4.7% *Lemna gibba*; 5.5% *Spirodela punctata*; 4.5% *Spirodela polyrhiza*; and 6.6% *Wolffia columbiana.* Mbagwu and Adeniji (1988) compared the fat contents of *Lemna paucicostata* obtained from three different locations in Nigeria, reporting a range of 4.00-4.40% fat (dry weight basis). In a study of aquatic plants collected near Calcutta, India, Banerjee and Matai (1990) determined a crude fat content of 3.8% (dry weight) for *Lemna minor*. Hassan and Edwards (1992) reported a crude fat content of 4.5% for *Lemna perpusilla* and 3.8% for *Spirodela polyrhiza*, on a dry weight basis. Nevertheless, like the protein and amino acid composition, the fatty acid composition of water lentil species can vary significantly due to the cultivation conditions (Yan et al., 2013 and Appenroth et al., 2016).

Table 7. Fat Composition of Water lentil Species (On Dry Basis)

Source	Fat	Reference
Wolffia arrhiza	5.0%	Bhanthumnavin and McGarry (1971)
Lemna gibba	4.7%	Rusoff et al. (1980)
Spirodela punctata	5.5%	Rusoff et al. (1980)
Spirodela polyrhiza	3.8%	Hassan and Edwards (1992)
Spirodela polyrhiza	4.5%	Rusoff et al. (1980)
Wolffia columbiana	6.6%	Rusoff et al. (1980)
Lemna paucicostata	4.00-4.40%	Mbagwu and Adeniji (1988)
Lemna minor	3.8%	Banerjee and Matai (1990)
Lemna perpusilla	4.5%	Hassan and Edwards (1992)

The concentration and nature of trace elements present in *Lemnaceae* also depends largely on the water source where water lentils grow. A summary of reported trace element contents found in *Lemnaceae* as reported by the literature is provided in Table 8. Overall, Macro-elements are characterized by low contents of Na^+ and high contents of K^+; while microelements contain high amounts of Cu^2+, $Fe^{2+/3+}$, Mn^{2+} and Zn^{2+}. As discussed, "the content of microelements depends heavily on the nutrient medium used for the cultivation of water lentils (Appenroth et al., 2016)."

Table 8. Percent Trace Elements in Water lentils (On Dry Basis)

Source	Ca	P	Na	K	Fe	Mg	Zn	Cu	Mn	Reference
Lemna minor	1.10	0.48	1.99	2.38	NR	NR	NR	NR	NR	Banerjee & Matai, 1990
Wolffia microscopica	6.0	7.04	0.30	83	240	3.1	30.8	3.52	755	Appenroth et al., 2016
Lemna gibba	1.9	0.9	1.8	NR	NR	NR	NR	NR	NR	Haustein et al., 1994
Lemna minor	0.71	0.62	0.14	4.92	0.27	0.17	0.01	0.002	0.17	Men et al., 2001
Lemna minor	1.9	0.5	NR	NR	NR	NR	NR	NR	NR	Men et al., 2002
Lemna paucicostata	0.65-1.03	NR	0.03-0.3	ND-4	0.02-0.24	0.5-0.6	NR	NR	0.04-0.07	Mbagwu & Adeniji, 1988
Lemna perpusilla	2.39	0.71	NR	NR	NR	NR	NR	NR	NR	Khandaker et al., 2007
Water lentils	2.58	0.17	NR	NR	NR	NR	NR	NR	NR	Islam et al., 1997
Lemna sp.	0.71	0.62	0.14	4.29	0.27	NR	0.01	0.002	0.17	Men et al., 1995
Lemna minor	NR	NR	NR	NR	NR	NR	0.01	0.0003-0.0004	NR	Hanczakowski et al., 1995

NR: Not Reported; ND: Not Detected

Water lentils, like many other plants, also contain carotenoids, including carotenes and xanthophylls. Haustein et al., (1990) validated that cultivated water lentils have high concentrations of pigments such as ß-carotene and xanthophylls, and indicated that the total carotenoids content in water lentils is 10 times higher than terrestrial plants. A summary of reported carotenoid contents found in the literature is provided in Table 9**.**

Table 9. Carotenoids in Water Lentil Species (On Dry Basis)

Source	Carotene	Xanthophyll	Total Carotenoids	Reference
Lemna sp.	535 mg/kg	NR	NR	Becerra et al., 1995
Lemna gibba	NR	0.9 g/kg	NR	Haustein et al., 1994
Lemna minor	1025 mg/kg	NR	NR	Men et al., 2001
Lemna minor	NR	261-359 mg/kg	416-554 mg/kg	Hanczakowski et al., 1995
Lemna minor	NR	NR	0.1 mg/100 g	Kalita et al., 2007

According to Appenroth et al. (2016), the main components of carotenoids found in Wolffia microscopica include (all-E)-lutein with ca. 70 mg per 100 g (dmb), followed by (all-E)-violaxanthin with 46 mg/100 g (dmb) and (all-E)-ß-carotene with 28 mg/ 100 g (dmb). In addition, (all-E)-Zeaxanthin was detected at a lower yet significant level for human consumption at 4.3 mg/100 g (dmb).

At last, nucleic acids are also naturally occurring macromolecules, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). They are ubiquitous cellular components in all animals and plants, including water lentils. Rusoff et al. (1980) reported nucleic acid compositions for four water lentils species. The nucleic acid content was found to be 6.0% for Lemna gibba, 6.2% for Spirodela punctata, 6.3% for Spirodela polyrhiza, and 6.4% for Wolffia columbiana.

b. Chemical Composition of Parabel's LENTEIN Complete (LC) and Degreened LENTEIN Complete (DGLC)

The chemical composition of Parabel's LENTEIN Complete and Degreened LENTEIN Complete is very similar and primarily consists of protein, with lesser constituents being fiber, fat, ash, and moisture. In addition, LENTEIN Complete contains micronutrients including minerals, carotenoids, vitamins, chlorophyll and polyphenols. Table 10 lists the typical chemical composition for LENTEIN Complete. Relevant certificates of analysis are provided in the provided CD within the CoA Data folder and in Appendix A. The typical amino acid content of each product, which is comparable to other plant-derived proteins, is shown in Table 11. Elements data on an average composition of LC is provided in Table 12.

Table 10. Typical Chemical Composition for LENTEIN Complete

COMPONENT	TYPICAL ANALYSIS LENTEIN COMPLETE (as-is)
Appearance	Fine free-flowing powder. Leafy green color
Taste	Sweet mild plant flavor
Odor	Leafy organic odor
pH	6.5-7.0
Crude Protein (dmb, N x 6.25)	40-50%
Water	1-8%
Fat (AH)	Max. 10%
Ash	4-8%
Total Dietary Fiber	30-45 %
Soluble Dietary Fiber	2-10 %
Insoluble Dietary Fiber	25-35 %
Lignin	2 %
Cellulose	18 %
Hemi-cellulose	2 %
NFD	Max. 35 %
ADF	Max. 22 %
Crude Fiber	Max. 15 %
Oxalic Acid	Max. 0.1 %
Sugars and Starches	
Starch	Max. 12%
Total sugars	Max. 0.35 %
Carotenoids (mcg/g)	
Total Reported Carotenoids	942.6
trans-Lutein	618
cis-Lutein/Zeaxanthin	39.2
trans-Zeaxanthin	10.4
alpha-Carotene	3.6
trans-ß-Carotene	169.1

cis-ß-Carotene	102.2
Total ß-Carotene	271.3
Xanthophylls (mcg/g)	
Total Lutein	686.8
trans-Lutein	584.9
cis-Lutein	101.9
Total Zeaxanthin	27.2
Chlorophylls (mg/100g)	
Total Chlorophyll	602
Chlorophyll a	486
Chlorophyll b	117
Ratio of a to b	80.6:19.4
Polyphenols (mg GAE/kg)	
Total Polyphenols	3,244
Fatty Acid (F.A) Profile (% F.A/Total Fat)	
Total Fat (Gas Chromatography)	6.21
Saturated Fatty Acid	20.5
16:1 Palmitic Acid	16.5
Monounsaturated Fatty Acid	4.80
18:1 Oleic Acid	1.98
Polyunsaturated Fatty Acid	74.7
18:3 Alpha-Linolenic Acid (Omega-3)	51
18:4 Octadecatetraenoic Acid (Omega-3)	4.5
Linoleic Acid (Omega-6)	17
3' Nucleotides from Natural Sources (%w/w) *	
Total Nucleotides	1.76
Adenosine-3-monophosphate	0.32
Cytidine-3-monophosphate	0.57
Guanosine-3-monophosphate	0.53
Uridine-3-monophosphate	0.34

Biogenic Amines (mg/kg)	
2-Phenylethylamine	1.28
Cadaverine	4.84
Histamine	<1
Putrescine	75.7
Spermidine	2.58
Spermine	1.07
Tryptamine	<5
Tyramine	1.28
Vitamins (per kg)	
Vitamin A (ß-Carotene)	562,000 IU
Vitamin E (α-tocopherol)	120 IU
Riboflavin (B2)	6.5 mg
Niacin (B3)	5.4 mg
Vitamin B6	2.8 mg
Folic acid (B9)	2.3 mg

*5' Nucleotides from fortification resulted at a content below the limit of quantification

Refer to Appendix A for all Lab Reports

Table 11. Typical Amino Acid Content of LC Compared to Aquatic Amino Acid Sources

Amino Acid	Percent of *Arthrospira platensis* Powder [a]	Percent of *Dunaliella bardawil* Protein [b]	Percent of *Chlorella vulgaris* Protein [b]	Percent of Total Protein in LC	Percent of Amino Acid in LC (per 100g protein as-is) [c]	Percent of Amino Acid in DGLC (per 100g protein as-is) [d]
Alanine	4.59	7.3	9.4	5.4	4.62	4.87
Arginine	4.31	7.3	6.9	5.3	5.41	5.62
Aspartic acid	5.99	10.4	9.3	8.4	7.46	7.64
Cysteine	0.59[c]	1.2	NR	1	0.99	2.90
Glutamic acid	9.13	12.7	13.7	10.5	8.93	9.27
Glycine	3.13	5.5	6.3	4.9	3.84	4.04
Histidine	1.00	1.8	2.0	2	1.86	1.93
Isoleucine	3.50	4.2	3.2	4.3	4.00	4.22
Leucine	5.38	11.0	9.5	7.6	7.29	7.72
Lysine	2.96	7.0	6.4	5.2	5.58	5.93
Methionine	1.17	2.3	1.3	1.7	1.78	See Cysteine
Phenylalanine	2.75	5.8	5.5	4.8	4.81	See Tyrosine
Proline	2.38	3.3	5.0	4.1	3.46	3.71
Serine	2.76	4.6	5.8	3.6	3.43	3.73
Threonine	2.86	5.4	5.3	4.7	3.63	3.81
Tryptophan	1.09	0.7	NR	2.1	2.34	2.42
Tyrosine	2.50	3.7	2.8	3.4	3.34	8.54
Valine	3.94	5.8	7.0	5.4	4.88	5.12

[a] From Gershwin and Belay (2008)
[b] From Richmond (2004)
[c] Mean of 4 commercial representative batches (Refer to MedFinalReport_FA_G3.2, EUNF-b1, b2, b5.pdf in CoA folder or Appendix A2)
[d] Batch DGLC 170116 or 2017-MED-0986-02 (See Appendix A7)
NR= Not reported

Table 12 provides the mineral and heavy metal composition of Parabel's LENTEIN Complete for five commercial batches. As identified in the results, Parabel's LENTEIN Complete complies with the maximum contaminant levels (mercury, lead and cadmium) in foodstuffs, set by EU Regulation 1881/2006 and No. 629/2008 (Commission of the European Communities, 2006b), which specifies a 0.3 ppm (wet-wt.) limit for lead in leaf vegetables; 0.2 ppm (wet-wt.)) for cadmium in leaf vegetables; and 0.1 ppm (wet-wt.)) for mercury in food supplements. Since Degreened LENTEIN Complete is produced from the same starting material as LC, the process resembles Parabel's LC (See Section II.D), and the chemical composition among the two products is very similar; Parabel believes there is no need to test for a thorough elemental analysis for DGLC for this notification. Nevertheless, Table 12 lists the results for Parabel's DGLC heavy metals.

Table 12. Element Data

TRAIT	UNITS	Method	BASIS	LENTEIN Complete (dmb) [a]	Degreened LENTEIN Complete (dmb) [b]
Aluminum	mg/kg	AOAC 993.14 mod	as is	**3.13**	N/A
Zinc	mg/kg	AOAC 993.14	as is	**69**	N/A
Calcium	mg/kg	AOAC 993.14	as is	**14,680**	N/A
Cadmium	mg/kg	AOAC 993.14 mod	as is	**<0.010**	**<0.01**
Lead	mg/kg	AOAC 993.14 mod	as is	**0.01**	**≤0.02**
Mercury	mg/kg	AOAC 993.14 mod	as is	**<0.015**	**<0.01**
Arsenic	mg/kg	AOAC 993.14 mod	as is	**0.05**	**≤0.02**
Chromium	mg/kg	AOAC 993.14 mod	as is	**0.41**	N/A
Manganese	mg/kg	AOAC 993.14 mod	as is	**245**	N/A
Selenium	mg/kg	AOAC 993.14 mod	as is	**<0.30**	N/A
Cobalt	mg/kg	AOAC 993.14 mod	as is	**<0.05**	N/A
Copper	mg/kg	AOAC 993.14 mod	as is	**2.80**	N/A
Iron	mg/kg	AOAC 993.14 mod	as is	**366**	N/A
Potassium	mg/kg	AOAC 993.14 mod	as is	**3,950**	N/A
Molybdenum	mg/kg	AOAC 993.14 mod	as is	**0.09**	N/A
Sodium	mg/kg	AOAC 993.14 mod	as is	**1,649**	N/A
Phosphorus	mg/kg	AOAC 993.14 mod	as is	**5,306**	N/A
Magnesium	mg/kg	AOAC 993.14 mod	as is	**3,539**	N/A
Tin	mg/kg	AOAC 993.14 mod	as is	**<0.15**	N/A

[a] Mean of 5 commercial LC representative batches (Refer to Eurofins Lab Report for each batch in Appendix A1)
[b] Mean of 5 DGLC representative batches (Refer to Eurofins Lab Report in Appendix A1)
N/A = Not Available

Secondary metabolites have been identified in water lentils across the literature, and analyzed by accredited laboratories on Parabel's LENTEIN Complete. Among those identified, include tannins, trypsin inhibitors, oxalates and phytates (Gilani et al., 2012; Hill, 2003). Some of these secondary metabolites are anti-nutritional factors (ANF) that are considered counteractive to optimum nutrition. Whether a component is an ANF is dependent upon the digestive process of a specific animal (Kumar, Date Unknown; Hill, 2003).

Maznah (1996) analyzed anti-nutritional factors tannins, trypsin inhibitor, nitrate and nitrite in water lentils species Spirodela polyrrhiza and Lemna perpusilla, and their protein concentrates. Results indicated that there is not a significant variation in the levels of these antinutritionals among the species, and concluded that "the low content of these anti-nutritional factors enable the plant to be used safely as a protein source for domestic animals as well as for human consumption".

Parabel analyzed a composite representative sample of Parabel's LENTEIN Complete and results indicate that the product does not contain significant levels of ANF's that would impair human and animal safety. Refer to Table 13 for data pertaining to antinutritionals on Parabel's LENTEIN Complete. For an additional discussion on these ANF, please refer to Section VI. Since Degreened LENTEIN Complete is produced from the same starting material as LC, the process resembles Parabel's LC (See Section II.D), and the chemical composition among the two products is very similar; Parabel believes it is unnecessary for this application to test for a thorough ANF analysis for DGLC. Nevertheless, as noted in Appendix A1 and Table 17, Parabel's DGLC has typically an oxalic content of <0.001% (as-is).

Table 13. LENTEIN Complete Anti-Nutritional Factors Data

ANF	UNITS	Method	Laboratory	BASIS	LENTEIN Complete
Phytate (Phytic Acid)	%	Analytical Biochemistry Vol 77:536-539:1977	Eurofins ECAL	as is	0.31 [a]
Trypsin Inhibitor	TIU/g	AOCS Ba 12-75	Eurofins ECAL	as is	<1,000 [a]
Protease Inhibitor	U/mg	USP 26-pancreatin assay for protease activity	Eurofins ECAL	as is	<0.170 [a]
Tannins	%	J. Agric. Food Chem. 1978, 26,1214	Eurofins ECAL	as is	<0.050 [a]
Oxalic acid	%	AOAC 986.13 mod	Medallion	as is	0.04 [b]

[a] Mean value from 2 composite samples (Refer to GRAS Comp ANF and EUNF-CSPBWL-COMP in CoA folder in USB-drive or Appendix A4) [b] Mean Value from 4 batches (Refer to Appendix A2)

Moreover, even though water lentils are aquatic plants, there are no concerns regarding the microbiological safety of water lentils grown in Parabel's controlled system, as pathogens including E. coli, Listeria, and Salmonella sp. are constantly monitored for every batch and lot processed. In addition, the production process of Parabel's LENTEIN Complete involves a killing step at proprietary washing to minimize the risk of microbial contamination. In addition, the water activity of the product in the powder form is <1%, which limits the potential for microbial growth (refer to stability data). Microbiological data as reported in Table 14 for Parabel's LENTEIN Complete and DGLC, indicate that there were no pathogenic organisms detected and the total viable counts were low, meeting the food ingredient product specifications.

Table 14. LENTEIN Complete Microbiological Data

TEST	UNITS	Method	Laboratory	BASIS	LC [a] and DGLC [b]
Aerobic plate count	cfu/g	AOAC 990.12	Eurofins ECAL	as is	≤10^6
Salmonella		AOAC 2003.09	Eurofins ECAL	as is	Neg/25g
Clostridium Perfringes	cfu/g	AOAC 976.30	Eurofins ECAL	as is	≤10
E. coli	MPN/g	FDA BAM Chapter 4	Eurofins ECAL	as is	≤3
Listeria		AOAC-RI 050903	Eurofins ECAL	as is	Neg/25g
Coliform	cfu/g	AOAC 991.14	Eurofins ECAL	as is	≤100
Yeast	cfu/g	FDA BAM Chapter 18	Eurofins ECAL	as is	≤100
Mold	cfu/g	FDA BAM Chapter 19	Eurofins ECAL	as is	≤100

[a] Specification limits set for LENTEIN Complete. Refer to 5 batch data in Appendix A1; lab reports also included in CoA folder in provided USB-drive)
[b] Mean of 5 DGLC representative batches (Refer to Eurofins Lab Report in Appendix A1)

According to Moyo et al. (2003) "contamination can be minimized or eliminated altogether by following environmental sanitation procedures including those of employee hygiene". In Parabel Crop Growth Area (CGA), cultivation of water lentils occurs in a completely contained environment, ensuring no leaching or sewage contamination, nor any heavy metal contamination. In addition, Parabel Ltd. manages the crop to prevent fungal infections and algal blooms. Similarly, as noted by Appenroth et al. (2016), "by selecting the required concentration in the nutrient medium, almost any concentration of trace elements in the plant material can be adjusted to the requirement for specific human nutrition." Therefore, Parabel carefully controls the media composition, monitoring the weekly tissue element data and water media data.

In addition, no pesticides are used during the cultivation of Parabel's water lentils nor at any stage of the production process of Parabel's LENTEIN Complete nor Degreened LENTEIN Complete. A composite sample of Parabel's LENTEIN Complete was sent to a third-party laboratory (Eurofins) for analysis. The material was screen for residues of over 600 pesticides using GC-MS and LCMS and no residues of pesticides were recorded above the limits of detection. Refer to Appendix A4.

Regarding mycotoxins, a composite batch of Parabel's LENTEIN Complete was analyzed for aflatoxin B_1, B_2, G_1, and G_2, as well as Fumonisin, HT-2 Toxin, Ochratoxin A, T-2 Toxin, Vomitoxin, and Zearalenone. The results as reported in Appendix A4, indicate no mycotoxins were recorded above the limits of detection. Similarly, Parabel has examined a representative batch of Parabel's LENTEIN Complete for presence of Microcystin data (cyanobacteria). The total microcystins levels were found to be very low and not detected above the limits of detection. Data from analyses for microcystins are attached in Appendix B.

Biogenic amines have been reported in a variety of foods including fish, meat, cheese, vegetables and wine. The most common biogenic amines found in foods are histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine, and agmatine. Biogenic amines can result in allergic reactions manifesting as difficulty in breathing, itching, rash, vomiting, fever or hypertension (Naila et al., 2010). Biogenic amines are also normally present in fermented foods at low concentrations and concentration is higher in improperly kept food. Biogenic amines occur in amounts of 5-4500 mg/kg in cheese, 5-130 mg/dm3 in wine, 2.8-13 mg/dm3 in beer, 110-300 mg/kg in sauerkraut, 2400-5000 mg/kg in improperly kept fish, and 10-700 mg/kg in improperly kept prepared meat (Karovicova and Kohajdova, 2003).

Biogenic amines have been considered carcinogens because they react with nitrates to form potentially carcinogenic nitrosamines (Shalaby, 1996). Histamine has been suggested to be the responsible amine for food poisoning, and can be enhanced by the presence of cadaverine, putrescine, and tyramine (Shalaby, 1996).

An intake of greater than 40 mg biogenic amines per meal has been considered potentially toxic (Ayres et al., 1980 in Shalaby, 1996). Levels of 50-100 ppm of histamine, 100-800 ppm of tyramine, and 30 ppm of β-phenethylamine, or a total of 100-200 ppm are regarded as acceptable (Shalaby, 1996).

While there are no reported biogenic amines found in water lentils in the literature, Parabel has analyzed a composite sample of Parabel's LENTEIN Complete for biogenic amines as shown on Table 10 (Refer to Appendix A4 for Lab Report). LENTEIN Complete contains a total of 86.75 ppm biogenic amines and approximately 17.24 mg/day at the EDI of 201 g/person/day total biogenic amines, which is significantly lower than the 40 mg/day toxicity level. Also, none of the common biogenic amines are at levels of concern and are significantly lower than the highest levels found in common foods.

According to results reported in Table 10, a few biogenic amines occur in Parabel's LENTEIN Complete:

i. Cadaverine: The highest levels of cadaverine measured in foods were from fermented soy products (634 mg/100 g), dry sausage (535 mg/100 g), and canned tuna (447 mg/100 g) (Shalaby, 1996). Parabel's LC was reported to contain 4.84 ppm cadaverine (0.48 mg/100g). This level is a much lower than found in various fermented foods (Shalaby, 1996).

ii. Putrescine: In a study conducted on human milk during the first week postpartum, putrescine levels were measured at 24 ± 3.5 nmol/dl (Buts et al., 1995). The highest amounts of putrescine recorded were for dry sausage (1506 mg/100 g), fermented soy products (1234 mg/100 g), and canned tuna (200 mg/100 g) (Shalaby, 1996). Parabel's LC was reported to contain 75.7 ppm putrescine (7.6 mg/100 g), which is generally lower in sausage and fermented soy products (Shalaby, 1996).

iii. Histamine: The highest amount of histamine in foods is found in anchovy paste (3440 mg/100 g), canned tuna (2000 mg/100 g), and smoked mackerel (1788 mg/100 g)

(Shalaby, 1996). The Nutritional codex of the Slovak Republic determined the maximal tolerable limit for histamine at 20 mg/kg in beer and 200 mg/kg in fish and fish products (Karovicova and Kohajdova, 2003). Parabel's LC was reported to contain <1 ppm histamine or below limit of detection.

iv. Phenethylamine: The highest levels of 2-phenethylamine measured in sausages were 13 mg/kg in Finnish sausage and 17.4 ± 43.7 mg/kg in Salsichon (Suzzi and Gardini, 2003). The limits proposed for fermented sauerkraut for β-phenylethylamine was 5 mg/kg, while for foods in general the tolerance level is 30 mg/kg (Shalaby, 1996). Parabel's LC was reported to contain 1.28 ppm phenethylamine (0.1 mg/100g).

v. Tyramine: According to Shalaby (1996), fermented soy products contain the highest amounts of tyramine, 3568 mg/100 g, and dry sausage is much lower at 151 mg/100 g, however many of the other products that are high in other biogenic amines did not have values for tyramine. Parabel's LC was reported to contain a low amount at 1.28 ppm tyramine (0.1 mg/100g).

C. Metabolism

Parabel's LENTEIN Complete and Degreened LENTEIN Complete are typically composed of 40-55% plant protein (on DM basis), with lesser amounts of fiber and other carbohydrates (35-45%) and fats (<10%). The metabolic fate of both versions of Parabel's LENTEIN Complete (LC and DGLC), the digestion and subsequent absorption, distribution, metabolism, and excretion of the ingredients are relevant to the metabolic fate of their macronutrient constituents such as dietary fiber, protein, and fat.

1. Protein component

Parabel's LC and DGLC are typically composed of 40-55% protein (on a DM basis) with lesser amounts of fiber, other carbohydrates, and fat. The major macronutrients in the Parabel's LC and DGLC fraction of ingredients are expected to undergo normal metabolism. Following consumption, the protein components of Parabel's LC and DGLC are expected to be denatured in the stomach by acid and/or cleaved by enzymes to release individual amino acids. These amino acids will be absorbed in the small intestine. Therefore, the metabolism of protein derived from Parabel's LC and DGLC do not raise safety concerns, and no systemic toxicity is expected following ingestion of Parabel's LC and its degreened version.

Based on a meta-analysis of 235 individuals from 19 studies, Rand et al. (2003) recommended new dietary reference values; an Estimated Average Requirements (EAR; median) and Recommended Dietary Allowance (RDA; 97.5th percentile) for healthy adults of 105 and 132 mg N/kg BW/d or 0.65 and 0.83 g protein/kg BW/d. For example, a 70-kg male would require at least 58 (0.83 x 70 kg) g protein/d. However, protein metabolism is influenced by a variety of factors including age, gender, diet, as well as exercise type, duration, and intensity. To maintain nitrogen balance, individuals who are physically active require more dietary protein than sedentary counterparts (Kredier and Campbell, 2009). Inadequate protein consumption results in negative nitrogen balance, losses in lean body mass, and may even delay recovery after exercise. To optimize skeletal muscle recovery after prolonged exercise through increased protein synthesis and myofiber repair (Kredier and Campbell, 2009; Phillips et al., 2007), protein consumption of 1.1 g/kg/d was recommended for low- to moderate-intensity endurance athletes and 1.4-2.0 g/kg/d for competitive or elite endurance athletes in intensive training. These levels are 50-150% higher than those recommended for sedentary adults.

2. Fiber components

As reported in Appendix A5, Parabel's LENTEIN Complete fibers are typically composed of water-insoluble hemicelluloses (1.9% as-is), cellulose (17.6% as-is), and lignin (2.1% as-is) as indicated by values for neutral detergent fiber (21.5 % as-is). In addition, Parabel's LC is composed of non-fibrous carbohydrate (NFC) or Neutral Detergent Soluble Carbohydrates (NDSC) which primarily include pectin, as well as some sugar (<0.35 as-is) and starch (1.2% as-is). In general, and according to the typical compositional data provided in Table 19, the cellulose fraction of Parabel's LC resembles that of other fiber sources. According to the literature, most plant cellulose is recovered in the feces as it remains largely undigested in the upper gastrointestinal tract and is not highly fermented in the colon (Spiller et al., 1980; Stephen, 1989), although natural plant celluloses and some water- soluble cellulose derivatives such as hydroxypropyl cellulose are fermentable. Cellulose in Parabel's LC is naturally occurring. Thus, consumption of a large quantity of fiber with a lower percentage of cellulose compared to many grain fibers (alfalfa, wheat bran, and sugar-beet pulp) would not result in gastrointestinal discomfort. Also, the cell walls of water lentils lack lignin, which is also represented by the low lignin content in Parabel's LENTEIN Complete, thus increasing the products digestibility (Leng et al., 1995). Therefore, the metabolism of cellulose derived from Parabel's LC does not raise safety concerns, following ingestion of Parabel's LC.

Table 15 compares typical fiber composition of Parabel's LC with other types of fibers. The compositional analysis shows that LC typically consists of approximately 22% NDF and 20% ADF (approximately 80% insoluble fibers). As shown in Table X, the composition of Parabel's LC is comparable to many grain fibers such as dehydrated alfalfa, wheat bran, and sugar-beet pulp, which are already recognized as safe feed and food ingredients. These insoluble fibers act as a bulking agent in the colon to relieve constipation and are fermented to SCFA and some gases although their degree of fermentation is less than that of soluble fibers.

Table 15. Fiber composition (% DM) of LENTEIN Complete and various grain components [1,2]

	LC[3]	Alfalfa	Wheat bran	Sugar-beet Pulp
NDF	20-35	46	45	46
ADF	10-22	34	11	22
CF	10-15	27	10	19
Total Dietary Fiber	30-45	48	46	68
Soluble fiber	2-10	6	1	13
Insoluble fiber	25-35	42	45	55

[1]NRC of Dairy Cattle: 7th Revised Edition, 2001; [2] Noureddine Benkeblia. 2014. Polysaccharides: Natural Fibers in Food and Nutrition. Table 17.1, page 408; [3] Refer to Appendix A1 and A5.

The metabolic fate of the hemicellulose moiety of Parabel's LC fiber fully resembles that of other hemicelluloses from rice bran and wheat bran. These insoluble fibers remain largely undigested in the upper gastrointestinal tract and are fermented in the colon to a varying extent, leading to production of gases and SCFA that lower pH of the colon (Tomlin and Read, 1988). Several health effects have been reported for SCFA, including improvement in bowel function, calcium absorption, lipid metabolism, and reduction of the risk of colon cancer (Scheppach et al., 2001). Any plant-derived fiber that comprises sources of fermentable fiber (arabinoxylans, hemicelluloses) lead to generation, absorption, and excretion of the same metabolites (H2, C02, SCFA) as those produced upon consumption of Parabel's LC. Therefore, the metabolism of hemicelluloses derived from Parabel's LC fiber does not raise safety concerns, and no systemic toxicity is expected following ingestion of Parabel's LC.

D. Manufacturing Process for Parabel's LENTEIN Complete

Parabel produces Lemnaceae in open field hydroponic crop growth areas that are above the soil. Parabel's water lentils are grown in a closed production system so all nutrients stay in the system and are recycled together with the water. The system doesn't require arable land or use of pesticides and herbicides. The crop growth areas are plastic-lined to prevent lixiviation or contamination of the environment (Figure 7), as well as preventing water loss due to permeation. The liner is impervious and therefore non-leaching. It provides the unique ability for Parabel to 'fertilize' water lentils without the run off due to rain or leaching into ground water systems.

From the growth systems, the Lemnaceae are harvested daily to provide the processing system a fresh supply of material. A typical Parabel facility ranges from one hundred to several hundred hectares of growth systems. This produces between 100 and 1500 metric tons of freshly harvested Lemnaceae each day.

Figure 7. Parabel's Hydroponic Crop Growth Area

Parabel manages the growth system and conditions of the Lemnaceae through rigorous nutrient and water chemistry analysis. Parabel also routinely monitors both the growth systems and water supply for contaminating components including pathogens and heavy metals. The water source used for crop irrigation and for the initial fill, is typically water from a deep well, but can also be filtered river water. The water source chosen for each Parabel facility depends upon local supply, permitting, chemistry, reliability, and general biosecurity. Once the growth systems are initially filled, the Lemnaceae reduces the effects of evaporation (as opposed to open water) and helps minimize the amount of water required for operation. The growth system is continuously monitored for pH, temperature, flow and aeration. The Lemnaceae are inspected and constantly monitored for health, crop density, and composition. The growth media consists of well water with the addition of soluble mineral fertilizers. Parabel carefully controls the level of the selected components of its proprietary growth media, which meet established specifications for heavy metals and/or potential contaminants. Micronutrients are monitored and maintained in optimal ranges, to maintain maximum product yields and crop health as per Parabel's quality control standards. All fertilizers or growth media components are sampled and tested for heavy metal contaminants as a preventative measure to ensure that the LENTEIN Complete and DGLC final products are maintained within the specification limits for any heavy metals.

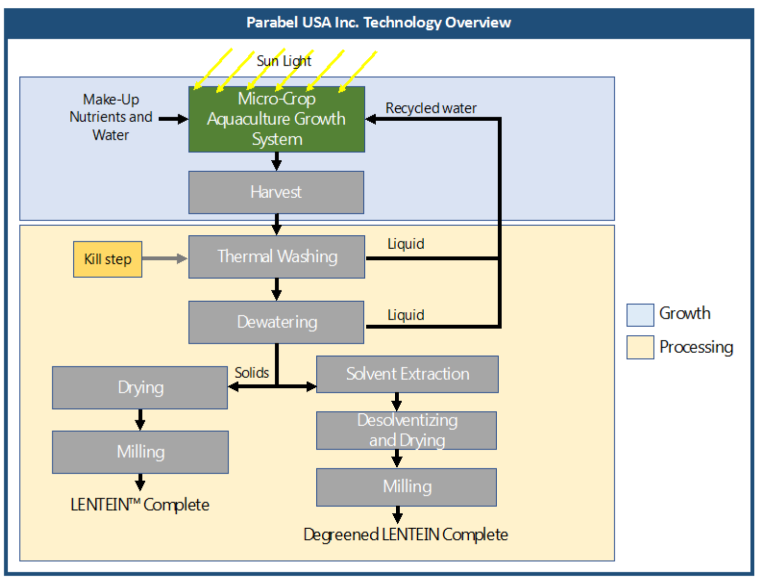
Parabel's manufacturing process involves transfer of freshly harvested material to the processing facility. The water lentils are initially pre-screened for removal of any foreign materials. The Lemnaceae (whole part of the plant) are then fed to a thermal washing system for microbial and pathogen elimination, deactivation of enzymes, and removal of undesirable components. This step is followed by a dewatering stage. The material is finally dried to a low moisture content, reducing the water activity level below the threshold which supports growth of spoilage microorganisms (<0.6).

Nevertheless, with regards to Parabel's Degreened LENTEIN Complete, the dewatered material is fed into a solvent extractor where fats and plant pigments are removed using 95% food-grade ethanol. The defatted and degreened material is then desolventized and dried before milling.

The dried products are further milled to a desired particle size prior to packaging in a controlled environment. Appendix D provides the details of the 25kg multilayer food-grade foil bag, currently used to pack either version of LENTEIN Complete (green or degreened). Potentially, Parabel will consider alternate packaging materials such as heat-sealable clear plastic bags (nylon material) for Parabel's LENTEIN Complete (green version), and Kraft bags lined with plastic for the degreened version. The bags are stored in carton boxes that have been tagged with a lot number. The cartons are stacked in a place designated for Parabel's LENTEIN Complete and DGLC in a separate finished product warehouse kept free of moisture and contamination.

Parabel's LENTEIN Complete and its degreened version are processed in a licensed food manufacturing facility in the State of Florida. In addition, both products will be manufactured under cGMP to meet SQF level 2 Category 19 and 21 CFR 110 Manufacture Standards for GMP, Hazard Analysis and Critical Control Point (HACCP) and food additive regulations established by the U.S. Food and Drug Administration (FDA). Refer to Appendix E to access Parabel's commercial facility' Annual Food Permit from the Florida Department of Agriculture, and review Parabel's contract with Eagle Certification Group. Figure 8 represents the manufacturing flow chart.

Figure 8. Manufacturing Process Flow Chart for Parabel's LENTEIN Complete and Degreened LENTEIN Complete



E. Product Specifications

1. General Physical Properties

Parabel's LENTEIN Complete is a green free-flowing powder extracted from the *Lemnaceae* family, that is not intended for use as a coloring agent, but as a nutritional ingredient it imparts coloring properties to food or beverages under the intended conditions of use. Refer to Table 10 and Section VI for natural pigment data and discussion on chlorophyll and carotenoids. The degreened version of LENTEIN Complete, is a beige free-flowing powder extracted from the *Lemnaceae* family, with a clean bland flavor and odor that is also intended for use as a nutritional ingredient.

2. Physical/Chemical Specifications

The product specifications for Parabel's LENTEIN Complete and Degreened LENTEIN Complete appear in Table 16 and 17. Appendix A contains the certificates of analysis for five nonconsecutive batches of LC, which demonstrate compliance with these specifications.

Table 16. LENTEIN Complete Specifications and Data from Five Non-consecutive Batches [a]

PARAMETER	UNIT	METHOD	LIMIT VALUES	BASIS	BATCH 1- G3.2 160922	BATCH 2- CSPBWL-170207	BATCH 3- CSPBWL-170213	BATCH 4- CSPBWL-170308	BATCH 5- CSPBWL-170310
Moisture	%	AOCS Ba 2a-38	**≤10%**	as is	5.61	2.86	5.51	1.6	2.33
Crude Protein	%	AOAC 990.03	**39-55%**	dmb	44.4	44.4	43.7	46.0	43.9
Fat (AH)	%	AOAC 922.06	**≤12%**	dmb	8.2	9.4	9.7	9.9	9.1
Ash	%	AOAC 923.03/ 32.1.05 16th Ed.	**≤10%**	dmb	5.2	5.3	4.8	4.4	4.2
Carbohydrates	%	By difference [b]	**≤12%**	dmb	4.1	3.5	3.7	5.8	10.8
Dietary fiber	%	AOAC 991.43	**30-45%**	dmb	38.0	37.5	38.2	33.9	31.9
Soluble	%	AOAC 991.43	**10-20% TDF**	dmb	4.8	2.6	3.7	2.3	5.7
Insoluble	%	AOAC 991.43	**80- 90% TDF**	dmb	33.3	34.9	34.5	31.6	26.2
Cadmium	mg/kg	AOAC 993.14 mod	**<0.05 ppm**	as is	<0.010	0.014	0.011	0.006	0.006
Lead	mg/kg	AOAC 993.14 mod	**<0.10 ppm**	as is	0.014	0.014	0.009	0.016	0.01
Mercury	mg/kg	AOAC 993.14 mod	**<0.05 ppm**	as is	<0.015	<0.015	<0.015	<0.015	<0.015
Arsenic	mg/kg	AOAC 993.14 mod	**<0.50 ppm**	as is	0.080	0.044	0.037	0.038	0.041
Oxalic acid	% (w/w)	AOAC 986.13 mod	**<0.1%**	as is	0.060	0.032	N/A	N/A	0.026
Aerobic Plate Count	cfu/g	AOAC 990.12	**≤100,000**	as is	5.80E+04	5.40E+04	4.70E+04	2.60E+04	7.60E+04
Salmonella	g	AOAC 2003.09	**Negative/25g**	as is	ND	ND	ND	ND	ND
E. coli	MPN/g	AOAC 988.19 or FDA BAM Chapter 4	**< 3**	as is	<3	<3	<3	<3	<3
Listeria	g	AOAC-RI 050903	**Negative/25g**	as is	ND	ND	ND	ND	ND
Clostridium perfringes	cfu/g	AOAC 976.30	**≤10**	as is	N/A	ND	ND	ND	ND
Coliform	cfu/g	AOAC 991.14	**≤100**	as is	100	<10	<10	<10	<10
Yeast	cfu/g	FDA BAM Chapter 18	**≤100**	as is	<10	10	40	<10	20
Mold	cfu/g	FDA BAM Chapter 19	**≤100**	as is	<10	10	10	<10	<10

[a] Refer to Appendix A for complete reports
[b] 100% - [protein (as-is) % + moisture % + fat (AH) % + ash % + dietary fiber %]
N/A = Not Available ND = Not Detected

Table 17. DGLC Specifications and Data from Five Non-consecutive Batches [a]

PARAMETER	UNIT	METHOD	LIMIT VALUES	BASIS	BATCH 1- DGLC-170523	BATCH 2 - DGLC-170614	BATCH 3- DGLC-170619	BATCH 4- DGLC-170621	BATCH 4- DGLC-170710
Moisture	%	AOCS Ba 2a-38	**≤10%**	as is	7.24	5.96	5.90	5.94	6.53
Crude Protein	%	AOAC 990.03	**39-55%**	dmb	39.2	47.3	47.0	46.4	45.0
Fat (AH)	%	AOAC 922.06	**≤10%**	dmb	1.2	0.9	0.9	0.7	1.0
Ash	%	AOAC 923.03/ 32.1.05 16th Ed.	**≤10%**	dmb	4.4	6.9	6.8	6.6	7.6
Carbohydrates	%	By difference [b]	**≤10%**	dmb	8	-4.3	-1.9	0.1	-1.4
Dietary fiber	%	AOAC 991.43	**35-50%**	dmb	47	49.3	47.3	46.1	47.8
Cadmium	mg/kg	AOAC 993.14 mod	**<0.05 ppm**	as is	<0.01	<0.01	<0.01	<0.01	<0.01
Lead	mg/kg	AOAC 993.14 mod	**<0.10 ppm**	as is	<0.02	<0.02	0.03	<0.02	<0.02
Mercury	mg/kg	AOAC 993.14 mod	**<0.05 ppm**	as is	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	mg/kg	AOAC 993.14 mod	**<0.50 ppm**	as is	<0.02	<0.02	0.02	0.02	0.02
Oxalic acid	% (w/w)	AOAC 986.13 mod	**<0.1%**	as is	0.068	<0.001	<0.001	<0.001	<0.001
Aerobic Plate Count	cfu/g	AOAC 990.12	**≤100,000**	as is	5.30E+02	5.70E+02	1.90E+04	2.60E+03	6.70E+03
Salmonella	g	AOAC 2003.09	**Negative/25g**	as is	ND	ND	ND	ND	ND
E. coli	MPN/g	AOAC 988.19 or FDA BAM Chapter 4	**< 3**	as is	<3	<3	<3	<3	<3
Listeria	g	AOAC-RI 050903	**Negative/25g**	as is	ND	ND	ND	ND	ND
Clostridium perfringes	cfu/g	AOAC 976.30	**≤10**	as is	ND	ND	ND	ND	ND
Coliform	cfu/g	AOAC 991.14	**≤100**	as is	<10	<10	<10	<10	<10
Yeast	cfu/g	FDA BAM Chapter 18	**≤100**	as is	<10	<10	<10	<10	<10
Mold	cfu/g	FDA BAM Chapter 19	**≤100**	as is	<10	20	<10	10	40

[a] Refer to Appendix A for complete reports
[b] 100% - [protein (as-is) % + moisture % + fat (AH) % + ash % + dietary fiber %]
N/A = Not Available ND = Not Detected

F. Stability Data for Parabel's LENTEIN Complete

LENTEIN Complete and its degreened version (DGLC) are expected to be stable for 2 years under recommended storage conditions that Parabel will provide to its customers, as follows:

> Recommended storage is cool temperature (below 25 °C/ 75 °F) with low relative humidity (< 60% humidity). Maintain the product in the provided packaging, air-tight sealed bags, and store away from direct light.

Parabel has initiated a six-month and 12-month accelerated shelf life stability study for Parabel's LENTEIN Complete, on four representative commercial samples. The parameters tested address physiochemical, biochemical and microbiological stability, including Peroxide Value, Hexanal, p-Anisidine value, Total Oxidation Value (TOTOX), and fatty acids Omega 3 and 6 to determine fat stability; crude protein, amino acid profile, pH, Aerobic Plate Count, Yeast and Mold Count, and visual appearance (color, texture and aroma). Refer to Appendix F for analytical results, lab proposal and summary of raw data. Results indicate that no significant difference is detected across the time periods among the different parameters, except for the Peroxide Value (PV) in sample CSPBWL-170308 which is recognized as an outlier. PV values in the two products is highly unlikely to develop under real time, ambient conditions of storage. Also, according to the sensory profile of the product, "no off odors" are detected which is an indicator that oxidation of the product is present (refer to Appendix F).

In addition, Parabel has conducted two internal studies to determine the real-time shelf-life of LENTEIN Complete (LC) powder from microbial, chemical, oxidative, and sensory stability data. Details as follows:

Study 1
LC samples from batch SPBWL160310 were packaged in aluminum foil zip lock bags and stored at 20 °C/50%RH. Samples were pulled at months 0, 6, 12 and 18 to conduct the following analysis:

- Chemical Stability
  - Proximate composition
  - Protein quality (Protein Digestibility Adjusted Amino Acid Score [PDCAAS])
  - Fatty acid composition
- Oxidative Stability
  - Peroxide value
  - TBA Rancidity
- Microbial Stability
  - Aerobic plate count (APC)
  - Yeast and Mold
  - *Clostridium perfringens*
- Sensory Stability
  - Color and odor

Results

LC samples packaged in aluminum foil zip lock bags and stored at 20 °C/50%RH remained stable and within product specifications for crude protein, fat, ash, and microbial after 18 months. Protein quality was not significantly altered during storage. Microbial growth during storage typically results in protein degradation. Being a low water activity (<0.6) product, microbial growth was not significant, so protein quality was unaltered. There was an increase in TBA rancidity between the 6th and 12th month, although peroxide values remained very low (< 2 meq/Kg fat). However, this increase in TBA rancidity, which is indicative of the formation of secondary lipid oxidation products, did not cause a detectable change in product sensory or odor, nor has any expected health implications. Furthermore, there was no perceptible change in product color. Direct exposure to light is known to alter LC color due to photo-bleaching of pigments like chlorophylls and carotenoids. The sample packaging excluded light exposure, therefore, product color was stable for up to 18 months under the given storage conditions. The percentage of omega 3 and 6 fatty acids, was maintained at ~70% total fatty acids and indicates there was little to no degradation of polyunsaturated fatty acids during the 18-month storage period.

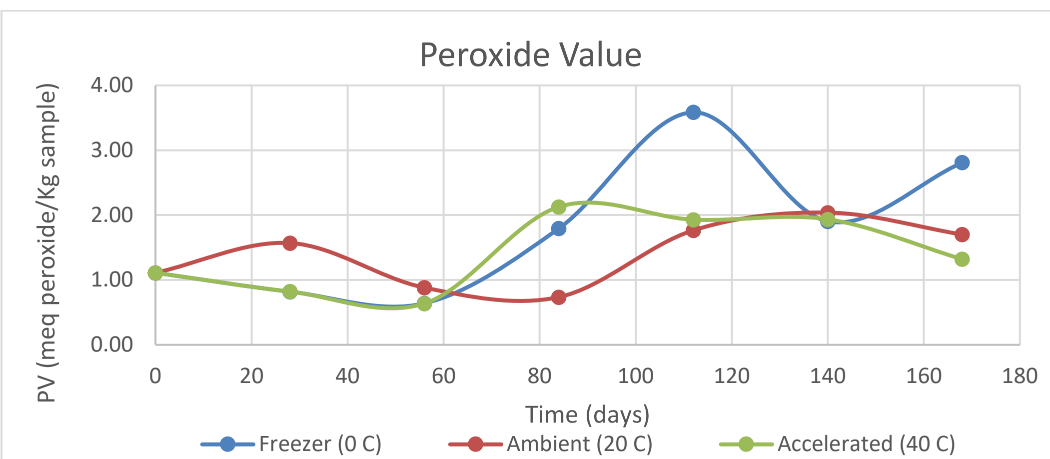
Analyte	Specification	0 month	6 month	12 month	18 month
Moisture (%)	<10	3.36	4.69	4.81	5.13
Crude Protein (%, dmb)	45-50	48.49	49.21	48.76	47.47
Protein Digestibility (%)		N/R	0.90	0.90	0.90
Amino Acid Score		N/R	0.969	0.974	1.168
PDCAAS		N/R	0.87	0.88	1.05
Crude Fat (%, dmb)	<10	N/R	8.77	8.61	8.07
Total Fatty Acids (% w/w)		6.99	N/R	6.59	6.01
Total Omega 3		3.61	N/R	3.54	4.2
Total Omega 6		1.18	N/R	1.19	1.39
Peroxide value (meq/Kg fat)		N/A	0.96	< 2.0	11
TBA Rancidity (mg/Kg)		N/A	2.5	11.3	9.3
Ash (%, dmb)	<10	5.01	4.91	4.96	4.84
Total Dietary Fiber (%, dmb)	30-45	N/R	33.25	N/R	
Water activity (measured in-house)		N/R	N/R	0.140	0.139
APC (cfu/g)	< 10^5	N/R	31000	23000	3,200
Yeast (cfu/g)	< 100	N/R	< 10	< 10	<10
Moulds (cfu/g)	< 100	N/R	20	< 10	10
C. perfringens (cfu/g)	< 100	N/R	< 10	< 10	<10

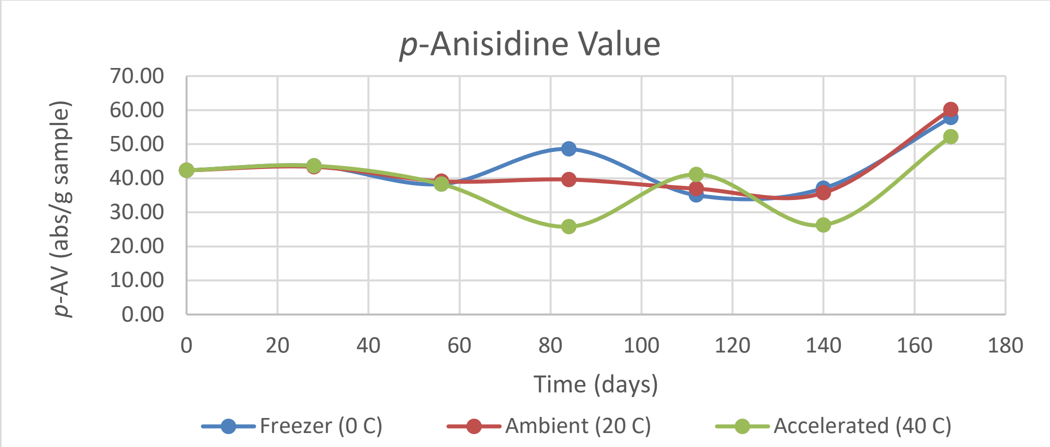
Color	Vivid green	Vivid green	Vivid green	Vivid green
Odor	Fresh, grassy	Fresh, grassy	Fresh, grassy	Fresh grassy

N/R = Data not recorded Refer to Shelf Life Data folder saved in provided CD

Study 2
LC samples from batch SPBWL160902 were packaged in aluminum foil zip lock bags and stored at 0°C (freezer), 20°C (ambient), or 40°C (accelerated). Samples were pulled at 0, 1, 2, 3, 4, 5, and 6 months for oxidative stability analysis (peroxide and p-anisidine value). Protein contents of samples were analyzed at 0 and 6 months.

Results
LC samples stored for 6 months at 0, 20, and 40 °C were analyzed for peroxide value (PV), *p*-anisidine value (*p*-AnV), and crude protein. The PV corresponds to hydroperoxides formed as primary oxidation products during lipid oxidation. The LC samples were stable to lipid oxidation under freezer, ambient, and accelerated conditions over the 6-month period. The highest PV (3.58 meq /kg) was observed for the 0 °C sample, at month 4, possibly due to slower breakdown of hydroperoxides. However, as stated previously, the PV did not increase significantly under storage conditions tested during 6 months. *p*-Anisidine value (*p*-AnV) is a measure of non-volatile secondary oxidation products from the degradation of hydroperoxides. The *p*-AnV for all samples were stable for the first 5 months but increased by the 6th month. The initial protein content of LC samples was 46.35% (dmb). After 6-month storage, protein content of samples stored at 0, 20, and 40 °C was 46.28, 46.02, and 45.53 % (dmb), respectively. The protein content of the LC sample was stable for 6 months under accelerated conditions. This can be extrapolated to a shelf stability of up to 12 months which is in agreement with data from Study 1. Refer to Shelf Life Data folder saved in the CD provided. At last, Parabel will continue to assess the stability of the product at real-time every six month, up to 48 months.





Conclusion
LC samples packaged in aluminum foil zip lock bags and stored at 20 °C/50%RH remained stable and within product specifications for crude protein, fat, ash, and microbial after 18 months. Under accelerated (40 °C) conditions, protein and peroxide values did not change significantly over the six (6) month storage period. Based on the data presented, LC is expected to be stable within 24 months from date of manufacture, when stored at ambient conditions in packaging that excludes light exposure.

III. DIETARY EXPOSURE

A. Intended Human Food Uses

Parabel's LENTEIN Complete and Parabel's Degreened LENTEIN Complete, both manufactured in accordance with cGMP as specified in 21 CFR 110, are intended for use as an ingredient at the level of 1.0-24 g/serving in the food categories listed below. Table 18 lists proposed food categories and uses for Parabel's LENTEIN Complete and DGLC. FDA's methodology was used to estimate mean and high total consumption using USDA survey data on daily consumption of various food types (FDA, 2006). FDA methodology is recognized as a method that overestimates consumption. By considering the food categories and use levels depicted in Table 19, the estimated upper daily intake for Parabel's LENTEIN Complete and its degreened version would be 201 g/person/day.

Parabel's LC may be added to the following categories of foods as defined in 21 CFR §170.3(n):

(1) Baked goods and baking mixes, including all ready-to-eat and ready-to-bake products, flours, and mixes requiring preparation before serving.
(3) Beverages and beverage bases, nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit- and vegetable-flavored gelatin drinks.
(4) Breakfast cereals, including ready-to-eat and instant and regular hot cereals.
(9) Confections and frostings, including candy and flavored frostings, marshmallows, baking chocolate, and brown, lump, rock, maple, powdered, and raw sugars.

(16) Fresh fruits and fruit juices, including only raw fruits, citrus, melons, and berries, and home-prepared "ades" and punches made therefrom.
(20) Frozen dairy desserts and mixes, including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties.
(23) Grain products and pastas, including macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables.
(25) Hard candy and cough drops, including all hard type candies.
(28) Jams and jellies, commercial, including only commercially processed jams, jellies, fruit butters, preserves, and sweet spreads.
(31) Milk products, including flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages, and other milk origin products.
(33) Plant protein products, including the National Academy of Sciences/National Research Council "reconstituted vegetable protein" category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins.
(35) Processed fruits and fruit juices, including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom.
(36) Processed vegetables and vegetable juices, including all commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends.
(37) Snack foods, including chips, pretzels, and other novelty snacks.
(38) Soft candy, including candy bars, chocolates, fudge, mints, and other chewy or nougat candies.
(40) Soups and soup mixes, including commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes.

This excludes foods that fall under USDA jurisdiction, such as catfish, eggs, meat and poultry products, and soups that include more than "relatively small portions" of meat and poultry within the products. Relatively small portions are defined by 9 CFR 381.15 and the 2005 USDA Food Standards and Labeling Policy Book: 3 percent or less of raw meat; less than 2 percent of cooked meat or other portions of the carcass; or 30 percent or less fat, tallow, or meat extract, alone or in combination. In the case of poultry: less than 2 percent of cooked poultry meat; less than 10 percent of cooked poultry skins, giblets or fat, separately; or less than 10 percent of cooked poultry skins, giblets, fat, and poultry meat (limited to less than 2 percent) in any combination; and soups that include more than "relatively small proportions" of egg (as defined in 9 CFR 590.5 (h), p. 660 under subtitle "Egg product".

Table 18. Proposed Food Uses for Parabel's LENTEIN Complete [a]

PROPOSED FOOD CATEGORY	
Beverages & Beverage Bases	Non-alcoholic, including special or spiced teas, soft drinks, coffee substitutes, and fruit- and vegetable-flavored gelatin drinks
Breakfast Cereals	Ready-to-eat and instant and regular hot cereals
Fresh Fruits & Fruit Juices	Raw fruits, citrus, melons, and berries, and home-prepared "ades" and punches made therefrom
Frozen Dairy Desserts & Mixes	Ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties
Grain Products & Pasta	Macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables
Milk Products	Flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages, and other milk origin products
Plant Protein Products	National Academy of Sciences/National Research Council "reconstituted vegetable protein" category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins
Processed Fruits & Fruit Juices	Commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom
Processed Vegetable & Vegetable Juices	Commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends
Snack Foods	Chips, pretzels, and other novelty snacks
Soft Candy	Candy bars, chocolates, fudge, mints, and other chewy or nougat candies
Soups & Soup Mixes	Commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes

[a] Based on food categories as defined in 21 CFR § 170.3 (n)

B. Consumer Dietary Intake Calculation

Since the LC and DGLC level in each food are not listed in the USDA food composition tables and the National Health and Nutrition Examination Survey (NHANES) databases, the current exposure levels from food sources were estimated by FDA's methodology. The methodology was used to estimate mean and high total consumption using USDA survey data on daily consumption of various food types (FDA, 2006). FDA methodology is recognized as a method that overestimates consumption. By considering the food categories listed above and use levels depicted in Table 19, the estimated upper daily intake for Parabel's LENTEIN Complete would be nearly 201 g/person/day. Since DGLC is intended to be applied in the same categories and food products, the estimated upper daily intake for Parabel's Degreened LENTEIN Complete would also be nearly 201 g/person/day. Nevertheless, upon considering the protein and essential amino acid contribution of LC and DGLC as noted on Section IV.C, and toxicity study results noted on Appendix O, 201g LC/person/day is an adequate and safe exposure for individuals over 10 years of age.

Table 19. Dietary Intake Calculation for Parabel's LENTEIN Complete or/and Degreened LENTEIN Complete

Food Category [a]	USDA Product Category [c]	Use Level LC or DGLC (%)	Use Level LC or DGLC (g)	Serving Size (grams)[c]	LC or DGLC inclusion per serving (g)	USDA Mean Grams of Food Consumed (All Individuals)	Mean Grams LC or DGLC Consumed (All Individuals)	Mean x 2 Grams LC or DGLC Consumed (All Individuals)	Reference
Sponge Cake	Cakes, light weight (angel food, chiffon, or sponge cake without icing or filling) 8	2.5	24	55	1.49	28	0.70	1.40	h
Gluten Free Cake	Cakes, light weight (angel food, chiffon, or sponge cake without icing or filling) 8	5	58.4	55	3.06	28	1.40	2.80	h
Noodles	Pastas, dry, ready-to-eat, e.g., fried canned chow mein noodles.	6.3	14	55	3.47	163	10.27	20.54	e
Shortbread	Cookies	5	17.7	30	1.95	40	2.00	4.00	e
Gluten Free Almond Cookies	Cookies	5	7.5	30	1.63	40	2.00	4.00	e
Muffin	Coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, toaster pastries.	5	24	55	3.13	28	1.40	2.80	h
Nutrition Bars	Grain-based bars with or without filling or coating, e.g., breakfast bars, granola bars, rice cereal bars.	5	19.4	40	2.16	45	2.25	4.50	b

English Bread	Breads (excluding sweet quick type), rolls	5	36.6	50	2.61	50	2.50	5.00	e
Pasta	Pastas, plain	6.5	7	55	4.4	163	10.60	21.19	e
Snack Cluster	Ready-to-eat cereal	4.3		25		16	0.69	1.38	e1
Chips	**subcategory**								e1
	Corn chips	4.3		26		4	0.17	0.34	e1
	Potato chips	4.3		14		4	0.17	0.34	e1
Cracker	Crackers	3.5		12		3	0.11	0.21	e1
Yogurt	Diary product	3.8	12.5	130	4.95	390	14.82	29.64	f
Protein Shake	Beverages: Carbonated and noncarbonated beverages	18	6	240	6	182	32.76	65.52	e1
Smoothie	Fluid milk (not consumed with cereal)	2	2	244	2	182	3.64	7.28	e1
Green Drink	shakes or shake substitutes	3.3	3.5	240	8.02	182	6.01	12.01	e1
3in1	Milk, milk-based drinks, e.g., instant breakfast, meal replacement, cocoa.	3.3	3.5	240	7.91	182	6.01	12.01	e1
Cold pressed juice (vegetable or fruit)	**subcategory**				12				e1
	Orange juice	2		30		50	1.00	2.00	e1
	Apple juice	2		186		17	0.34	0.68	e1
Sausage	Substitute for luncheon meat, meat spreads, Canadian bacon, sausages and frankfurters	2	10.3	55	1.43	19.1	0.38	0.76	g
Trail Mix	Special Category [a]	4.3		31		31	1.33	2.67	h
TOTAL							**100.54**	**201.08**	

a 21 CFR § 170.3 (n)

b Mixtures mainly grain: Includes mixtures having a grain product as a main ingredient, such as burritos, tacos, pizza, egg rolls, quiche, spaghetti with sauce, rice and pasta mixtures; frozen meals in which the main course is a grain mixture; noodle and rice soups; and baby-food macaroni and spaghetti mixtures. Information found at http://www.specialk.com/en_us/products/nutrition-bars/dark-chocolate-nut-delight.html

c Food categories and Serving sizes for similar food categories provided in 21CFR 101.12

d Mean grams of food consumed for all individuals taken from Reference 2 or calculated from Reference 1

e Food commonly eaten in the United States, 1989-1991 and 1994-1996: Are portion sizes changing?

e1 Foods Commonly Eaten in the United States, Quantities Consumed Per Eating Occasion and in a Day, 1994-96, Appendix B (All Individuals), http://www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/Portion.pdf

f Consuming the daily recommended amount of dairy products would reduce the prevalence of inadequate micronutrient intake in the United States: diet modeling study based on NHANES 2007-2010

g http://cebp.aacrjournals.org/content/21/10_Supplement/B44.abstract

h http://www.statista.com/statistics/436557/quantity-biscuits-and-cakes-consumed-in-the-united-kingdom/

i Information found at http://www.planters.com/varieties/nutrition-information.aspx?Site=1&Product=2900007880

C. History of Use of Water Lentils in Human Diet

Water lentils are consumed by many species including poultry, fish, and herbivorous animals, as well as by humans in many parts of the world (Rusoff et al., 1980). The earliest official scientific literature that was found mentioning the use for human food of Lemnaceae (Wolffia arrhiza) is by K. Bhanthumnavin on Aug. 13, 1971, mentioning the use as a human food to exist already for many generations.

Water lentils are a dietary staple of a variety of animals, including ducks and other waterfowl, fish, and muskrats (Hillman, 1961; Leng, 1999). For over 35 years, water lentils have been investigated as a dietary source of protein for fish (Fasakin et al., 1999; Gaigher et al., 1984; Bairagi et al., 2002; Hassan and Edwards, 1992; van Dyke and Sutton, 1977; El-Shafai et al., 2004 a, b), poultry, ruminants, and swine.

In southern Asia, water lentils are a traditional part of the human diet, and is a common food in the traditional and small farmer groups (van der Spiegel et al., 2013). Burmese, Laotians, and the people of northern Thailand have used water lentils "Khai-nam" as a nutritious vegetable for generations (Bhanthumnavin and McGarry, 1971). In Thailand, *Wolffia globosa* is referred to as "Khai-nam" or "eggs of the water," and is considered highly nutritious (Appenroth et al., 2016; FAO, 1999; van der Spiegel et al., 2013). For example, in Thailand, several studies indicate the consumption of water lentils is common in the Thai diet (Uttama, 2012; Deepanya, 2012; Siripahanakul et al., 2012). A study conducted by Sansanee Uttama (2012), investigated methods to develop a set of five water meal's standard recipes, to promote water lentils consumption in the Thai community and province.

There are numerous ways of W. globosa consumption and a variety of recipes, using it either as a main ingredient (such as Wolffia crisps or "kaeng pum" - a popular vegetable dish in northeastern Thailand) or incorporating it in other foods (e.g. Wolffia-meat ball, fermented Wolffia-meat sausage, Wolffia rice noodle, Wolffia cookies, Wolffia bread, and various soups and salads). Along with its long history as a food source in Southeast Asia, it is recognized as an edible vegetable for humans in several databases, including the United States Department of Agriculture (USDA 2014) GRIN database and a database dedicated to tropical species.

Regarding the use of Wolffia in Thailand, the specie has been generally used in savory local northern and north-eastern dishes (Siripahanakul et al., 2013). Recent survey conducted by the research group from Loei Rajabhat University, on Wolffia consumption in Loei province in Thailand, showed that the locals still consume Wolffia in traditional ways, such as an ingredient in spicy soup and spicy salad. The survey also indicated that there was a frequent demand of Wolffia incorporated in other local foods, especially Wolffia-pork ball and fermented Wolffia-pork sausage. (Siripahanakul et al., 2013).

As reported by Sree and Appenroth (2016), water lentils, specially species from the Wolffia genus, have been traditionally consumed globally as a component of the human diet, typically applied as a salad, soup or omelet. In northern Thailand, Wolffia can easily be purchased in local markets. According to the report, "the market price of Wolffia is 10-15 Thai baht (29-43 cents according to the current exchange rate to US Dollar) per kilogram when it is in season and is 50-80 Thai baht ($1.4 to $2.3) during off-season." Refer to selected water lentil (duckweed) forum reports in Appendix K for additional information on recipes and form of uses.

In addition, in the 17th century benefits of water lentils were extensively described in the first Dutch medicinal plant guide: the "Cruydt book" (Rembertus Dodonaeus, 1644). According to the book, water lentils can be successfully applied to treat a variety of illnesses.

Water lentils are also suggested for use as food ingredient in many western dishes, such as Wolffia muffins, Wolffia-tomato sandwiches, Wolffia dip, and Wolffia pies (Armstrong, 2001). Water lentils are also commercially available at rural farmers' markets, (Godwin, 2014). Two species of water lentils, Lemna minor and Spirodela polyrhiza, are also included in a field guide for edible plants (Foraging Texas, 2014). Water lentils (species unknown) is also used as spice in another foraging guide (Urban Outdoor Skills, 2014).

D. Nutritional Requirement for Protein

The 2015-2020 Dietary Guidelines Recommendations are based on data from the Institute of Medicine (IOM) which used the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996,1998 to estimate the background dietary intakes of protein for the US population. The mean adult protein intake ranged from 56 - 104 g/day, depending on age group. At the 90th percentile, adult protein intakes ranged from 76 g/day to 142 g/day.

Insufficient dietary protein intake has been associated with adverse effects in human health and development. In 2005, IOM set a Recommended Dietary Allowance (RDA) value for protein of 0.8 g/kg bw in adult males and females (IOM, 2005). An adequate intake (AI) for infants aged 0 to 6 months was set at 1.52 g/kg bw/day. IOM concluded that there were insufficient data to set Tolerable Upper Intake Levels (UL) for total protein or individual amino acids.

E. Adequacy of LENTEIN Complete and DGLC as a Protein Source

Food products containing Parabel's LENTEIN Complete or its degreened version will be targeted towards individuals over 10 years of age. Parabel considers the upper range of these high-level intakes unrealistic (i.e., that an individual would consume two servings of all food categories or products containing Parabel's LENTEIN Complete or/and DGLC), because it implies an unrealistic and unhealthy diet and it would imply Parabel to have 100% market share."

Protein intake data in the U.S. population is available from the NHANES database (NHANES, 2012). In the U.S., the average protein intakes among adults range from 80.0 to 110 g/day for men and from 58.8 to 75.5 g/day for women, with average values of 98.8 g/day for men and 68.1 g/day for women. According to the estimated dietary intake and considering the typical protein content in Parabel's LENTEIN Complete and its degreened version (40-55% dmb), the total protein intake of adult "high" consumers may be estimated as follows:

100 g LC or DGLC is on average 45 g crude protein
Proposed Parabel's LENTEIN Complete or DGLC EDI = 201g/individual/day
201g * 0.45g = **90.45 g dietary protein per day for an adult**

In the general population, about 75 % of protein intake is derived from meat and meat products, grain and grain-based products, as well as milk and dairy products (EFSA NDA Panel, 2012). Another 7-8 % is ingested with seafood, eggs and egg products. The remaining 18 % of protein intake, corresponding to 0.15 g/kg bw per day, may in part represent protein isolates such as soy protein isolates which are added to processed foods. The latter may represent a higher proportion of the diet of vegans. As a result, the estimated average intake of Parabel's LENTEIN Complete or its degreened version (90.5 g dietary protein/individual/day) in comparison to Dietary Reference Values for protein (NHANES, 2012), should not cause any implications for human safety.

F. Estimate of Exposure to Undesirable Substances based on LC data

1. Lead (Pb) Calculations

PTWI for Pb = 0.025 mg/kg BW (established by JEFCA in 2003)
1Kg LC = 0.01-0.02 mg Pb (ppm)
Average adult BW = 70 kg → (70*0.025) = 1.75 Pb/week
1.75/0.02=87.5 kg LC/week

Provisional Tolerable Daily Intake for lead would yield to a daily intake allowance of 12.5 kg of Lentein Complete.
Proposed LC EDI = 201 g/individual/day (0.20 Kg)
Lead Exposure = (.20 Kg LC* 0.02 ppm) → 0.004 mg/kg

Results indicate that even at an unrealistic chronic intake of Parabel's LENTEIN Complete of 201 g/individual/day of an adult, the intake of lead, an undesirable substance and heavy metal, would fall far below the calculated Provisional Tolerable Daily Intake for lead (12.5 kg LC/day). Same calculations and conclusions apply to DGLC due to similar chemical composition.

Refer to Table 31 for an additional assessment on mineral and heavy metal consumption based on the EDI for Parabel's LENTEIN Complete of 201 g/individual/day.

2. Oxalic Acid (OA) Calculations

A low-oxalate diet, prescribed for people with calcium oxalate kidney stones, limits the consumption of moderate-oxalate foods (2 to10 mg of oxalate per serving) to three servings per day, but high-oxalate foods (>10 mg/serving) should be avoided entirely (University of Pittsburgh Medical Center (UPMC), 2006). Therefore, the maximum exposure for oxalic acid is set at:

10 mg OA * 3 servings/day = 30 mg OA/day

Parabel's LENTEIN Complete typical value of OA is 0.04g (4mg) in 100g LC.
If Parabel's LENTEIN Complete is consumed by an adult at an unrealistic chronic intake of 201 g/day, the individual would consume 8.96 mg of OA/day:

(4 mg OA * 201 g LC)/100 = 8.04 mg/day

Therefore, even at an unrealistic high anticipated dietary intake of 201 g/individual/day, a high-level consumer required to follow a low oxalate diet, would continue to meet the recommended dietary requirements for oxalic acid. See Section VI.G for a complete safety analysis on antinutritional factors in Parabel's LENTEIN Complete.

Identical calculations and conclusions apply to DGLC due to lower oxalic acid values (<0.001%).

G. Precautions and Restrictions of Use

Based on the estimate of exposure results for undesirable substances on Section III. E and F, Parabel does not see the need for proposing precautions and restrictions of use following the proposed daily anticipated dietary intake of LC or/and DGLC at 201 g/individual/day for the target population.

H. Human Consumption of Carotenoids Found in Parabel's LC and DGLC

1. Trans-Lutein and Cis-Lutein

The free and esterified form of lutein is found in green leafy vegetables, yellow-orange fruits, yellow-orange vegetables, and egg yolks (Sies and Stahl, 2003). The combined mean daily intake of lutein and zeaxanthin varies from 0.8 to 4 mg per day, depending on the population studies (Rock et al., 2002; Bone et al., 2003). LC contains about 687 mg/kg of lutein.

Levels of lutein up to 13.4 mg/day are generally considered as safe (GRAS). In a recent submission of four GRAS notices, the FDA responded with a "no questions" letter (up to 13.4 mg/day in specified food categories). Similarly, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated a group acceptable daily intake (ADI) of 0-to 2-mg/kg body weight/day for lutein from *T. erecta* and zeaxanthin (FAO, 2004). In addition, the

European Food Safety Authority panel (EFSA) also reviewed the use of lutein by infants and young children, and concluded that there is no concern of safety for recommended use of lutein at the use level of 250-µg lutein/L of infant formula (EFSA, 2008). LC would supplement the human diet as it contains about 585 mg/kg trans lutein and 102 mg/kg cis-lutein. At an unrealistic EDI of 201 gram/day this equates to 138 mg/day lutein.

2. Zeaxanthin

Dietary sources of zeaxanthin include yellow corn, red pepper, orange juice, honeydew, mango, and chicken egg yolk (Sajilata et al., 2008). The mean daily intake of lutein and zeaxanthin combined varies from 0.8 to 4 mg per day, depending on the population studies (Rock et al., 2002; Bone et al., 2003). LC contains about 27.2 mg/kg zeaxanthin, or approximately 5.47 mg/day using a 201 g/day exposure.

3. ß-Carotene

Beta-carotene is a fat-soluble carotenoid pigment, found naturally in many fruits and vegetables such as green plants, carrots, pumpkin, sweet potatoes, squash, spinach, apricots, cantaloupe, pink grapefruit, and green peppers. The Institute of Medicine has reviewed beta-carotene, but recommendations for daily intake were lacking, citing a lack of sufficient evidence. Consuming five servings of fruit and vegetables daily provides 6-8mg of beta-carotene (Natural Standard, 2014). LC would supplement the human diet as it contains about 271 mg/kg β-carotene. This equates to 54.5 mg/day with 201 g/day exposure.

Degreened LENTEIN Complete is expected to have significant lower levels of Carotenoids compared to LC, because the fat-soluble components and natural pigments within water lentils are extracted during the manufacturing process of Parabel's Degreened LENTEIN Complete (See Section II.D). As a result, no health and safety implications are expected from DGLC's carotenoid content and DGLC consumption.

I. Human Consumption of Chlorophyll Found in Parabel's LC and DGLC

Chlorophyll a and chlorophyll b are natural, fat-soluble chlorophylls found in plants. All forms of Chlorophyll can be found in green leafy vegetables (broccoli, Brussels sprouts, cabbage, lettuce, and spinach), algae (Chlorella and Spirulina), wheatgrass, green tea, and numerous herbs (Natural Standard, 2014). These compounds were first identified in the chloroplasts of the photosynthetic parts of plants. The two green components are named chlorophyll a and b (Schwartz and Lorenzo, 1990).

There is no readily available data on the average dietary intake of chlorophyll in humans and there is no data on maximum levels of chlorophyll that is considered safe. However, The European Union has allowed chlorophyll as a food additive (E number is E140). LC was analyzed for total chlorophyll content and it was reported to be 602 mg/100g

Degreened LENTEIN Complete is expected to have significant lower levels of Chlorophyll compared to LC, as the fat-soluble components and natural pigments within water lentils are extracted during the manufacturing process of Parabel's Degreened LENTEIN Complete (See Section II.D). As a result, no health and safety implications are expected from DGLC's chlorophyll content and DGLC consumption.

IV. SELF LIMITING LEVELS OF USE

Protein is a macro component or ingredient in food. At high levels of protein, food products become bitter and unpalatable. Additionally, because of the physical properties of LENTEIN Complete and DGLC, and their high water-binding properties, excessive levels can make the food product dry, dense and difficult to manufacture. Levels exceeding those provided in Table 19 of this dossier are not anticipated due to the potential for unpalatability or the technological impracticality of higher use levels.

The belief that the projected use levels are representative is supported by the protein levels in current market products with high protein claims and published literature. Table 20 provide examples of commercial high protein products.

Table 20. Examples of Commercial High Protein Products

Product Type	Brand Name	Protein Source(s)	Protein / serving	% Protein
Bread	See: Mizrahi et. al. 1967	Soy	2-10	
Dairy alternative	SoDelicious-Vanilla Frozen Dessert[1]	Soy protein	2 g	2.5
Dairy alternative	Stonyfield-O'Soy vanilla yogurt[2]	Soy protein	7 g	4.1
Dairy alternative	Silk – vanilla yogurt[3]	Soy protein	6 g	4
Donuts	See: Singh et. al. 2008	Soy	3 – 3.5	
High Protein Cookie	Nashua – ProteiDiet[4]	Gelatin, soy, whey, egg	15 g	35.7
Meat Analogues	See: Asgar et. al. 2010	Soy, whey, egg, legume	4-20	
Nutritional Beverage	Power Bar – Protein Shake[5]	Casein, Whey	30 g	6
Nutritional Beverage	Boost- High Protein[6]	Soy, Casein	15 g	6
Pasta	Barilla-Protein Plus Spaghetti[7]	Bean flour, egg	10 g	17.8

Sport Nutrition	Power Bar-Clean Whey[8]	Whey	20 g	30
Sport Nutrition	Power Bar-Protein Plus[9]	Soy, Casein, Whey	20 g	30
Sport Nutrition	Gatorade – Whey Protein Bar[10]	Whey	20 g	25
Weight Management	Nashua-Health Smart Protein Bar[11]	Soy, Whey, Casein	14 g	35
Weight management Cereal	NutriWise Cinnamon Diet Protein Cereal[12]	Soy protein Isolate	15 g	51.7 (reduced to 5.6 when eaten with skim milk)
Weight management Soup	NutriWise – Instant Cream of Chicken[13]	Whey protein	15 g	5.6

http://sodeliciousdairyfree.com/products/soy-milk-frozen-desserts/creamy-vanilla
http://www.stonyfield.com/products/yogurt/osoy/vanilla
https://silk.com/products/vanilla-dairy-free-yogurt-alternative
http://www.nashuanutrition.com/store/snacks-and-treats/protidiet-cookies-cranberry-lemon-7-box.html
https://www.powerbar.com/protein/protein-shake
https://www.boost.com/products/high-protein
https://www.barilla.com/en-us/products/pasta/proteinplus/proteinplus-spaghetti
https://www.powerbar.com/chocolate-chip-cookie-dough
https://www.powerbar.com/protein/20-30g-proteinplus%E2%84%A2
https://shop.gatorade.com/sports-fuel/whey-protein-bar
http://www.nashuanutrition.com/store/protein-bars/healthsmart-protein-bar-chocolate-mint-7-box.html
https://www.bariatriclifestylediet.com/product/nutriwise-bariatric-cinnamon-protein-cereal/
https://www.bariatriclifestylediet.com/product/nutriwise-bariatric-cream-of-chicken-soup/

V. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Parabel Ltd. is unaware of any use of LENTEIN Complete, Degreened LENTEIN Complete or water lentil protein powder prior to 1958. However, as mentioned in Section III.C, the micro aquatic plant was not identified as a human food source until the 1970's. The earliest official scientific literature that was found mentioning the use for human food of Lemnaceae (Wolffia arrhiza) is by K. Bhanthumnavin back on August 1971, mentioning the use of water lentils as a human food to exist already for many generations. As a result, for over 35 years, water lentils have been investigated as a dietary source of protein for many animal species including fish, poultry, ruminants, and swine. Section VI summarizes these investigations through a literature review of safety data based on published animal studies.

VI. NARRATIVE

Parabel has investigated the published literature addressing the safety of the substance under review and other similar materials to focus on toxicologically relevant constituents. Water lentils are viewed as promising source of protein to meet future challenges of food supply. Thus, Lemnaceae have been identified has a potential protein source for both animals and humans, and many studies have been conducted worldwide to address the safety of Lemnaceae and Lemnaceae derived products. In addition, the FAO has actively followed the development of water lentils as a food source (Leng, 1999). As reported in

Section III.C, there is a history of use of water lentils for human food in South Asia, Burma, Laos and northern Thailand (van der Spiegel, 2013 and Leng, 1999).

The protein fraction of water lentil species has been well characterized in the literature. The protein has a high nutritional quality and is a good source of many amino acids. Due to its relatively high protein content and digestibility (See Appendix A6), whole or dried water lentils have been used successfully as animal feed material. A FAO document (Leng, 1999) noted that water lentils are widely used by farmers in Vietnam as feed for ducks and fish as well as in Taiwan as feed for pig and poultry. As demonstrated by the numerous studies reviewed and summarized later in this section, water lentils are already widely used around the world as a protein source for food-producing animals.

The Amino acid composition of various species of water lentils is also well established in literature. Data on both LENTEIN Complete and on its degreened version demonstrates the products have similar amino acid composition to that found in the literature on other water lentils species as well as other FDA GRAS-notified aquatic proteins sources (Appenroth et al., 2016).

As described in Section II.C, Parabel's LENTEIN Complete and DGLC proteins will be digested like any other protein, by normal metabolic processes. Proteins are an essential part of the daily human diet and are an integral part of many food products. After ingestion, proteins are hydrolyzed in the gastrointestinal tract by proteolytic enzymes derived from the pancreas resulting in the release of dipeptides, tripeptides and free amino acids (Grimble 1994). Carrier systems specific for the transport of either the amino acids or the di- and tripeptides are responsible for the efficient transport across the intestinal wall. The amino acids resulting from the digestion of foods are used as building blocks for formation and maintenance of body proteins.

The protein from Parabel's LC and DGLC, provides protein containing all the essential amino acids (EAAs) meeting FAO standard (Table 21). Parabel's LENTEIN Complete and its degreened version conserve the amino acid composition as found in water lentils. The amino acid composition in Parabel's LENTEIN Complete, which is very similar to its degreened version (see Table 11) is comparable with spinach and broccoli. It should be stressed that in no case the content of EAAs in Parabel's LENTEIN Complete is lower than those recommended by WHO (2007). In Parabel's LENTEIN Complete, the content of cysteine plus methionine, threonine, phenylalanine plus tyrosine, and leucine which are commonly insufficient in plant protein, are above the recommended limitations. The lysine content in Parabel's LENTEIN Complete protein is above the level in maize, rice, and sorghum. In addition, the amino acid composition of Parabel's LENTEIN Complete protein can successfully be compared to flour-form legumes like chickpea and soy.

Protein Digestibility Corrected Amino Acid Score (PDCAAS), is a quality metric for proteins developed and endorsed by the Food and Drug Administration, United Nations and World Health Organization; that weighs the protein's essential amino acid concentration against its ability to digest it. A protein quality index, such as PDCAAS, characterizes the protein in relation to its ability to achieve defined metabolic actions. The PDCAAS is the current internationally approved method for protein quality assessment (WHO, 2007). PDCAAS is a score which depends on the limiting EAAs divided by the requirement for these amino acids for a pre-school children, corrected by digestibility (Yong and Pellett, 1994). Even though the PDCAAS of Parabel's LENTEIN Complete and DGLC were determined in vitro, the data from each product shows that the in vitro protein digestibility compares favorably with those obtained by in vivo method applied in rats (Akeson and Stahmann, 1964; Maga et al., 1973; Saunder et al., 1973). The analytical result performed by Medallion Laboratory indicates the amino acid score of both Parabel's LENTEIN Complete and DGLC is 1.02 and protein digestibility is 91%, consequently the PDCAAS Parabel's LENTEIN Complete and DGLC is 0.93 (See Appendix A6). Thus, the PDCAAS of both Parabel's LENTEIN Complete and DGLC is comparable to beef and soy protein isolate, and is much higher than pulse and grain products (refer to Table 22); indicating the high quality of the protein in Parabel's LENTEIN Complete and DGLC.

Table 21. Comparison of essential amino acid composition of protein form different sources

% [a]	Spinach [b]	Broccoli [b]	Water lentils [b]	Maize (whole meal) [c]	Rice (milled polished) [c]	Sorghum [c]	Soya Flour [d]	Chickpea Flour [d]	LENTEIN Complete [e]	DGLC [e]	WHO Recommendation
Tryptophan	1.6	1.8	2.0	0.7	2.7	1.3	N/A[g]	N/A[g]	2.1	2.4	0.5
Threonine	4.9	3.9	5.0	3.7	3.4	3.1	4.1±0.1	3.9±0.2	3.7	3.8	1.1
Isoleucine	5.9	3.9	4.5	3.8	4.4	4.1	4.2±0.2	4.1±0.2	4.4	4.1	1.5
Leucine	8.9	6.3	9.0	12.8	8.6	13.8	7.7±0.4	7.7±0.3	7.7	7.6	2.1
Lysine	7.0	7.4	6.5	2.7	3.8	2.1	6.0±0.1	7.0±0.3	6.0	5.9	1.8
Methionine	2.1	1.8	2.1	2.0	2.2	1.4	1.4±0.1	1.6±0.1	2.9	2.9	2.0
Cysteine	N/A[g]	N/A[g]	N/A[g]	1.6	1.6	1.6	1.5±0.1	1.7±0.1			
Tyrosine	N/A[g]	N/A[g]	N/A[g]	3.9	3.3	2.8	9.0	8.6	8.8	8.5	2.1
Phenylalanine	5.2	4.8	5.8	5.0	5.0	5.1					N/A[g]
Valine	6.5	5.7	5.9	5.0	6.0	5.2	4.4±0.4	4.2±0.2	5.3	5.0	1.5
Histidine	2.6	2.5	2.2	2.8	2.4	2.2	2.7±0.2	2.7±0.1	2.0	1.9	1.5

[a] Value calculated as percent of total amino acid (g/100g protein x100)
[b] Edelman and Colt, 2016
[c] adapted from Day, 2013
[d] Jahreis et al., 2016
[e] Data for LENTEIN Complete were determined by Medallion Lab for LENTEIN Complete produced by Parabel USA Inc.
[f] WHO, 2007
[g] N/A = no data

Table 22. Protein digestibility corrected amino acid score (PDCAAS) values of individual plant protein, compared with selected animal protein

Protein source	PDCAAS value
Animal protein	
Casein	1.00[a]
Egg white	1.00[a]
Beef	0.92[a]
Plant protein	
LENTEIN Complete and DGLC	0.93[b]
Whole wheat	0.42[e]
Wheat gluten	0.25[a]
Rice	0.47[c]
Maize	0.46[c]
Sorghum	0.20-0.30[d]
Soy protein isolate	0.92[e]
Pea flour	0.69[e]
Pea protein concentrate	0.73[c]
Chickpeas	0.71[c]
Black bean	0.75[a]
Peanuts	0.52[a]

[a] Hoffman & Falvo 2004
[b] Data for LENTEIN Complete and DGLC was determined by Medallion Laboratory's in vitro enzyme digestion model for rapid screening of food prototypes: US Pat Appl No. 14/599,050: in vitro method for estimating in vivo protein digestibility, Plank, DW. (Appendix A6)
[c] Day, 2013
[d] Anyango et al., 2011
[e] FAO/WHO, 1991

Moreover, there are many studies in the literature and others conducted and published by Parabel in which the nutritional value of several water lentil species (identified as wild raw duckweed in some publications) and Parabel's LPC (an earlier non-commercialized Parabel Lemnaceae product, described next page) were investigated in numerous animal species including poultry, cattle, pigs, sheep and fish, at inclusion levels above 20% up to 100%. As a result, based on the scientific literature review of the available published studies and the scope of animal studies conducted by Parabel on LPC, there is confidence that LENTEIN Complete and its degreened version provide adequate nutrition at high levels in several animal species without any overt toxic effect. In addition, there is a significant body of data present from which conclusions and a presumption of safety can be made. In most studies, adequate levels of growth and maintenance of health was achieved. In some studies, better growth and performance were shown with optimum diets used in agriculture by more developed countries. Many of the studies used high levels of water lentils in the diet without adverse effects and it is important to note the lack of adverse effects given that the water lentils used in many of these studies were not a refined material such as Parabel's LPC, LC or DGLC. For example, the protein was not concentrated in most of the studies and the conditions of growth were often not clearly defined in the literature. In addition, agriculture in less developed countries relies on feed sources that are readily available so while diets containing water lentils may not have been considered optimal the experimental diets were usually considered adequate by the investigators. Lastly, there were several studies done on breeding animals. No adverse effect was seen on reproductive outcomes in these studies. Also, in the studies using Lemna Protein Concentrate (Rojas et al., 2014; Parabel, 2015a; Parabel 2015b; Garcia-Gonzalez et al., 2015; Kenny et al., 2015) there were no adverse effects noted and LPC preparations were recommended for use as feed for fish, pigs and poultry. As a result, these studies are considered corroborative to the principal published safety data, and are summarized in this section as follows.

A. Literature Review of Safety Data based on Published Animal Studies

1. Overview animal trials conducted on Parabel Lemna Protein Concentrate (LPC)

Parabel's Lemna Protein Concentrate (LPC) is a plant protein concentrate powder produced from Parabel's Lemnaceae aquatic plants. LPC contains 60-70% protein with a profile and content of amino acids that resemble that of Parabel's LENTEIN Complete. The objective of the following animal trials was to assess LPC as a protein alternative and commercial feed material, and to demonstrate that no health implications or toxicity is expected from the consumption of Parabel's processed water lentils. Appendix G contains LPC specification sheet. Table 23 represents the similarities between both products chemical composition.

Table 23 Parabel's LPC and Parabel's LC Typical Chemical Composition

Component	LPC	LENTEIN Complete
Moisture	5-10%	2-6%
Crude Protein (dmb)	60-70%	40-50%
Total Fat (dmb)	6-12%	6-10%
Ash/Minerals (dmb)	8-15%	4-6%
Carbohydrates calc. (dmb)[a]	0	3-11%
Dietary Fiber (dmb)	7-9%	30-45%
Sodium (dmb)	6,305	1,634 mg/kg*
Sugars (dmb)	<1.5%	<0.35%
Starch (dmb)	<0.8%	0.4-12%

[a] 100% - [protein (as-is) % + moisture % + fat (AH) % + ash % + dietary fiber %]
*Mean value from 5-batch data (See Appendix A1)

a. Swine Studies

In a trio of experiments, Rojas et al. (2014) at the University of Illinois assessed the nutritional value of Parabel lemna protein concentrate (LPC) for piglets. The experiments were conducted to determine: (1) the supply of digestible and metabolizable energy (DE and ME, respectively), (2) the apparent (ATTD) and standardized (STTD) total tract digestibility of phosphorus, and (3) the apparent (AID) and standardized (SID) ileal digestibility of amino acids (AA). These values were compared to those obtained for corn, fishmeal (menhaden) and soybean meal (SBM). In the first experiment, 32 barrows were allocated to 1 of 4 diets, with 8 replicate pigs/diet, in a block design. Four corn-based diets were provided on an as-fed basis: (1) basal diet, (2) LPC (25.0%), (3) fishmeal (25%), and (4) SBM (35.0%). Diets were supplemented with vitamins and minerals to meet/exceed the needs of weanling pigs. Results are summarized in Table 24. No significant differences were noted in ME among ingredients ($P > 0.05$), but DE was greatest for LPC and SBM vs. corn. For a second experiment, 24 barrows were allocated to 3 diets with 8 replicate pigs/diet. Diets were prepared by mixing corn starch and sugar with LPC (25%), fishmeal (15%), or SBM (40%). Vitamins and minerals, except phosphorus, were supplemented accordingly, thus making the test ingredients the sole source of phosphorus in the diets. The authors found the STTD of phosphorus to be 73% in LPC, 66% in fishmeal, and 63% in SBM. There was a trend for the STTD of phosphorus to be greater in LPC than in fishmeal or SBM ($P = 0.07$). In a third experiment, eight barrows with a T-cannula in the distal ileum were randomly assigned to treatments in a replicated 4×4 Latin square design. Four corn starch based diets were formulated: a nitrogen-free diet, and 3 diets where LPC (20%), fishmeal (15%), and SBM (40%), respectively, were the sole sources of amino acids. The AID of AA was comparable for both LPC and fishmeal ($P > 0.05$) while there was a trend ($P = 0.07$) for higher SID for fishmeal. Overall, the incorporation of LPC into the diet of pigs did not affect the absorption of energy or AA vs. fishmeal while there was a tendency for a higher absorption of phosphorus.

Table 24. Concentration of digestibility and metabolizable energy, and digestibility of phosphorus and amino acids in LPC and reference ingredients for piglets

	Corn	LPC	Fishmeal	SBM	SEM	*P*
DE[1], kcal/kg DM	3943[c]	4342[ab]	4314[b]	4523[a]	70.0	< 0.01
ME[2], kcal/kg DM	3855	3804	3904	4184	106.2	0.08
ATTD P[3], %	-	59.5	61.9	-	2.99	0.33
STTD P[4], %	-	72.8	65.6	-	2.99	0.07
AID AA[5], %	-	75.9	76.2	-	1.93	0.91
SID AA[6], %	-	80.3	85.6	-	1.93	0.07

[1] Digestible energy; [2] Metabolizable energy; [3] Apparent total tract digestibility of phosphorus; [4] Standardized total tract digestibility of phosphorus; [5] Apparent intestinal digestibility of amino acids; [6] Standardized ileal digestibility of amino acids
[abc] Means with a different subscript differ from each other (P < 0.05)

Another trial was conducted in a commercial pig farm to assess LPC as a fishmeal replacer for weaning piglets. One hundred and twenty newly weaned crossbred piglets averaging 6.6 kg were allocated to two treatment diets, each diet with 4 replicate pens of 15 piglets per pen. The trial comprised a first (weeks 1st-2nd post weaning) and second phase (weeks 3rd-4th). Two treatment diets were formulated with either fishmeal or LPC (in both cases at 4% and 2% for first and second phase, respectively) The experiment was conducted in an open-sided house with solid concrete floor pens. Each pen measured 2.0 m x 6.0 m and was equipped with a feeder and 3 nipple water drinkers. Feed and water were provided ad libitum. All diets were used in pellet form. Feed consumption as pen basis and individual body weight were measured at the beginning and end of both phases 1 and 2. Body weight, daily feed intake, average daily gain, feed conversion ratio, and livability were calculated and were subjected to analysis of variance as a block design. The performance of piglets fed LPC diet was superior to those piglets fed fishmeal diet (Table 25): animals showed same intake but average daily gain and final body weights were higher for piglets fed with LLPC, resulting in an improvement of feed conversion ratio by 7%. LPC can therefore be used to replace fishmeal in post-weaning piglet diets with a positive impact in piglet performance (Parabel, 2015a)

Table 25. Effect of LCP on performance of piglets (week 1-4 post-weaning)

	LPC	Fishmeal	SEM	*P*
Initial body weight, kg	6.56	6.56	-	-
Final body weight, kg	20.3	18.9	0.23	0.02
Avg. daily gain, kg	0.49	0.44	0.008	0.02
Daily feed intake, kg	0.70	0.68	0.010	0.24
Feed conversion ratio	1.44	1.56	0.015	0.01
Livability, %	100	100	0.0	1.0

b. Poultry

A trial was conducted to examine the performance of chicken fed with LPC vs. fishmeal. Two hundred and forty newly hatched male broiler chicks of commercial strain Ross 308 were randomly allocated to pens of 10 chicks each, and pens were then randomly allocated to each of 3 treatments (test diets). Eight replicates were run per treatment. Three test diets for each growing phase (starter, 0 to 10 d; grower, 11 to 24 d; finisher, 25 to 35 d) were formulated: control, fishmeal and LPC. Fishmeal or LPC were included at 2% in the starter phase and 1% in the grower phase. After day 24, birds in all treatment groups were fed the same commercial finisher diet until the end of finisher period (35 days of age). The experiment was conducted in a closed house with tunnel ventilation and evaporative cooling system. Birds were raised on solid-concrete-floor pens using rice hull as bedding material. Each pen measured 1.0 m x 1.0 m and was equipped with a self-feeder and two nipple water drinkers. Feed and water were provided ad libitum. All experimental diets were processed under conditioning temperature of 82°C and pelleted to 3.2 mm diameter. Feeds were provided to birds in crumble form during starter phase, and in pellet form thereafter. Feed consumption and body weight were measured per pen at the beginning and end of each phase and the following traits were calculated: individual body weight gain (average final body weight – average initial body weight); feed conversion ratio (pen feed intake / pen weight gain); and individual feed intake (average body weight gain x FCR). Livability was calculated as 100 – (% death + % culls). Results are shown in Table 29. The overall performance 0-35 days of birds was not different among groups. These results indicate that LPC can replace fishmeal in broiler diets (Parabel, 2015b)

Table 26. Effect of LCP on performance of broilers (0-35 d of age)

	Control	Fishmeal	LPC	SEM	*P*
Initial body weight, kg	0.049	0.049	0.049	-	-
Final body weight, kg	2.45	2.45	2.45	0.023	0.99
Weight gain, kg	2.40	2.40	2.40	0.023	0.99
Feed intake, kg	3.71	3.73	3.75	0.039	0.81
Feed conversion ratio	1.55	1.55	1.56	0.010	0.60
Livability, %	97.5	100	97.5	1.44	0.39

c. Aquaculture (tilapia and shrimp)

There is an increasing demand for alternative high quality, nutritious and sustainable feed ingredients. Parabel's Lemna Protein Concentrate (LPC) contains 65% of protein rich in essential amino acids (EAA) that resemble that of fishmeal, and is produced under highly efficient, sustainable conditions.

A trial was conducted at the University of Idaho (Hagerman, ID, USA) to study the digestibility of LPC in post juvenile Nile tilapia (Oreochromis niloticus). Two diets (diet 1, reference; diet 2, 30% LPC) containing 0.1% yttrium oxide as inert marker were fed to duplicate groups of fish (80 fish/400 L tank) up to apparent satiation for 3 weeks. Tanks were cleaned of feed residue and fecal material after the last meal of the day. Feces were collected by netting the following morning. Feces were pooled by tank, dried and analyzed for nutrient composition. Results are shown in Table 4. The apparent digestibility coefficient of each essential AA was over 85% for tilapia (Garcia-Gonzalez et al., 2015)

Another trial was conducted to study the performance of tilapia fed LPC. Six isocaloric, isoaminoacidic diets were formulated with graded levels of LPC substituting for fishmeal (Table 5) to meet the NRC 2011 nutritional requirements of tilapia. Each dietary treatment was randomly assigned to one tank in each of the three-recirculation systems at the University of Idaho (Hagerman, ID, USA) in a randomized block design. Water temperature was maintained at 26 °C and dissolved oxygen levels by aeration. Tanks were stocked with 30-mixed sex and mixed species hybrid juvenile tilapia (Oreochromis sp.). Fish were fed twice per day to apparent satiation. After 12 weeks, all tanks from one recirculation system were discontinued and then restocked with fish from the other two tanks of the same treatment, for a final density of 20 fish/tank, and the trial continued for another 12 weeks. At the end of the trial, samples of fish were removed from each tank for whole-body analyses and for measuring hepatosomatic index. Results are summarized in Table 27. Weight gain, specific growth rate, feed conversion ratio, and protein and energy retentions were calculated. Data was analyzed as a block design. Tilapia growth, feed intake, and feed conversion ratio were not significantly different among the treatments ($P>0.05$); hepatosomatic index, fish whole-body composition, and protein and energy retention were unaffected by diet. The data show that LPC readily substitutes for up to all the fishmeal in customary tilapia feed formulations with no performance and fish quality implications (Kenny et al., 2015). The abstract for this study was recently published at the World of Aquaculture Society (WAS) conference in Jeju, Korea, in the year 2015.

Table 27. Amino acid composition and apparent digestibility coefficients (ADC) of LPC and menhaden meal for shrimp and tilapia

	Shrimp				Tilapia
	g/100g as-is		ADC, g/100g		ADC, g/100g
	LPC	Menhaden	LPC	Menhaden	LPC
Arginine	4.39	3.94	84.3 ± 3.4	85.1 ± 1.7	93.4
Histidine	1.66	1.60	79.8 ± 2.9[a]	92.1 ± 1.5[b]	87.6
Isoleucine	3.22	2.82	82.1 ± 2.1	77.5 ± 1.9	86.8
Leucine	5.90	4.40	81.8 ± 2.4[b]	73.2 ± 1.6[a]	88.7
Lysine	3.48	4.86	87.9 ± 2.0	86.9 ± 1.3	94.2
Methionine	1.48	1.63	82.8 ± 3.4	87.4 ± 2.1	85.3
Phenylalanine	3.84	2.88	84.8 ± 1.8[b]	72.6 ± 3.0[a]	88.8
Threonine	3.98	3.87	82.4 ± 2.2	83.3 ± 2.6	86.9
Valine	4.78	3.41	82.2 ± 1.8	75.8 ± 1.8	88.0

[a, b] Different superscripts indicate significant difference between means ($P<0.05$)

A study was conducted to determine the digestibility of nutrients of Parabel's Lemna Protein Concentrate (LPC) by Nile tilapia and to evaluate LPC as an ingredient in feeds for Nile tilapia. Six experimental diets were formulated to contain 32% crude protein and 7% lipid. The LPC accounted for 0, 8, 16, 24, 32, and 40% of the diet, and it replaced 0, 20, 40, 60, 80, or 100% of fishmeal protein in the experimental diets. Each diet was fed to three replicate tanks (containing 40 fish) in a randomized block design. Fish were fed three times daily to apparent satiation for 9 weeks. Twenty fish were sacrificed from the original group in order to perform proximate analysis. Every three weeks, approximately 30 fish from each experimental diet were bulk weighed and averaged. At the end of the study, all fish were weighed and sacrificed for chemical analysis. Apparent Digestibility Coefficients were calculated by standard methods. For Lemna Protein Concentrate, the apparent digestibility coefficient was equivalent to fishmeal for dry matter, crude protein, lipid, and energy content. The apparent digestibility coefficient for phosphorus was significantly higher than fishmeal, indicating that LPC contains a non-phytate source of phosphorus. The amino acid Apparent Digestibility Coefficient of Lemna Protein Concentrate (84-94%) was comparable to that of fishmeal. The 20% LPC diet had the highest weight gain (889%), and fish fed no fishmeal and the highest level of LPC had the lowest weight gain (587%). These diets were not significantly different, however, from the control diet (7% fishmeal and no LPC). Overall, the results of this study indicate that replacement of 60% of fishmeal protein with LPC resulted in the best growth rate, feed conversion ratio, and protein efficiency ratio in tilapia. Therefore, Lemna Protein Concentrate is a suitable protein source for commercial tilapia feed. Moreover, body composition was not altered by the substitution of LPC for fishmeal; the protein and amino acids are highly available to fish, and high phosphorus digestibility was observed (Hardy, 2010).

Hardy (2014) evaluated the digestibility of Parabel's protein product using Rainbow Trout. Post-juvenile Rainbow Trout (Oncorhynchus mykiss) were divided into seven treatment groups, two tanks per treatment group with 50 fish per tank. The treatments included two control diets: casein-gelatin diet and USDA Agricultural Research Service (ARS) diet (containing soy protein concentrate, wheat gluten, and squid meal), and five treatments that included Parabel product: Casein diet with LPC (64% protein) and ARS diet with four different batches of LPC of different protein concentrations (ranging from 59 to 64% protein). Fish were fed the diets twice a day for two weeks. Fecal material was collected and analyzed, along with diets and protein powder, for crude protein, crude lipids, energy content, amino acids, and minerals to calculate apparent digestibility coefficients (ADC). Results indicated that ADC protein values, dry matter values, and energy values were slightly lower in the Parabel diet compared with fishmeal. ADC values of lipids, phosphorus, lysine and methionine were high compared with fishmeal. The authors conclude that ADC values indicate that Parabel products have significant potential as part of fish and livestock feed, but that the crude protein content of the Parabel product would need to be raised to be desirable. Subsequently, Parabel has increased the purity concentration of LPC from 60% crude protein up to 70% protein.

Shrimp (Litopenaeus vannamei) averaging 16.8 g were stocked into 550 L tanks (1 kg shrimp/tank) at the Oceanic Institute (Waimnalo, HI, USA). Three diets containing 1% chromic oxide as inert marker were fed to triplicate tanks (diet 1, reference; diet 2, 30% LPC; diet 3, 30% menhaden). Tanks were cleaned and mortalities were recorded every morning before feeding. After 7 d of acclimation, shrimp were fed 7 times daily within 2 h at a rate of 3% body weight, and leftover feed was removed. Fecal samples were collected hourly from a collection bottle for 4 h each day for 10 d. Apparent digestibility coefficients were calculated following standard methods. LPC was higher in essential AA content and showed equal or greater apparent digestibility coefficients than menhaden meal for all the essential AA except histidine for shrimp (Table 28). This data shows that LPC is a rich and highly digestible source of amino acids for shrimp and tilapia diets (Garcia-Gonzalez et al, 2015). The abstract for this study was recently published at the World of Aquaculture Society (WAS) conference in Jeju, Korea, in the year 2015.

Table 28. Growth performance, feed utilization, fish whole-body composition, and nutrient retention of tilapia juveniles fed diets containing graded levels of LPC for 24 weeks[1]

	Treatments (fishmeal protein replacement)					
	0%	20%	40%	60%	80%	100%
LPC inclusion	0%	2%	4%	6%	8%	10%
Fishmeal inclusion	12%	10%	8%	5%	3%	0%
Initial weight, g/fish	59.0 ± 0.2	59.6 ± 0.5	59.5 ± 0.4	58.8 ± 0.3	59.4 ± 0.0	59.4 ± 0.4
Final weight, g/fish	463 ± 20	422 ± 31	477 ± 4	426 ± 8	426 ± 19	442 ± 25
Mean weight gain, %	685 ± 33	607 ± 47	702 ± 12	624 ± 11	617 ± 32	643 ± 36
Specific growth rate, %/d	1.23 ± 0.03	1.16 ± 0.04	1.24 ± 0.01	1.18 ± 0.01	1.17 ± 0.03	1.19 ± 0.03
Feed intake, g/fish	544 ± 15	507 ± 26	563 ± 13	511 ± 9	517 ± 15	537 ± 20
Feed intake, % BW/d	1.24 ± 0.02	1.26 ± 0.02	1.25 ± 0.04	1.25 ± 0.01	1.27 ± 0.02	1.28 ± 0.02
FCR, g/g	1.35 ± 0.03	1.41 ± 0.05	1.35 ± 0.05	1.39 ± 0.02	1.41 ± 0.04	1.41 ± 0.04
Fish crude protein, %	16.0 ± 0.3	16.7 ± 0.3	16.1 ± 0.3	16.7 ± 0.3	16.1 ± 0.4	16.4 ± 0.4

Fish crude fat, %	7.8 ± 0.8	7.1 ± 0.8	7.7 ± 0.8	7.9 ± 0.6	6.4 ± 0.4	8.2 ± 0.4
Fish crude ash, %	3.8 ± 0.3	4.1 ± 0.1	3.8 ± 0.1	4.5 ± 0.0	4.3 ± 0.3	4.0 ± 0.1
Hepatosomatic index, %	1.28 ± 0.09	1.20 ± 0.16	1.15 ± 0.05	1.27 ± 0.05	1.27 ± 0.11	1.20 ± 0.09
Protein retention, %	33.0 ± 1.5	32.5 ± 1.6	32.5 ± 1.1	32.9 ± 0.9	31.4 ± 1.7	32.0 ± 0.3
Energy retention, %	27.2 ± 2.1	26.0 ± 1.5	27.3 ± 1.8	26.7 ± 1.5	23.5 ± 1.5	26.0 ± 0.4

[1]Mean ± SE (n=3) There was no significant difference among the treatments for any of the traits (*P*>0.05)

A study was conducted to evaluate LPC as an ingredient and potential protein source for Pacific white shrimp (Litopenaues vannamei), as well as to determine the effectiveness of LPC as a replacement for menhaden meal. In seven of the test diets, fishmeal was replaced by LPC at increasing levels: 0 (control), 16.7, 33.3, 50, 66.7, 83.3, or 100%. The control diet contained 18% menhaden, 25% soybean meal, and 5% squid meal as the major protein sources. A reference diet containing 40% protein and 9% lipid was also used. Growth of the shrimp was measured for a period of 8-weeks. Results indicated that LPC can replace up to 50% menhaden meal in feed for Pacific white shrimp without any adverse effect on growth performance and feed conversion ratio. A higher inclusion of LPC (>50%) negatively affected feed conversion ratio, however the replacement of LPC did not affect the composition, gross energy and sensory attributes (flavor, color, texture) of shrimp tail muscle. The authors concluded that LPC can replace up to 50% of fishmeal in the diet of Pacific white shrimp (Aquatic Feeds and Nutrition Department, Oceanic Institute, 2013).

2. Published Studies Conducted on Various Preparations of Water Lentils

a. Studies in Poultry

It has been suggested that dried water lentils may be useful in poultry feed due to the quality of protein present at high levels and its high xanthophyll content, especially in regions which are conducive to growing this water plant (Haustein et al., 1990, 1992, 1994). The levels of the essential amino acids surpass the FAO reference pattern, except for methionine, which met 61.4% of the recommended value (Mbagwu and Adeniji, 1988). The protein from various species of water lentils has comparable amino acid compositions to L. minor.

Several studies have examined the effects of inclusion of preparations of water lentils from various biological and cultivation sources in poultry diets, primarily as a substitute for standard meals used as protein sources (Goopy and Murray, 2003; Haustein et al., 1990, 1992; O'Neill et aI., 1996).

The first publication by the research group led by Haustein (Haustein et al., 1990) demonstrated that birds (layers and broilers) have high growth rates when fed on high levels (200 g/kg) of Lemna gibba. Three studies were conducted. In the first study, one hundred and fifty 43-week old Topaz layers were divided into three treatment groups. The birds were acclimated for 2 weeks and then started on one of three test diets: control (0% Lemna gibba), 15% Lemna gibba or 15% Wolffia arrhiza. The water lentils were manually harvested, sundried to approximately 40% moisture, dried to 10% total moisture in a forced air oven and then stored in bags until used. The dried water lentils were ground prior to preparation of the diets. All diets used in the study were isonitrogenous (17% crude protein) and isocaloric (2,800 metabolizable energy (ME) kcal/kg). The base diets contained ground yellow corn, wheat middling, fishmeal, fish oil, limestone, dicalcium phosphate and premix. The control diet also contained soybean meal and iodized salt. The birds were on the experimental diets for a total of 90 days following a 14-day adaptation period, during which time all birds received the control diet and no experimental data were collected. The group with the Wolffia had a shorter period due to limited supply of this water lentil species. Feed and water were supplied ad libitum. Egg production, feed consumption, feed conversion, mean egg weight, mean weight gain, number of eggs per hen per week, and yolk pigmentation were measured. There was no significant difference in egg production, feed conversion, egg weight, egg number or mean egg weights between the experimental groups and the control.

In a second experiment in Haustein et al. (1990), 100 of the birds from the first experiment were used in a two-month feeding study that included a control diet and treatment diets that contained 25% Lemna gibba (2800 ME kcal/kg), 25% Lemna with a higher ME (2900 kcal/kg), and 40% Lemna (2800 ME kcal/kg). Egg production, feed consumption, feed conversion, mean egg weight, mean weight gain, number of eggs per hen per week, and yolk pigmentation were measured. There was no significant difference between the control and the 25% Lemna diets in any of the measured variables. Higher levels of Lemna in the diet produced significant incremental positive changes in yolk pigmentation.

In a third experiment in Haustein et al. (1990), two hundred 41-week old Leghorn Hyline hens were fed isocaloric diets consisting of 0% Lemna gibba (control), 15% L. gibba, and 25% L. gibba, (ME of all diets was 2800 kcal/kg) for three months, including a two-week adaptation period prior to data collection. Egg production, feed consumption, feed conversion, mean egg weight, mean weight gain, number of eggs per hen per week, and yolk pigmentation were measured. There was no significant difference in feed production, feed conversion, and mean egg weights between the control and any of the Lemna diets. There was a significant decrease in egg production in the 25% Lemna diet compared to the control after 10 weeks, but no significant difference in egg production between the 15% Lemna diet and the control after 10 weeks. There was a decrease in egg number and total egg weight in the 25% Lemna group as compared to the control group after 10 weeks. There was a significant increase in yolk pigmentation with an increase in Lemna in the diet. The authors conclude that using sewage-grown water lentils can successfully be used at a protein source for layer hens. The optimal level of Lemna in a diet for chickens was 15%, but even at a level of 40%, egg quality was not affected, and egg production was only affected in one of two periods.

In a Haustein et al. (1994) study, 96 Titan broiler chickens were fed standard diet for a 28-day pre-experimental period, after which they were randomly divided into groups and fed diets containing water lentils (Lemna gibba) in proportions of 0, 10, 15, and 25% for 3 weeks. All diets contained 12.1 mJ ME/kg and equal protein content was maintained across treatments by varying amounts of wheat middling, yellow corn, soybean meal and fishmeal. Lysine, methionine, cysteine, and calcium were kept constant across all treatments via supplementation. There was no significant difference in weight gain of broilers fed up to 15% in comparison to birds fed no water lentils in their diet. There was a significant decrease in growth rate in the 25% water lentils diet group in comparison to the chickens in the 15% water lentils diet group. Feed consumption was significantly lower in chickens fed 25% water lentils diet compared to all other treatment groups.

In a second study (Haustein et al. 1994), roughly 390 21-day old Titan and Arbor Acres broiler chickens were grouped by sex and breed and fed diets either containing 0 or 5% water lentils (Lemna gibba) for 28 days. Prior to experimental diets, birds were fed a standard diet for 3 weeks. Using the same methods as first experiment in Haustein et al. (1994), proteins, metabolizable energy and amino acids were maintained at a constant for the control and treatment groups. Female birds (Titan and Arbor Acres) fed diets containing 5% water lentils showed significantly increased final weights when compared to controls, but the same relationship was not demonstrated for male birds in the study.

A study conducted by Chantiratikul et al (2010), compared the effect of replacing the protein from soybean meal with protein from Wolffia Meal (Wolffia globosa L.) in the diet of laying hens. The effects on performance and egg production were investigated. A total of 180 Rohman laying hens, 71 weeks old, were allocated into 5 groups. The 5 treatment diets were a control diet (100% of protein from Soybean meal), and then 4 diets with 25%, 50%, 75% and 100% of the crude protein from the soybean meal replaced with crude protein from the Wolffia meal (crude protein content was 29.61 g/100 g dry matter). The fresh Wolffia meal was purchased, dried in the sunlight for 1-2 days, ground through a 2-mm screen and stored in airtight bags. The lysine and methionine content of the Wolffia meal was 5.00 and 1.45 g/100g crude protein, respectively and it was lower in crude protein, lysine, isoleucine, histidine and arginine but comparable in the remaining amino acids examined as compared to the soybean meal. All diets met the nutrient requirements of laying hens and all diets were supplied ad libitum for 8 weeks. Feed consumption and egg production were recorded daily and the feed conversion rate was calculated as kilograms of feed consumed per kilogram of eggs produced. The hens were weighed at the beginning and end of the study to determine BW change. Eight eggs from each group/week were used to determine egg weight, yolk color, Haugh units and eggshell thickness. Daily feed and metabolizable energy intake was significantly reduced when the crude protein was from Wolffia meal. However, this did not significantly affect the crude protein intake and feed conversion rate per kg of eggs. The complete replacement of crude protein from soybean with that of Wolffia meal significantly decreased egg production but the egg weight; Haugh units and eggshell thickness were not affected. Egg yolk color was significantly increased with the replacement of soybean meal with Wolffia meal. The results of this study showed that egg production was negatively affected; however, egg quality was not when Wolffia meal was used as a protein substitute. The authors concluded that 75% of the crude protein from soybean meal could be replaced with the crude protein from Wolffia meal in the diet of laying hens.

Samnang (1999) conducted a study to compare the effect of fresh water lentils (species not specified), soya bean meal and broken rice on the live weight growth in scavenging chickens. One hundred and twenty native chickens (mixed sexes) were allocated to 6 treatment groups (three treatments in both of two locations, an experimental station and a farm). Birds scavenged for 11 hours/day prior to being individually penned for the evening and offered the supplements during this time. During the day, the animals scavenged in areas with fruit trees, a biodigester and water lentils ponds. The supplements offered were either 50 g broken rice + 50 g water lentils, 50 g broken rice + 50 g soya beans or 50 g broken rice. The BWs of the chickens were taken every 14 days and the feed offered/refused was measured each morning. Mean feed intakes were calculated for the successive 14-day periods in the study and feed samples were collected every 14 days and analyzed for dry matter and nitrogen. The average amount of broken rice consumed was similar across groups and locations. The average amounts of fresh water lentils were 36 ± 0.35 and 49 ± 0.22 g/day and of ground soya bean were 27 ± 0.35 and 28 ± 0.22 g/day for the station and farm, respectively. There was a large variation in the protein intake between the two supplement groups. The protein content in the dry matter of water lentil was 27% as compared to the 38% in the soya beans and this, along with the much lower dry matter content of the water lentils (6% versus 90% for the soya bean) contributed to the lower intake of protein in the groups receiving water lentils. There were significant differences in final live weight between supplements and locations. The best growth occurred in the group receiving broken rice and soya beans and the worst for those receiving only broken rice. The relative ranking for performance between water lentils and soya bean differed according to location. At the experimental station, the soya bean supplemented chickens grew faster whereas on the farm those receiving water lentils grew faster. The authors concluded that offering 30-40 g/day of fresh water lentils to scavenging chickens improved their growth rate when they had access to broken rice as well.

Kusina et al. (1999) reported that supplementation of water lentils at a 10% (w/w) level in the feed did not affect the growth performance or carcass composition of the broiler chicks. In this study, 160 male 3-wk old broiler chickens were fed one of 4 diets (40 chicks per groups): control containing no water lentils, or 10, 20, or 30% water lentils in the diet. The authors concluded that water lentils could be incorporated into broiler finisher diets up to 10% without affecting growth rate, feed conversion efficiency, or carcass composition.

Islam et al. (1997) conducted an experiment to investigate the effects of replacing fishmeal protein with water lentils and soybean meal in diet for broilers. One hundred and twelve 7-day-old broiler chicks were divided into 4 groups, and given different isoenergetic and isonitrogenous diets. Fresh water lentils (Lemna minor) was collected, dried in the sun to residual moisture content of 10%, and then ground for use in the diets. The control diet was a commercial broiler diet and contained 12% fishmeal with no water lentils or soybean meal. The fishmeal protein was replaced with varying levels of water lentils and soybean meal in the other three diets as follows: 3% water lentils with 13.5% soybean meal; 6% water lentils with 11.5% soybean meal; and 9% water lentils with 10% soybean meal. Daily feed consumption, weekly BW gain, water consumption, mortality, and production cost was recorded. At the end of the 49-day test period, four males and four females from each group were slaughtered to determine the dressing yield, and breast muscle was taken for analysis of chemical composition. The broilers in the control diet had higher feed consumption, higher live weight gain, and better feed efficiency than the broilers on all other diets. The broilers on the 3% and 6% water lentils diets had significantly greater feed intake, live weight gain, and feed conversion efficiency compared to those on the 9% water lentils diet. The growth of the broilers was best with the control diet, followed by the 3%, 6%, and 9% water lentils diets. Survivability was similar across all treatment groups. The broilers in the control group had significantly higher average carcass weights compared to all other groups; however, the dressing yields were similar for all groups but tended to decrease as the proportion of water lentils and soybean meal in the diet increased. The chemical composition of the breast meat was not significantly influenced by the composition of the diet. It was concluded that Lemna minor, together with soybean meal, can be used at up to 9% of a boiler ration with no adverse effects on chick performance (Islam et al., 1997).

In a 16-week feeding trial, 42 Cross Star laying pullets of similar age and same genetic origin were fed of sun-dried water lentils (Lemna minor) at 0, 50, 70, 110, 130 and 150 g/kg for 16 weeks. All diets were isocaloric and isonitrogenous with equal amount of critical amino acids. BW, feed consumption, hen-day egg production, change in BW, average egg count, egg mass output, and feed efficiency were measured. There was no significant difference in BW or egg weight between the treatments and control. Feed consumption was significantly lower in the 130 and 150 g/kg water lentil treatments as compared to the control. Egg mass in the 150 g/kg treatment was significantly lower than the control, while none of the other treatments was significantly different from the control. The authors conclude that including Lemna minor up to 150 g/kg in the diet had no adverse effect on the birds (Akter et al, 2011).

b. Studies in Ducks

One hundred and sixty local ducklings were assigned to one of 6 treatments in a production function experiment to evaluate whether taro silage could replace water lentils and part of the rice bran in the diet of growing ducks without affecting their growth rate. Ducks selected at one day of age were fed a commercial feed for the first 30 days, after which they underwent a gradual adaptation to the experimental diets for one week prior to the experimental period. Water lentils (species not specified) was harvested from natural ponds and wilted overnight. Taro foliage was harvested from ponds, chopped into small pieces, and wilted for 3-4 hours under sunlight. Rice bran and water lentils were mixed and fed at dosages amounting to 70 g of dry matter/kg live weight. Following rice bran and water lentils consumption, Taro silage was offered at 0, 15, 30, 45, 60, and 75% of the dry matter of the diet. As the amount of taro leaf in the diet increased, the amounts of rice bran (67, 57.5, 48, 38.5, 29, and 19.5%, respectively) and water lentils decreased (31, 25.5, 20, 14.5, 9, and 3.5%, respectively). Feeds were given three times daily, and water was available ad libitum; feeds offered and refused were recorded daily. Ducks were weighed as a group, every 10 days throughout the 60-day experiment. The replacement of rice bran and water lentils with taro silage led to a curvilinear decrease in dry matter intake and a decrease in the crude protein content of the dry matter consumed. However, improvements in live weight gain and dry matter feed conversion were also observed.

Therefore, the authors concluded that the taro silage was of a higher nutritive value compared to the combination of rice bran and water lentils (Ty et al., 2011).

Hamid et al. (1993) studied the effects of feeding diets containing Lemna trisulaca meal to growing ducklings. One hundred and twenty 4-week-old female growing ducklings were divided into 4 groups. The Lemna trisculaca meal (LTM) was prepared by collecting fresh Lemna trisulaca and allowing it to dry in the sun before it was ground to make the meal. Commercially available fishmeal was also used in the diets. On a dry matter basis, the protein content of LTM was 200 g/kg and the fishmeal contained 400-g/kg protein. The four test diets contained either 0, 80, 120, or 160 g LTM/kg, which replaced 0, 40, 60, and 80 g fishmeal/kg in the diet, respectively. The replacement was on a protein basis. The birds had ad libitum access to the test diets and water throughout the 16-week test period. BW, food supply, sexual maturity, and mortality were recorded and the information used to calculate weight gain, food consumption, food conversion efficiency, and viability. No significant differences were noted in BW gain between the control diet and the diets containing 80 and 120 g LTM /kg. In the diet containing 160 g LTM/kg, there was a significant reduction in weight gain as compared to all other groups. Food consumption in the three LTM groups was significantly lower as compared to control, but the food conversion efficiency was found to be comparable but slightly improved in the diets with 80 and 120 g LTM/kg. The inclusion of LTM in the diet of growing female ducklings had no effect on the age when sexual maturity occurred; however, the BW gain at sexual maturity in the group, which had 160 g LTM/kg, was significantly reduced as compared to all other groups. The authors concluded that LTM could feasibly be incorporated into the diet at levels between 80 and 120 g/kg.

Anh and Preston (1997) used a duckling growth assay to evaluate the protein quality of water lentils. Twenty-five, 5-day-old ducklings were divided into five groups and were studied for 12 days. The protein free basal diet consisted of brown sugar and cassava root meal (50:50 ratio). The water lentil species were supplied in fresh form at ratios (fresh basis: basal diet) of 1:1, 2:1, 3:1, 4:1, and 5:1, with a crude protein basis, Nitrogen x 6.25% in dry matter of 3.27, 5.17, 6.82, 8.26, and 9.54, respectively. The basal diet and water lentils were mixed together and offered ad libitum. Ducklings were individually weighed at the start of the study and then every four days, and the feed consumed was recorded daily. Samples of the diet were taken daily, bulked over 4 days and then refrigerated until the nitrogen level and dry matter could be determined. The live weight and daily gain of the ducklings at 17 days of age were linearly related to the crude protein from the water lentils (r2 = 0.927 and 0.93, respectively). The authors concluded that water lentils can be used as the sole source of protein in the diet for ducks.

Men et al. (1995) conducted an experiment to determine the effects of feeding water lentils as a replacement for roasted soya beans in crossbred meat ducks. Two hundred crossbred ducklings were divided into five groups and fed the test diets from 28 to 63 days of age. The diets were based on broken rice, and the control diet was supplemented with 27 g/day of roasted soya beans and no water lentils. The four other diets were the broken rice basal diet (ad libitum) along with 19, 15, 12, and 0 g/day of roasted soya beans and ad libitum fresh water lentils. No details were supplied regarding the harvesting and handling of the water lentils other than that it was supplied fresh. A premix with trace minerals was mixed into the control diet but not to other diets. The soya beans and water lentils had a crude protein basis, Nitrogen (N) x 6.25% of 44.0 and 38.6 as a % of dry matter, respectively. All groups readily consumed the water lentils and the intake of the broken rice diet was slightly depressed in the group fed 19 g/day soya beans, and slightly increased for the group with the diet containing no soya beans. The intake of water lentils increased as the amount of soya beans in the diet decreased. The total protein intake was highest in the group offered 19-g/day soya beans with the water lentils and lowest on the control and no soya bean diets. The rate of live weight gain was significantly higher on the diets supplying 19 and 15 g soya beans/day as compared to the control group, and the group receiving no soya beans showed slightly better growth as compared to the control group. The mean weights of the chest and thigh muscle tended to be higher on the control diet as compared to the other groups but there were no differences in the weights of the other components of the digestive tract, heart, and liver. The authors concluded that water lentils can completely replace roasted soya beans and a vitamin-mineral premix in a broken rice based diet for fattening ducks without negatively affecting growth performance and carcass traits. The poorer feed conversions seen in the diets containing water lentils were not considered important for economic reasons.

In another study by Men et al. (1996), one hundred and twenty 4-week-old local Muscovy ducks were divided into three groups (equal numbers of males and females) with males managed separately from the females. The ducks were fed a basal diet of broken rice and protein supplementation was provided using roasted soya beans to supply either 100% of the requirements, 40% of the requirements, or no soya beans. A salt and mineral/vitamin premix was added to the control diet only. The ducks were fed the experimental diets from 28 days of age to 70 days of age for the females, and to 84 days of age for the males. The diets and fresh water lentils (Lemna sp.) were fed ad libitum. The water lentils were harvested twice daily, washed with tap water, and drained for one hour before feeding. The soya beans and water lentils had a crude protein basis, N x 6.25%, of 44.0 and 38.6 as a % of dry matter, respectively. The ducks were weighed individually at the beginning of the study, at weekly intervals, and then again at the end of the trial. The consumption of water lentils increased as the level of soya beans decreased with males consuming more than females. For males, the live weights at the end of the study and the growth rates during the study were highest in the control group. No differences in the growth rates for either males or females were noted in the groups receiving 0 or 40% of their protein requirements from soya beans; however, feed dry matter conversion was poorer for all water lentils diets as compared to controls. There was a trend for decreased carcass yield on the water lentils diets that was not significant, and there were no differences in the weights of the chest and thigh muscle, heart, and liver. The authors were again evaluating the economic benefits as well as performance and carcass traits and concluded that water lentils can be used as a substitute for soya beans in the broken rice diets as a high-quality protein source.

Men et al. (2001) investigated the effects of replacing ground, roasted soya beans (a commercial protein supplement) in the diets of crossbred meat ducks (female Pekins ×

Cherry Valley males from Czechoslovakia) with fresh water lentils (Lemna minor) by evaluating the physical traits and organ weights of the ducks. Two hundred, 4-week-old ducklings were randomly allocated to one of 5 dietary treatments with 4 replicates/diet and 10 ducklings (balanced for sex) per replicate. Initial weights of the ducklings were between 830 and 860 g. The ducklings received the diets from 28 to 63 days of age. Broken rice was offered ad libitum (2× daily) in all diets, while roasted soya beans were offered ad libitum (2× daily) to balance the protein in four of the diets, at doses of 30, 21, 16, and 12 g/day. Fresh water lentils (4.7% dry matter) was offered ad libitum (3× daily) in 4 of the diets, except for the control diet, which consisted of broken rice, 30 g/day of ground, roasted soya beans, and a vitamin/mineral complex.

Men et al. (2001) found that total dry matter intake on the water lentils diets was significantly higher than for the control diet, indicating a preference for the palatability of the water lentils. As the soya bean intake was restricted, water lentils consumption increased to a maximum of 566 g/day (fresh weight) for the rice/water lentils diet. The ducks met their energy requirements by increasing their water lentils consumption, as no significant differences in total metabolizable energy intake were observed. Feed conversion ratios were slightly higher for diets containing fresh water lentils. No significant differences were found for carcass yields, chest and thigh muscle weights, or internal organ weights between the control-fed ducks and the water lentils-fed ducks. It was concluded that fresh water lentils can completely replace ground, roasted soya beans and a vitamin/mineral premix in broken rice-based diets for crossbred duckling growth without any reductions in growth performance or carcass traits.

Men et al. (2002) conducted an experiment to determine the effects of using water lentils (Lemna minor) as a replacement for commercial protein supplements in the diets of local and Cherry Valley breeding ducks. One-day-old ducks (n=180) were selected and reared to the point of lay on a commercial diet up to 28 days of age, at which point they were fed restricted amounts of a grower diet up to six and seven months of age. Ducks were randomized to one of five treatments, with three replicates and six breeding ducks per replicate for both local and exotic Cherry Valley ducks. Local breeding ducks were fed the diets from 7 to 12 months of age; exotic breeding ducks (Cherry Valley) were fed the diets from 8 to 13 months of age. The five treatment diets consisted of rice by-products supplemented with roasted soya bean meal and dried fishmeal at levels of 100%, 75%, 50%, 25% and 0% of the protein in the control diet (corresponding to 18, 15, 13, 10, and 8% of the crude protein in the diets, respectively). Water lentils were grown on ponds enriched with nutrients from the Cantho University experimental pig farm wastewater, collected twice daily, rinsed, and dried. Diets and fresh water lentils were provided ad libitum for all treatments. Water lentils were offered in the morning, afternoon, and evening; refusals were collected and weighed to calculate actual intake. Laying rates, and daily intake of the concentrate and water lentils were measured as means of the group; the proportion of fertile eggs and hatchability were also calculated. Water lentils were found to have a dry matter content of 5.1%, of which 38.1% was crude protein. Local breeding ducks had a higher water lentils intake (DM intake per kg of BW) compared to Cherry Valley ducks (18.2 and 14.0 g, respectively). A reduction in daily crude protein intake was observed in both breeds as the soya bean and fishmeal levels were progressively reduced. There were no significant differences in the laying rates or hatchability of local ducks; however, in Cherry Valley ducks, laying rates and hatchability were significantly lower in ducks fed the diet without any protein supplement. The authors therefore concluded that fresh water lentils can replace protein supplements in the diets of local laying ducks without affecting reproductive performance. No adverse effects were reported (Men et al., 2002).

In a study by Ngamsaeng et al. (2004), a randomized trial was conducted in 24 Muscovy ducks (approximately 3 months of age) to evaluate the protein quality in water spinach (Ipomoea aquatic), water lentils (Lemna minor), or a mixture of water spinach and water lentils as the only supplement to a basal diet of broken rice for growing ducks. Ducks were weighed prior to the start of the experiment and every 5 days during the study. Ducks were randomly assigned to one of the three treatments, with two replicates per treatment. Diets consisted of 20% (on fresh basis) broken rice and 80% (on fresh basis) water spinach, water lentils, or a mixture of water spinach and water lentils (35:45). Ducks were fed twice daily ad libitum and water was freely available; feed offered and refused was recorded daily (Ngamsaeng et al., 2004). Total dry matter intake was significantly higher for water lentils (83.9 g/day) than for water spinach (55.9 g/day). Average daily gain was highest for ducks fed water lentils (22.4 g/day) and lowest for ducks fed spinach (6.2 g/day). Study results also showed a growth rate linear to total crude protein intake derived from the three different diets. Feed conversion was significantly higher for diets containing water lentils than for diets with water spinach. While this study did not compare the intake of water lentils to a common duck feed protein source, it did demonstrate that total DM intake, total crude protein intake, live weight gain, and feed conversion to be superior in ducks fed water lentils compared to those fed water spinach (Ngamsaeng et al., 2004).

c. Studies in Cattle

The chemical composition, and rate and extent of digestion of dry matter and crude protein of three water lentils genera (Spirodela, Lemna, and Wolffia, species not specified) in the rumen were evaluated in cattle. Three rumen cannulated bulls (317.0 +/- 15.0 kg) were fed ad libitum a diet of straw and green grasses supplemented with a concentrate mixture containing 279 g water lentils per kg. The three genera of water lentils that were used were Spirodela, Lemna, and Wolffia. Feeds, refusals, and fecal samples were analyzed to determine chemical composition and rumen degradation characteristics of the three water lentils genera. On average, water lentils were consumed at a rate of 10% of live weight. The average daily weight gain of the bulls was 1,135 g/day. The degradable fractions of dry matter were measured as 823 g/kg for Wolffia, 712 g/kg for Spirodela, and 426 g/kg for Lemna. The rate of degradation of dry matter was 5.73 %/h for Wolffia, 2.22 %/h for Spirodela, and 3.63 %/h for Lemna. The degradable fractions of crude protein were measured as 765 g/kg for Wolffia, 560 g/kg for Spirodela, and 452 g/kg for Lemna. The rate of degradation of crude protein was 6.05 %/h for Wolffia, 5.14%/h for Spirodela, and 4.22%/h for Lemna. The authors concluded that the dry matter and crude protein of the water lentils mixture were highly degradable in the rumen and therefore may be fed to cattle mixing with concentrates (Huque et al., 1996).

A cross-over study comparing alfalfa and Lemna minor meal was conducted in 36 multiparous Holstein and crossbred (Holstein x Montbeliard x Swedish Red) dairy cows wherein they were assigned to either the total mixed ration (TMR) control (with alfalfa pellets) or treatment group (with Lemna meal pellets). Cows underwent a 7-day adaptation period prior to the experimental period for adaption to the diet. Treatment periods were 21 days in length. On a dry matter basis, 25% of either alfalfa or Lemna meal was used to produce pellets of equal nutrient composition. Alfalfa and Lemna meal pellets replaced 22% of the dry matter in the TMR. Diets were provided ad libitum, and contained adequate net energy for lactation and metabolizable protein for a cow weighing 650 kg and producing 40 kg of milk with a fat concentration of 3.5%. The control and test diets were isocaloric, isonitrogenous, and contained the same amount of forage neutral detergent fiber. Feed offered and feed refused were measured daily and recorded electronically. Cows were milked twice daily (0100h and 1300h) and fed once daily (1000h). Milk weights were recorded daily during each period, and samples were obtained from consecutive a.m. and p.m. milkings weekly (Litherland et al., 2011).

Dry matter intake (DMI) did not differ between treatments, nor did DMI expressed as percentage of body weight. Milk yields and fat-corrected milk values were similar among treatments. Fat concentration and yield were not different among treatments. The authors hypothesized that lower than anticipated levels of milk fat concentration observed in both treatment groups may have been due to the physical form or composition of the diets, which may have affected rumination time, rate of passage, and digestibility. The low fat-yield gave rise to a low fat: protein ratio; however, this ratio was similar between the two treatments. Nutritional composition of milk did not differ among treatments. Polyunsaturated fatty acids in milk were low and identical between treatments. Overall, this experiment demonstrated that cows fed diets containing 5.5% of diet dry matter, as alfalfa or Lemna meal, result in similar dry matter intake and milk yield (Litherland et al., 2011).

A study was conducted to determine if feeding Lemna meal pellets (25.7% of diet DM) either as a total mixed ration (TMR) or top-dress alters dry matter intake (DMI), growth or feeding behavior compared with a control TMR with alfalfa hay. Thirty-six Holstein and Swedish Red-Montbeliard cross-bred heifers were divided into nine pens with four heifers per pen for the 30-d study. Pens were balanced by age (166.3 ± 7.4 d) and body weight (BW) (BW = 205.0 ± 1.6 Kg). The three treatments were; TMR with Lemna pellets (LTMR); Top-dressed Lemna pellets (TTMR); (14.7% CP; 43.3%NDF; 0.43 Mcal/Kg NEg) Alfalfa hay TMR (ATMR) (15.2% CP; 43.4%NDF; 0.40 Mcal/Kg NEg). LTMR and TTMR were identical except for method of feeding Lemna pellets. Calves were adapted to the diets for five d prior to data collection. Total gain 22.9, 21.6, 19.7 ± 1.6 Kg/d and average daily gain (ADG) (0.79, 0.74, 0.68 ± 0.05 kg/d) were similar among treatments for LTMR, TTMR, ATMR. There was no difference among treatments for pen DMI 6.9, 6.7, 6.6 ± 1.8 Kg/d for LTMR, TTMR, ATMR. Feeding and ruminating behavior was observed once weekly for four h after feed delivery. Feeding time was higher ($P < 0.05$) for ATMR compared with LTMR or TTMR and averaged 110.3, 90.3, 79.2 ± 7.5 min/4 h respectively. Ruminating time was similar and averaged 56.9, 48.6, 50.6 ± 5.0 min/4 h for LTMR, TTMR, ATMR. Greater feeding time is likely due to the physical form of ATMR. Lemna Meal pellets are an attractive alternative to alfalfa meal for dairy heifers during the growing phase. Top dressing did not alter growth rates (McDonald et al. 2012).

d. Studies in Pigs

A study was carried out to confirm that sows can be fed with a lower protein level in combination with ensiled cassava roots, and to determine if water lentils (Lemna sp.) could be used to replace traditional protein sources. Sixteen local Baxuyen sows mated to Yorkshire boars by insemination were studied for two reproductive cycles. The basal diet consisted of ensiled cassava root during the first reproductive cycle and of cassava root meal during the second reproductive cycle. The 2*2 factorial design consisted of the following two factors: protein level during gestation and partial substitution of protein supplement by water lentils. During pregnancy, sows were given a protein supplement (50% fishmeal and 50% soya bean meal) at 150 or 200 g/day (treatments without water lentils) and 75 or 100 g/day (treatments with water lentils). During gestation, cassava roots were provided at 2.7 – 3.0 kg/day and ad libitum during lactation. Water lentils were fed at 2.7 kg/day during gestation and 4 kg/day during lactation. Water lentils significantly improved the measured traits (litter size, litter weight, and total feed dry matter during lactation per unit weight piglet); protein level, however, had no effect on these parameters. Overall, study results indicate that half the conventional protein sources (soybean meal and fishmeal) can be replaced with fresh water lentils and may improve the reproductive performance of sows (Men et al., 1997).

A randomized block design study consisting of two treatments and 3 replications was conducted in pigs to compare a diet based on ensiled cassava root to the conventional cereal-based diet, and to evaluate the effect of partially replacing conventional protein supplements with water lentils (Lemna spp). Twenty-four pigs (Yorkshire x Landrace-Baxuyen) were randomized to the control diet (60% broken rice, 33% rice bran, 5% fishmeal, and 2% soya bean meal) or a diet consisting of ensiled cassava root (69%), water lentils (8.6%) and supplement (22.4%) for 120 days. Pigs fed the traditional diet had slightly higher growth rates (5%) compared to the cassava and water lentils diet; however, conversion rates were similar for both treatments. Pigs fed the cassava and water lentils had thinner back-fat as compared to control pigs. No serious adverse events were reported. The authors concluded that a diet of ensiled cassava root with water lentils partially replacing the protein supplement can be used in place of the traditional diet used to fatten pigs in Vietnam (Van et al., 1996).

Ensiled cassava leaves and water lentils were evaluated as protein supplements for growing fattening pigs fed on local resources at smallholder farms in the Thuy Xuan village of Vietnam. Traditional feed consists of dried cassava root, rice bran, and brewery by-products and has a crude protein content of 9% of the dry matter. At each farm household, two pigs received the conventional diet, and two pigs received the conventional diet plus either fresh water lentils or ensiled cassava leaves. The fresh water lentils (Lemna minor) and cassava leaves replaced fresh sweet potato vines. Forty-four crossbred pigs (mainly Large White x Mong Cai), of about 3 months of age and 23-25 kg initial BW, were allocated to one group of 6 families (ensiled cassava) or one group of 5 families (water lentils). Each household housed 4 pigs. Pigs were fed thrice daily and feeds offered and refused were recorded for each meal. The pigs were weighed monthly. The mean values for the effects of supplementation on weight gain; back-fat thickness, and area of loin eye muscle were compared among treatments using ANOVA. Cassava leaves provided 9% of the dietary dry matter and 15% of the total protein; the sweet potato vines provided 9% of the control diet matter and 13.4% of the protein. Water lentils provided 5% of the dietary dry matter and 12.9% of the total protein; the sweet potato vines provided 7% of the control diet matter and 11.2% of the protein. Protein content of the dry matter was similar for both diets (Hang, 1998).

Study results indicated that fresh water lentils intake at an average of 1.5 kg/day had a stimulating effect on live weight gain (an increase of 37%). Compared to control, pigs fed water lentils were significantly heavier at the end of the trial, gained weight more rapidly, and had better feed conversion. At the end of the study, 6 pigs were slaughtered from each treatment group. There were no effects on the carcasses attributable to water lentils or cassava supplementation. Moreover, this study demonstrates that water lentils afforded a highly significant improvement in growth rate and feed conversion as a replacement of sweet potato vines in the traditional diet (Hang, 1998).

The ileal digestibility of the nutrients of three different macrophytes was compared in an experiment in pigs fed a non-conventional diet supplemented with protein and fiber. Nine 45-kg pigs underwent ilea-rectal anastomosis and were randomly assigned to one of three diets in a 3 x 3 Latin square design. Following an adaptation period, pigs were fed a basal diet (sugar cane molasses and soybean meal) supplemented with one of three macrophytes: water hyacinth (Eichhornia crassipes), water lentils (Lemna minor), or azolla (Azolla sp. A). Macrophytes were grown on liquid pig slurry, sun dried, milled, and progressively substituted in diets at 0, 100, or 200 g/kg of food as an alternative source of nitrogen. Pigs were fed a standard diet of sugar cane molasses type "B" and soya bean meal for one week prior to surgery, after which animals recovered for one week before being randomized to one of three macrophytes. Diets were offered twice daily, and water was given ad libitum. Dry matter, ash content, energy, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were compared among the three treatments. As water lentils have a high crude protein concentration, its inclusion increased the N content of the diets. Water lentils content did not affect gross energy concentration. Treatment with water lentils resulted in decreased dry matter and energy digestibility up to the terminal ileum. No effects on the digestibilities of organic matter, nitrogen, crude fiber and NDF were seen. No serious adverse events were reported with respect to treatment with water lentils (Dominguez et al., 1996).

A study was conducted in Mong Cai pigs to evaluate whether they would eat greater amounts of water lentils (Lemna minor) and use it more efficiently than exotic pigs (Large White), and to evaluate the use of water lentils as a source of supplementary protein in a low-protein basal diet of sugar cane juice. Four Mong Cai piglets, four Large White piglets, and four Mong Cai x Large white piglets were fed fresh sugar cane juice and fresh water lentils for an unspecified period of time. Water lentils were harvested from experimental ponds from a farm fertilized with biodigester effluent. For the first five days of the adaptation period, pigs were offered both cane juice and water lentils ad libitum; for the remainder of the experiment, pigs were fed sugar cane juice and water lentils in discrete meals. The juice was removed between meals and during the night, and water lentils were continuously available. Intake of sugar cane juice and water lentils were measured daily by weighing amounts offered and refused.

e. Studies in Sheep

Twelve Pelibuey male lambs were randomly assigned to three diets designed to evaluate the effect of supplemental water lentils on voluntary intake, in vivo digestibility, nitrogen balance, concentration of ruminal nitrogen, volatile fatty acids, and ruminal pH. Water lentils (Lemna sp.) were harvested daily, dried in a greenhouse, and used the following day. Taiwan grass was harvested and mixed with water lentils. The diets consisted of 100:0, 80:20, and 70:30 ratios of Taiwan grass: water lentils. The study consisted of three periods, wherein each experimental period consisted of 8 days of adaptation to the diet, 8 days for feces collection and rumen liquor sampling, 2 days for urine collection, with a total of 60 days for the whole experiment. The intake of water lentils decreased dry matter intake significantly, and significantly improved dry matter digestibility, organic matter digestibility, crude protein digestibility, neutral detergent fiber digestibility, acid detergent fiber digestibility, nitrogen retention, and ammonia nitrogen concentration. Minimal changes in the ratio of acetate: propionate and ruminal pH were observed. Overall, these findings indicate the safe intake of Lemna sp. by lambs (Zetina-Cordoba et al., 2013).

The use of water lentils as a dietary protein source for fine-wool Merino sheep was investigated to observe whether sheep would readily ingest water lentils, and to investigate its post-ingestion effects on live-weight gain and wool production. A 2-week pre-experimental phase was undergone wherein 36 Merino sheep (6 months of age) became accustomed to their environment and a diet of oaten chaff (600 g/day) and lucerne chaff (50 g/day). For the following 7 weeks, a diet of 50 g/day dried water lentils (Spirodela punctata) and 700 g/day oaten chaff was provided. Animals were then allocated to one of 4 groups (n=9) based on live weight, and mean fiber diameter of their wool. Group 1 was provided 700 g/day oaten chaff; Group 2 was provided 630 g/day of oaten chaff and 50 g/day sun-dried water lentils; Group 3 was provided 540 g/day of oaten chaff and 100 g/day sun-dried water lentils; Group 4 was provided 630 g/day of oaten chaff and 1000 g/day fresh water lentils. At the end of the experimental period (6 weeks), sheep were weighed and dye-bands were analyzed. There were no differences in the measures of wool (yield, rate of fiber elongation, or fiber diameter) between treatments. There were occasional refusals of feed during the pre-experimental period when fresh water lentils were mixed in the diet; the amounts refused were small, and the sheep became accustomed to the diets within a matter of a few days. Water lentils were completely ingested during the experimental period. No clinically ill effects associated with water lentils intake were observed (Damry et al., 2001).

Damry et al. (2001) conducted a second experiment to evaluate the effects of water lentils ingestion on the yield and characteristics of wool with 21 of the 36 sheep, drawn from the previous experiment. During the pre-experimental phase, sheep were provided 800 g/day of oaten chaff and 50 g/day of lucerne chaff mixed with 8 g/day of urea. Sheep were then allocated to 3 isonitrogenous groups of 7 sheep based on mean wool fiber diameter and live weight at the end of the previous experiment. The three diets provided 800 g/day oaten chaff plus 8 g/day urea, 800 g/day oaten chaff plus 60 g/day cottonseed meal, and 800 g/day oaten chaff plus 100 g/day sun-dried water lentils. Dye-bands were used to analyze the effects of the diets on the characteristics of wool. The distances between dye-bands were used to calculate the length of wool grown during the different periods of the experiment. The water lentils and cottonseed meal diets resulted in a greater rate of wool fiber elongation compared to the diet containing urea. Fiber diameter and volume of wool produced were lower for the diet containing urea compared to the water lentils and cottonseed meal diets. As in the previous experiment, no clinically-ill effects associated with water lentils intake were observed. Together, these two experiments demonstrate that fresh and sun-dried water lentils were well accepted by Merino sheep and had a comparable effect to that of cottonseed meal on wool production. (Damry et al., 2001).

f. Studies in Rats

Hanczakowski et al. (1995) determined the nutritive value of dried water lentils (Lemna minor L.) in albino rats (strain not specified) by measuring true digestibility and biological value of proteins using the Thomas-Mitchell balance method. Net protein utilization (NPU) was calculated based on true digestibility (TD) and biological value (BV) [NPU= (TD x BV)/l00]. Two groups of rats, one for each sample of water lentils, were used. Six male 40-day-old albino rats weighing about 90 g received 1 g protein/d from 10 g of fresh food (9.2-9.4 g protein, DM basis) for 5 d. Diets also contained 20% sucrose, 6% soybean oil, cellulose, and mineral and vitamin mixtures. Wheat starch was used to complete the diet composition. The nutritive value of water lentils protein was found to be comparable to that of meat and bone meal (NPU= 25-33; TD=56.5; BV=50), but lower than that of soybean meal (NPU=49). The authors concluded that water lentils could be a source of dietary protein. No adverse effects were reported (Hanczakowski et al., 1995).

Dewanji and Matai (1996) evaluated the nutritional quality of the proteins extracted from the leaves of plants, including Lemna minor, in rat diets. Plant samples were collected, washed, drained, and hand fed into a specialty pulper. The pulper separated the outflow discharge from the juice of the pulped material. The protein in the juice was precipitated using steam injection; the subsequent protein coagulum was filtered, washed, pressed, and freeze-dried. The six experimental diets were prepared with 10% crude protein: a wheat flour diet; three diets of wheat flour supplemented with one of three different leaf proteins

(Alternanthera philoxeroides, Lemna minor, and Pistia stratiotes); one leaf protein diet; and one reference diet using casein. In the leaf protein diets, the leaf protein supplied 3% of the crude protein and the other 7% was from the wheat. Vitamins, salt, oil, cellulose and starch concentrations were kept constant across all diets. Forty- eight weanling albino Wistar rats were divided into 6 groups and housed individually. Water and food were given ad libitum for the 28-day experimental period. Food intake was recorded daily and weight gain recorded weekly.

The protein efficiency ratio (PER) was calculated at the end of the 28-day period. Feces were collected daily during the final week and used to calculate the apparent digestibility. The animals were euthanized at the end of the study period and blood samples were collected. Serum was analyzed for total protein and albumin content. The liver was removed, weighed, dried to a constant weight, ground, and analyzed for nitrogen content. Higher weight gain and improved PER were observed with the addition of L. minor leaf protein as compared to the wheat flour-fed group. In the group fed with L. minor supplemented diet, there was an increase in liver weight and nitrogen content as compared to the wheat flour-fed group. Serum protein and serum albumin were lower when compared to the casein group but did not differ between the leaf protein supplemented groups. The addition of leaf protein from L. minor significantly improved the protein quality of the diet as compared to the wheat flour diet. The authors suggest that leaf protein extracted from L. minor could be used as a supplement for traditional cereal-based feeds to help alleviate nutritional deficiencies (Dewanji and Matai, 1996).

Phuc et al. 2001 investigated the nutritive effects of eight crop products, including Lemna minor L., as replacements of casein protein in the diet of rats. A total of 102 male Wistar rats were randomly divided into three blocks of 34 rats each, with two rats allocated to each of the 17 experimental diets in each of the blocks, resulting in a total of 6 rats given each experimental diet. Diets consisted of maize starch, sucrose, cellulose, minerals and vitamins, and a sun-dried plant protein source from various species including Lemna minor L. In the experimental diets, either 0, 25, or 50% of the crude protein (as casein) was replaced with the plant protein source. Diets were calculated to be isocaloric and isonitrogenous, rats were fed 10 g air dry feed and 150 mg N once a day and water was available ad libitum for 4 days. Food intake, weight gain, digestibility, and nitrogen utilization were measured. There was no significant difference in food intake of L. minor in either the 25% or 50% treatments as compared to the casein control. There was no significant difference in weight gain between the 25% L. minor treatment and the control, but the 50% L. minor treatment resulted in a significant decrease in weight gain compared to the control. There was a significant decrease in digestibility of crude protein and in nitrogen utilization in the 25% and 50% L. minor treatments compared to the casein control. The authors conclude that the occurrence of anti-nutritive components may influence digestion and metabolism, and that crude protein digestibility and the amino acid profile may affect crude protein utilization. However, they also state that L. minor, along with a few other species, show promise for inclusion in diets of monogastric animals.

g. Studies in Fish

Fishmeal is the major source of protein for farmed fish worldwide and is in limited supply (Goopy and Murray, 2003). Fresh water lentil (Lemnaceae) is a good food source for tilapia as it contains about 35–45% CP with excellent amino acid and mineral profiles (El-Sayed, 1999; Mbagwu et al., 1990).

In a study by Hassan and Edwards (1992), tilapia in static-water concrete tanks were fed two species of water lentils, Lemna perpusilla and Spirodela polyrrhiza, at levels of 0, 10, 20, 30, 40, 50, or 60 g water lentils dry matter (DM) per kg wet weight of fish. The water lentils (approximately 24% crude protein) produced a linear increase in weight gain and improvements in feed conversion efficiencies when they included Lemna perpusilla and Spirodela polyrrhiza at up to 30 g DM/kg in the diet of Nile tilapia (Oreochromis niloticus). At higher water lentils level, weight gain decreased, food conversion efficiencies decreased, and there was a significant increase in mortality. The Spirodela was poorly consumed, whereas fish rapidly ingested Lemna. The authors concluded that water lentils were used very efficiently by tilapia and carp up to 30 g DM/ kg wet weight of fish.

Similarly, a study examining the culture of tilapia fed varying levels of S. polyrrhiza found that weight gain and food conversion ratios were unchanged from the control group at inclusion rates up to 30%, but above this level, growth rates were impaired (Fasakin et al., 1999).

Gaigher et al. (1984) evaluated the use of water lentils (Lemna gibba) as feed for tilapia (Oreochromis niloticus × O. aureus) in a recirculating unit. A total of 141 tilapias in three tanks were fed either only water lentils once a day or a combination of water lentils once a day and commercial pellets twice a day for 89 days. Intake rate, feed conversion, growth rate, and assimilation efficiency for each tank were calculated. When fed water lentils alone, 65% of the water lentils consumed was assimilated and 26% was converted to fish. When tilapias were fed by water lentils and commercial pellets, the rate of water lentil consumption decreased compared to the treatment of water lentils alone; 70% of the diet was assimilated, 21% was converted to fish, and there was a higher relative growth rate (1.46%) compared to the diet of water lentils alone. Therefore, assimilation efficiency was similar between the two treatments, but intake rate and growth rate were low in the sole water lentil treatment group; crude protein assimilation percentages and conversion efficiencies were similar on both diets. The authors conclude that water lentils could replace traditional feed for tilapia culture; and that adding water lentils to the diet of tilapia in intensive farms could help reduce cost of commercial fish pellet feed.

In a study by Bairagi et al. (2002), 8 isonitrogenous and isocaloric diets containing water lentils (Lemna polyrhiza) were fed to rohu (Labeo rohita) fingerlings for 80 days. Treatment diets included raw and fermented water lentil meal at 10, 20, 30, or 40% of diet and were compared to a fishmeal based reference diet. Weight gain and feed intake were measured, while fish body composition (upon termination of the experiment) was determined for moisture, crude protein, crude lipid, and ash. Results indicated that the diet containing 30% fermented Lemna leaf meal resulted in the highest growth response, food conversion ratio, and protein efficiency ratio. In general, fish had higher percent weight gain and specific growth rate when fed fermented water lentils in comparison to raw water lentils. The authors conclude that up to 30% Lemna leaf meal can be used in diets for L. rohita fingerlings without adverse effects impacting safety.

Thy et al. (2008) evaluated water lentils as fish feed for tilapia (Oreochromis niloticus), common carp (Cyprinus carpio) and mrigal (Cirrhinus mrigal) for 120 days. Treatments were effluent (E), effluent plus water spinach (EWS), or effluent plus water lentils (EDW) for 12 ponds. Effluent was applied at a level of 120 kg N/ha, and at 3 to 5% water spinach and water lentils dry matter of fish BW were given daily. A total of 360 fingerlings were used and composition of fish in each pond was 40, 35 and 25% for tilapia, common carp and mrigal, respectively. The survival rate was similar for all species, ranging from 88.3 to 92.4%. However, compared to 2 other feeds, survival rate was lower by 20% in ponds fed water lentils (80 vs. 96%). The daily weight gain was significantly higher with water lentils. The authors concluded that water lentils and water spinach can be used as a supplement for polyculture fish, but the fish also showed better growth with the water lentils supplement.

Other researchers also reported that water lentils are as safe to use as fishmeal (El-Sayed, 1999). The literature review by El-Sayed (1999) reported the following: 1) Mbagwu et al. (1990) found that when fingerling *Sarotherodon galilaeus* were fed with a 33% crude protein diet containing water lentils as a partial protein source, they exhibited better growth and feed utilization than those fed a 40% crude protein standard diet; 2) Similarly, Arrivillaga (1994) and Essa (1997) reported that *Wolffia* and *Lemna*, respectively, replaced up to 50% of commercial feeds (35% crude protein) of Nile tilapia without adverse effects on fish growth or body composition; 3) Appler (1985) found that up to 20% of fishmeal could be replaced by another aquatic plant, *Hydrodictyon reticulatum*, in diets fed to *Oreochromis. Niloticus* and *Tilapia zillii* without adverse effects on fish growth; 4) Chiayvareesajja et al. (1990) fed moist diets containing dry coontail (*Ceratophyllum demersum*), rice bran, and FM at ratios of 4:3:1 and 4:2:2 to Nile tilapia (mean weight 88–111 g) reared in floating cages for 90 days. They found that the 4:3:1 ratio was most appropriate; 5) Essa (1997) found that up to 25% of commercial Nile tilapia feed (35% crude protein) could be replaced with *Potomogeton*, *Pectinatus* or *C. demersum*; and 6) in another study (Okeyo et al., 1988), 3 levels (20, 30, and 40%) of coontail and chuut-nuu (*Eleocharis ochrostachys*) were included in Nile tilapia diets at 3 dietary protein levels (16, 25, and 35%) for 11 wk. At the same protein level, fish fed plant diets grew at similar rates, while the growth rose with an increasing dietary protein level. A diet containing either plant at 35% crude protein produced the best performance and least cost/kg fish produced (Klinnavee et al., 1990).

A study by Abdelhamid et al. (2010) evaluated water lentil meal as a feed for Nile tilapia (Oreochromis niloticus) fingerlings. A total of 182 fingerlings in 26 aquaria were fed twice daily at 3% of BW for 16 weeks. Diets were isonitrogenous and isocaloric and were a control diet of fishmeal, or diets where fishmeal was replaced by 25, 50, 75, and 100% of either water lentils meal, crayfish meal, or a 1:1 combination of water lentils and crayfish meal. Dry matter, crude protein, ether extract, ash, crude fiber, and nitrogen free extract were analyzed for diet and fish body at the beginning and end of the experiment; gross energy, protein/energy ratio, and metabolic energy were calculated. The highest values of final BW, BW gain, daily BW gain and relative growth rates were seen in the treatment group with the diet of 25% crayfish and the diet of 50% crayfish/water lentils combination. There was no significant difference in specific growth rate or percent survival in the water lentils groups as compared to the control. Relative growth rate among fish in all water lentils treatments was significantly lower than the control group. The authors conclude that fishmeal could be replaced with either 25% crayfish meal or 50% mixture of crayfish meal and water lentils meal.

Van Dyke and Sutton (1977) explored the digestion of water lentils by the grass carp. Fourteen three-year-old carp (Ctenopharyngodon idella Val.) were used in the study, with four to nine individuals at a time being fed a mixture of Lemna minor and Lemna gibba. Feces were collected and dried along with dried samples of water lentils, which were analyzed for gross energy, dry matter, crude protein, neutral detergent fiber, acid detergent fiber and lignin. Apparent digestibilities were 80%, 61%, and 58% for crude protein, gross energy, and organic matter, respectively. The authors conclude that grass carp digested water lentils effectively.

B. Safety of Protein Intake

The proteins found in most human diets are derived from animal, vegetable, or microbial sources. The Institute of Medicine designated the average minimum requirement of good-quality protein to be about 0.6 g/kg BW/day in normal healthy adults, but wide variations above this level have been compatible with good health (IOM, 2002/2005). It is possible to live healthily on a diet entirely of meat as long as the meat contains enough fat so the protein levels constitute less than 40% of the consumed energy. The Institute of Medicine defines an acceptable range of protein intake for adults as 10-35% of energy intake (IOM, 2002/2005). In addition, the Institute maintains that there is insufficient evidence to support the hypothesis that diets high in protein are associated with health conditions such as osteoporosis and cardiovascular disease. However, some investigators such as Eisenstein et al. (2002) state that the consumption of protein greater than two to three times the US Recommended Daily Allowance (more than 20-30% of energy intake) contributes to bone loss and urinary calcium loss, and individuals who are predisposed to nephrolithiasis or kidney disease should use caution with high protein intake.

On the other hand, insufficient dietary protein intake has been associated with adverse effects in human health and development. In 2005, IOM set a Recommended Dietary Allowance (RDA) value for protein of 0.8 g/kg BW in adult males and females (IOM, 2005). An adequate intake (AI) for infants aged 0 to 6 months was set at 1.52 g/kg BW/day. IOM concluded that there were insufficient data to set Tolerable Upper Intake Levels (UL) for total protein or individual amino acids.

Enriched plant proteins are available commercially in the form of protein concentrates and protein isolates. Enrichment of the raw material results from the extraction of the protein constituents (protein concentrate) or from extract and further subsequent separation (protein isolate) (Belitz et al., 2009). In a scientific opinion on safety of alfalfa protein concentrate containing 45-60% protein (EFSA, 2009a), the Panel concluded that the use of alfalfa protein concentrate as a food supplement at the proposed use level of 10 g per day is of no safety concern. The Panel notes that people may consume up to 2.2 g protein/kg BW per day, of which a significant part may come from rapeseed protein. In a recent scientific opinion on safety of rapeseed protein isolate, EFSA (2013) concluded that rapeseed protein isolate (90%) is safe.

There is a long history of recognized GRAS status for several types of protein preparations, including concentrates and hydrolysates, derived from a wide variety of sources including plants, fungi, fish, poultry, dairy, yeast, pork, and beef. Based on available information from FDA's GRAS notice inventory[1] website as of December 4, 2014, the agency has written 17 "no questions" letters on GRAS notices on protein preparations. A summary of these filings is presented in Table 29. Of these, 5 notices which are detailed in Table 30 are from aquatic sources. Table 11 compares the typical amino acid profile of these aquatic-sourced proteins to LC and DGLC and the protein profile of LC and DGLC is comparable to the protein sources from algae, Arthrospira platensis (Spirulina), Chlorella protothecoides (green algae), and Dunaliella bardawil (green algae). Chacon-Lee and Marino (2010) state that microalgae such as spirulina, Chlorella vulgaris and Dunaliella salina, when properly processed, have been shown to be safe with no known negative effects, based on human studies; however, FDA noted that, in certain cases, the ingredient might require a color additive listing.

Table 29. FDA's GRAS Notice Inventory on Protein Preparations [a]

Company	FDA GRAS Identifier	Protein Source	Intended Food Uses
Manildra Group	GRN 026	Wheat	Food emulsifier, foam stabilizer; thickening agent, etc.
American Dairy Products Institute (ADPI)	GRN 037	Whey	Food emulsifier, foam stabilizer; thickening agent, etc.
Avebe	GRN 086	Potato	Food emulsifier, foam stabilizer; thickening agent, etc.
Marlow Foods, Ltd.	GRN 091	*Fusarium venenatum* Fungus	Protein
Kyowa Hakko	GRN 134	Soy	Food Ingredient

[1] Last updated on 8/3130/2014. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices (Accessed 12/4/14).

Company	FDA GRAS Identifier	Protein Source	Intended Food Uses
Proteus Industries, Inc.	GRN 147	Fish	Protein
Proteus Industries, Inc.	GRN 168	Poultry	Protein
Martin Vialatte	GRN 182	Pea	Wine making
Snow Brand Milk Products Co., Ltd.	GRN 196	Bovine Milk	Protein
Calpis Co., Ltd.	GRN 199	Fermented or Enzyme Treated Milk	Food ingredient
DSM Food Specialties	GRN 284	Bakers Yeast	Wine making
Proteus Industries, Inc.	GRN 313	Beef	Water binding agent
Proteus Industries, Inc.	GRN 314	Pork	Water binding agent
Archer Daniels Midland Company	GRN 327	Canola/Rapeseed	Food Ingredient
Senmi Ekisu Co., Ltd.	GRN 360	Hydrolyzed Sardine	Food Ingredient
BioExx Specialty Proteins, Ltd.	GRN 386	Canola	Food ingredient
Solanic (Avebe)	GRN 447	Potato	Food ingredient & Protein

[a] GRN 504, addressing concentrated milk proteins, was submitted by American Dairy Products Institute/U.S. Dairy Export Council and filed by FDA on March 20, 2014, and is presently under review by FDA. GRN 519, addressing *Chlorella protothecoides* strain S106 flour with 40-75% protein, was submitted by Solazyme, Inc. and filed by FDA on June 2, 2014, and is presently under review by FDA.

Table 30. FDA's GRAS Notice Inventory on Aquatic Food Sources

Company	FDA GRAS Identifier	Source	Typical Composition
Cyanotech Corporation and Earthrise Nutritionals, Inc.	GRN 127	*Arthrospira platensis* (spirulina)	53-62% protein 17-25% carbohydrates 4-6% lipids 8-13% minerals 3-6% water
Nikken Sohonsha Corporation	GRN 351	*Dunaliella bardawil* (green algae)	<30% protein <50% carbohydrates <30% fat <6% moisture
RFI, Inc.	GRN 394	*Arthrospira platensis* (spirulina)	≥ 60% protein

Company	FDA GRAS Identifier	Source	Typical Composition
E.I.D. Parry (India) Ltd.	GRN 417	*Arthrospira platensis* (spirulina)	59-69% protein 15-25% carbohydrate 5-6% lipids 2.5-6% moisture
Solazyme Roquette Nutritionals, LLC	GRN 469	*Chlorella protothecoides* (green algae)	2-15% protein 40-70% lipids 10-50% fiber < 10% moisture

In addition, Parabel has received feedback from the Food Safety Authorities of Australia and New Zealand (FSANZ). On April 4th, 2017, the Advisory Committee for Novel Foods (ACNF) has concluded that Parabel's LENTEIN Complete although a non-traditional food in ANZ, is not considered to be a Novel Food under the ANZ Food Standard Codes, hence the product does not have to undergo a pre-market assessment (Refer to Appendix J). Parabel's LENTEIN Complete is considered compliant with the ANZ Food Standards code and therefore can be marketed as a Food Ingredient up to 24 grams per serving in the ANZ region.

C. Safety of Trace Element Content and Pesticides

Parabel produces LENTEIN Complete in modern hydroponic ponds where environmental contaminants such as pathogens and pesticides are not present and closely monitored (Parabel's LENTEIN Complete was analyzed using a full FDA pesticide screen and none were detected; refer to Appendix A4). Similarly, Parabel monitors the concentration of various metals in Lemnaceae and the final product. Residual levels of metals measured in LC and the levels of metals contributed by the consumption of 201 g of LC are shown in Table 31 and are compared to maximum intakes recommended by expert bodies or levels consumed daily due to background levels in the food supply. Each element results below recognized benchmark levels summarized in Appendix H. Moreover, since Degreened LENTEIN Complete is produced from the same starting material as LC, the process resembles Parabel's LC (See Section II.D), and the chemical composition among the two products is very similar; Parabel believes there is no need to test for a thorough elemental analysis for DGLC for this notification. Nevertheless, as noted on Table 12, Parabel's DGLC heavy metal content is identical to LENTEIN Complete. Table 31 also indicates that the maximum daily consumption of the heavy metals that could be consumed in selected levels of Parabel's LC and DGLC are below maximum recommended intakes. Therefore, no health and safety implications is expected from the consumption of Parabel's LC and DGLC.

Table 31. Levels of Various Elements in Parabel's LENTEIN Complete Compared with Dietary Reference Exposure Levels

Element	Level in LC (ppm)	Amount of element in 201 g LPC (mg)	Reference Exposure Level in Diet (mg/person/day)	Reference
Aluminum	3.13	0.63	7-9	ATSDR, 2008
Arsenic (total)	0.05	0.01	0.13	WHO, 2011
Cadmium	<0.010	0.00	0.055	
Calcium	14,680	2950.68	GRAS	21 CFR Part 184
Chromium	0.41	0.08	0.029	UK, 2009
Cobalt	<0.05	0.00	0.011	ATSDR, 2004a
Copper	2.8	0.56	1	ATSDR, 2004b
Iron	366	73.57	45	IOM, 2001
Lead	0.01	0.00	0.001	ATSDR, 2007
Magnesium	3,539	711.34	GRAS	21 CFR Part 184
Manganese	245	49.25	0.7-10.9	ATSDR, 2012a
Mercury (inorganic)	<0.015	0.00	0.12	WHO, 2011
Molybdenum	0.09	0.02	0.1-0.3	WHO, 2011
Potassium	3,950	793.95	GRAS	21 CFR Part 184
Selenium	<0.30	0.00	0.4	WHO, 2011
Sodium	1,649	331.45	2300	IOM, 2005
Zinc	69	13.87	18-60	WHO, 1982

D. Allergenicity of Parabel's LENTEIN Complete and Degreened LENTEIN Complete

In the recent thorough review (van der Spiegel et al., 2013), the human safety issues associated with popular sources of alternative proteins for use in food were examined. The authors discussed in detail the real possibility of allergenicity issues for several protein sources, but not for water lentils. The absence of discussion of allergenicity in this review is indicative of the lack of any reports of allergy or any scientific basis for suspicion of any allergic hazard from water lentils' protein. In a tabulated summary of the prime safety issues of alternative sources of protein in the review, allergenicity concerns for water lentils protein were conspicuously absent.

Additionally, Parabel has commissioned an extensive global literature search on the potential allergenicity of water lentils and water lentil proteins. On Parabel's behalf, GRAS Associates LLC has investigated this allergenicity question together with allergen expert Dr. Steven Taylor of the Food Allergy Research and Resource Program of the University of Nebraska. Refer to Appendix I for details on the expert's conclusions.

E. Safety of Carotenoid Content

1. Trans-Lutein and Cis-Lutein

FDA responded with no questions regarding the use of lutein in specified food categories in four GRAS notices for use levels of up to 13.4 mg/day. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated a group acceptable daily intake (ADI) of 0 to 2 mg/kg body weight/day for lutein from T. erecta and zeaxanthin (FAO, 2004). In addition, the European Food Safety Authority panel (EFSA) also reviewed the use of lutein by infants and young children, and concluded that there is no concern of safety for recommended use of lutein at the use level of 250 µg lutein/L of infant formula (EFSA, 2008). LC contains about 585 mg/kg trans lutein and 102 mg/kg cis-lutein. At 201 gram/day this equates to 138 mg/day, which is under the maximum level in the GRAS notices.

2. Zeaxanthin

In its opinion of the safety of synthetic zeaxanthin, The European Food Safety Authority (EFSA, 2012) panel concluded that based on the available data, intakes of 0.75 mg/kg bw per day for synthetic zeaxanthin, corresponding to a daily intake of 53 mg for a person with a body weight of 70 kg, do not raise safety concerns. LC contains about 27 mg/kg zeaxanthin, this equates to 5.47 mg/day using a 201 g/day exposure.

3. β-carotene

The safety of β-carotene has been reviewed by Bendich (1988), and additional information on the absorption and metabolism of beta-carotene was published by Wang (1994). Due to detailed toxicity trials, β-carotene is approved as a color additive and is considered Generally Recognized as Safe by FDA for use in foods as a nutrient (Diplock, 1995). LC contains about 271 mg/kg β-carotene. This equates to 54.5 mg/day with 201 g/day exposure.

Degreened LENTEIN Complete is expected to have significant lower levels of Carotenoids compared to LC, because the fat-soluble components and natural pigments within water lentils are extracted during the manufacturing process of Parabel's Degreened LENTEIN Complete (See Section II.D). As a result, no health and safety implications are expected from DGLC's and LC's carotenoid content and the products consumption.

F. Anti-nutritional Factors in LENTEIN Complete

1. Phytic Acid (Phytates)

Phytic acid is a simple sugar that occurs naturally in bran and germ of many plant seeds, and in nuts, legumes, and grains. Phytic acid can chelate mineral ions (i.e. Ca2+, Mg2+, Cu3+, Fe3+, etc.), limiting bioavailability (Dolan et al., 2010). When phytic acid is bound to the mineral in a seed it is referred to as phytate. Phytates can also form complexes with proteins, limiting protein digestibility (Francis et al., 2001).

Phytate-mineral complexes are insoluble in the intestinal tract (Dolan, 2010), inhibit the bioavailability of iron and zinc, and play an important role in the deficiencies of iron and zinc (Ma, 2005). Vegetarians that consume large amounts of tofu and bean curd are susceptible to mineral deficiencies or decreased protein and starch digestibility as a result of phytate consumption (Dolan, 2010). Phytate has been suggested as a therapeutic for management of diabetes and it may be useful as an antioxidant (Dolan, 2010).

Table 32 lists the phytic acid values of foods as reported in the literature. Phytate percentage can range from 0.1% in rice to 1.8% in rice bran (AB Vista, 2013). Phytate ranged from 0.06 to 6% in cereal, cereal products, and cereal-based foods (Reddy, 2002). In a study conducted on traditional Kuwaiti foods, phytate content ranged from 32.6 mg/100 g in labnah (strained yoghurt) to 2835 mg/100 g in rahash (Dashti et al., 2001). In different varieties of bread, phytate content ranged from 0 to 0.2% (Mameesh and Tomar, 1993), and in different varieties of uncooked rice, phytate content was between 0.05 and 0.22% (Mameesh and Tomar, 1993). In a study conducted on tropical foods, phytic acid contents were highest for gingelly (3-87%), gingelly meal (3-76%), and rice bran (3-65%) (Ravindran et al., 1994). In a study on foods commonly consumed in China, the phytate content of 60 foods ranged from 0 to 1878 mg/100 g (Ma, 2005). The phytate intake was between 648 and 1433 mg/day, with urban residents consuming much less phytate than their rural counterparts (781 vs 1342 mg/day) (Ma, 2005).

Since water lentils are taxonomically recognized as a leaf and not a seed, legume or grain, the resulting phytic acid content is reported to be a very small constituent of Lemna minor (Kalita et al., 2007) as two batches of Parabel's LENTEIN Complete were tested and found to have mean of 0.31% phytic acid. This is more than 20 times lower than phytic acid found in white rice. It is also ten times less phytic acid than the high levels reported for soybeans and cashews, and approximately five times less than the phytic acid content found in wheat and corn. Therefore, no safety concern should be expected regarding the phytic acid content of Parabel's LENTEIN Complete.

Table 32. Phytic Acid Content of Select Edible Plant and Plant Products

Plant	Phytic Acid (%)
Rice, Polished, White	0.3
Rice, Wild	2.2
Wheat	0.6
Corn	0.7
Soybean	2.6
Cashew	2.0
LENTEIN Complete*	**0.31**

Data taken from Gilani et al., 2012
* Mean value from 2 composite representative batches (Refer to Appendix A4)

In addition, on June 1, 2011 Tsuno Food Industrial Corporation, Inc. filed a GRAS notification (GRN 381) for phytic acid to be used as "an antioxidant, chelating agent, and antimicrobial agent in beverages and beverage bases, milk products, processed vegetables, and vegetable juices at a level of 0.2%." FDA responded with a "no questions" letter on June 6, 2012 (Tsuno Food Industrial Corporation, 2011).

2. Protease and Trypsin Inhibitors

Protease inhibitors are molecules that inhibit the function of proteases, which help break down proteins. Protease inhibitors are found in many plants and animals, including legumes, cereals, oilseeds, nuts, fruits, vegetables, eggs, and potatoes (Kennedy, 1993). Soybeans can contain as much as 6% of the protein content as protease inhibitor. Protease inhibitors in Parabel's LENTEIN Complete were reported as not detected or below detection limit of 0.170 (U/mg). Therefore, no safety issues should be expected regarding the trypsin inhibitor content of Parabel's LENTEIN Complete.

Protease inhibitors can be classified by the type of protease they inhibit. Trypsin inhibitors are subtype of protease inhibitor. Trypsin inhibitors prevent protein digestion by inhibiting the enzymes trypsin and chymotrypsin. They are found in common foods such as lima beans, peas, peanuts, wheat, barley, and potatoes (Hill, 2003). Soybeans have high trypsin inhibitor content, ranging from about 16 to 122 (Gilani, et al., 2013).

Trypsin inhibitor causes pancreatic heterotrophy and poor growth in animals (Leiner, 1979). However, the human pancreas is likely insensitive to trypsin inhibitor due to the relative size of the human pancreas and because heating and processing of soybeans reduces the amount of trypsin compared to the raw soybean (Leiner, 1979).

According to Kalita et al. (2007), the concentration of TI in Lemna minor is 1.47% This represents a lower concentration than soybeans, as soybeans are considered the richest source of dietary TI among common food and feed products. Contents in raw soybean vary from 8.6-48.2mg/g (0.86-4.8%) or 20.3-122.6mg/g protein (Gilani et al., 2012). Commercially available soy-protein products contain 5 to 20% residual trypsin inhibitor activity (Erdman and Fordyce, 1989). Parabel's LENTEIN Complete was reported to contain less than 1000 trypsin inhibitor units (TIU)/gram. Therefore, no safety concern should be expected regarding the trypsin inhibitor content of Parabel's LENTEIN Complete.

3. Tannins

Tannins are high molecular weight polyphenols. They interfere with digestion by binding proteins or minerals, or digestive enzymes. Tannins also limit vitamin B12 absorption (Francis et al., 2001). Phenolic compounds have recently garnered interest for their beneficial actions on human health (D'Archivio et al., 2010). Kalita et al. (2007) report 0.9% tannins (equivalent to 9 g/kg tannins) in Lemna minor. Parabel analyzed the content of tannins in Parabel's LENTEIN Complete and found the typical level to be below detection limit of 0.050 %. A summary of typical values for tannins in common foods is provided in Table 33.

Table 33. Tannin Content of Select Edible Plant and Plant Products

Plant	Tanning (%)	Reference
Chick pea	0.6-2.7	Jansman & Longstaff (1993)
Cowpea	1.4-10.2	Jansman & Longstaff (1993)
Pea	0.6-10.5	Jansman & Longstaff (1993)
Kidney beans	5.3-17.55	Jansman & Longstaff (1993)
Barley	5.5-12.3	Jansman & Longstaff (1993)
LENTEIN Complete	<0.050 %	Mean value from 2 composite representative batches (Refer to Appendix A4)

Tannins are nutritionally undesirable because of their ability to form complexes with protein, starches and enzymes to reduce the nutritional quality of foods. They have been shown precipitate proteins, inhibit digestive enzymes, and affect the utilization of vitamins and minerals (Chung et al., 1998). Tannins have been implicated in high levels of cheek and esophageal cancers in the Far East, where Betel nuts containing 11-26% tannins are often consumed after dinner. The incidence of esophageal cancer in Caribbean islanders has been correlated to the consumption of tea and other tannin-rich food items such as sorghum. Tannins were found to cause tumors in experimental animals when applied to burns or injected subcutaneously (Chung et al., 1998). Therefore, tannins and tannic acid are listed by OSHA as Category I carcinogens. The carcinogenicity of tannins may be more related to their ability to cause cellular damage rather than DNA mutation. A study, however, showed that tannic acid at 0.25 and 0.5% in distilled water for more than two years did not increase the incidence of tumors (Onodera et al., 1994). In addition, in Dietary Tannins: Consequences and Remedies, Salunkhe and Chavan (1989), state that "although tannins have been implicated in carcinogenesis, the data appear to be sketchy." Also, the International Agency for Research on Cancer (IARC) evaluation of tannic acid and tannins reports that tannic acid is carcinogenic in rats and mice following its subcutaneous injection, producing liver tumors, but that no adequate published studies involving oral administration of tannins in animals were available to the Working Group. Pertaining to human carcinogenicity data, no case reports or epidemiological studies were available to the working group (IARC, 1998).

On the other hand, tannins have also been shown to have antimutagenic, anticarcinogenic and antimicrobial properties. Tannic acid was shown to protect against chemically-induced tumor initiation in mice (Das et al., 1989; Athar et al., 1989). Tannins in Lemna minor were reported to be 0.8% (Kalita et al., 2007). Parabel's concentration of tannins in LENTEIN Complete from Lemnaceae is below detection limit of 0.050 %. This is significantly lower than the amounts found in the foods containing high levels of tannins, such as barley (0.5-1.2%) or kidney beans (0.5-1.8%), which have much higher daily consumption. Therefore, no safety concern should be expected regarding the tannins in Parabel's LENTEIN Complete.

In addition, in the FAO report on Sorghum and Millets in Human Nutrition (FAO, 1995) states that while tannins may adversely affect the grain's nutritional quality and several studies in rats, chicks and livestock have shown that high tannin in the diet adversely affects digestibility of protein and carbohydrates and reduces growth, feeding efficiency, metabolizable energy and bioavailability of amino acids there is no direct evidence regarding anti-nutritional effects of dietary tannins in human subjects.

4. Oxalic Acid

Many plant species contain oxalic acid, including beets, asparagus, celery, and parsley (Santamaria et al. 1999). Oxalate in spinach was measured at a mean of 0.54%, with data ranging from 0.23 to 1.0% (Santamaria et al., 1999), and soybeans were measured to contain oxalic acid ranging from 0.67 to 3.5% (Massey et al., 2001). A low-oxalate diet, prescribed for people with calcium oxalate kidney stones, limits the consumption of moderate-oxalate foods (2 to10 mg of oxalate per serving) to three servings per day, but high-oxalate foods (>10 mg/serving) should be avoided entirely (University of Pittsburgh Medical Center (UPMC), 2006). Even though water lentils grown in the wild are known to contain oxalic acid, often in the form of calcium oxalate raphides (FAO, Data unknown; Goopy and Murray, 2003); Parabel's LENTEIN Complete and DGLC typically contains less than 0.04% oxalic acid (see Appendix A2 for laboratory certificate of analysis). Table 34 provides oxalic acid values for a variety of plant species, including water lentil species.

Table 34. Oxalic Acid Content of Selected Edible Plant and Plant Products

Plant	Oxalic Acid (%, as-is)	Reference
Beetroot	0.07[a]	Santamaria et al., 1999
Swiss chard	0.21[a]	Santamaria et al., 1999
Alfalfa	0.36[a]	Hintz et al., 1984

Spinach	0.65 0.54[a]	Duke, 2014 Santamaria et al., 1999
Soybean	1.665[a] 0.67-3.5[a]	Mwangi et al., 2012 Massey et al., 2001
Buckwheat	11.1	Duke, 2014
Lemna minor	3.5	Kalita et al., 2007
LENTEIN Complete	0.04	Mean value from 4 composite representative batches (Refer to Appendix A2)
Degreened LENTEIN Complete	0.001-0.07	Refer to Table 17 and Eurofins Lab Report in Appendix A1

[a] As oxalate

Although oxalic acid has been reported in the literature to be a constituent of water lentils, most of the oxalic acid is removed in the manufacturing process of Parabel's LENTEIN Complete and its degreened version. Residual levels in Parabel's LC and DGLC are found to be at approximately 0.04 %. As shown in Table 25, other foods have higher levels of oxalic acid including, soybean (1.665%) and buckwheat (11.1%). As described in Section III.F, at a maximum daily intake of 201 g of Parabel's LENTEIN Complete or DGLC, the amount of oxalic acid contributed in the diet would be 8.96 mg/day, which is considered a low level of oxalic acid consumption (University of Pittsburgh, 2006).

G. Safety of Biogenic Amine Content in LENTEIN Complete

Biogenic amines have been considered carcinogens because they react with nitrates to form potentially carcinogenic nitrosamines (Shalaby, 1996). Histamine has been suggested to be the responsible amine for food poisoning, and can be enhanced by the presence of cadaverine, putrescine, and tyramine (Shalaby, 1996).

Even though there are no reported biogenic amines found in water lentils in the literature, Parabel has analyzed a composite sample of LENTEIN Complete for biogenic amines as shown on Table 10 (Refer to Appendix A4 for Lab Report). LENTEIN Complete contains a total of 86.75 ppm biogenic amines and approximately 17.24 mg/day at the EDI of 201 g/person/day total biogenic amines, which is significantly lower than the 40 mg/day toxicity level. Also, none of the common biogenic amines are at levels of concern and are significantly lower than the highest levels found in common foods.

H. Safety studies on components of Parabel's LC fiber

Toxicity studies of cellulose (a major fiber component in Parabel's LC fiber) showed no adverse effects (Anderson et al., 1992).

1. Safety studies of cellulose, a major component of Parabel's LC

Summary: In 1973, industrially purified cellulose from exogenous sources including wood (apart from natural plant materials and cereals) was recognized as a GRAS substance (Anderson et al., 1992; LSRO, 1973). The comprehensive review of Anderson et al. (1992) concluded that human exposure to cellulose would not result in any adverse health effects. Details of the review are described below and in Table 6. Overall, many celluloses are recovered in feces as it remains largely undigested in the upper gastrointestinal tract and may be poorly fermented in the colon to gases and SCFA. Thus, gastrointestinal discomfort is not expected with consumption of cellulose. Consumption of a large quantity of cellulose also did not affect gastrointestinal morphology. The only possible effect might be on body weight mostly due to its caloric dilution effect. Therefore, the metabolism of cellulose does not raise safety concerns and no systemic toxicity is expected following ingestion of cellulose.

2. Effects of cellulose on tumor formation

The review of Anderson et al. (1992) found that chronic ingestion of purified cellulose (from wood and softwood) over the lifespan of rats and mice did not increase spontaneous disease or neoplasia (in the mammary glands, colon, or bladder of rats), nor did it significantly alter the absorption or metabolism of dietary components. Several studies evaluated the role of exogenous cellulose on tumor incidence based on the assumption that diets containing exogenous cellulose should lead to an increased cancer rate when administered subsequent to a dose of a site-specific carcinogen. In various models, cellulose did not display tumor-promoting activity at large doses. In rats, 10-30% cellulose in the diet was administered for 24 weeks after exposure to azoxymethane (AOM) to induce large bowel tumors (Nigro et al., 1979). Tumor rates in animals ingesting cellulose were lower compared to fiber-free diets. Diets containing up to 46% purified cellulose, sourced from exogenous sources including wood, were protective against mammary tumor promotion for 120 or 134 d after exposure to 7,12-dimethylbenz[α]anthracene (DMBA; Kritchevsky et al., 1984).

3. Effects of cellulose on reproductive performance and growth

No adverse effects were noted on reproduction or neonate development in rats using 30% of purified exogenous cellulose in the diet (Anderson et al., 1992; LSRO, 1973). Similarly, a 5% purified cellulose diet did not cause any abnormal reproductive performance such as pups per litter, pup survival, or pup growth in mice and rats (Anderson et al., 1992; Bieri et al., 1977). Teratogenic effects were not observed.

Evaluation of effects of pure cellulose from exogenous sources including wood and microcrystalline cellulose on growth revealed that cellulose had no adverse effects in rats and mice. Growth rates for up to 18 weeks after weaning were not affected in either rats or mice when a 5% of purified cellulose diet was administered (Anderson et al., 1992; Bieri et al., 1977; Hove and King, 1979). Dietary concentrations of 20% microcrystalline cellulose for 4 week did not retard growth of rats (Hove and King, 1979). Rat pups consuming a diet containing 28.6% cellulose had decreased carcass fat due to lower calorie intake related to caloric dilution effects of dietary cellulose (Wojcik and Delorme, 1983). Also, rat pups consuming 10% and 20% microcrystalline cellulose diets gained less body weight due to caloric dilution of the diet and no increased food consumption to compensate caloric dilution (Sundaravalli et al., 1971). However, feeding 40% levels of dietary cellulose in isocaloric, isonitrogenous diets did not affect growth when compared to a diet with 5% dietary cellulose (Anderson et al., 1987). As discussed in the section of "Subchronic toxicity study of Lemna meal in rats", effects of total dietary fiber and cellulose on body weight are mostly due to caloric dilution and are considered as positive attributes.

4. Effects of cellulose on the gastrointestinal tract

As the gastrointestinal tract is directly exposed to ingested cellulose, which remains intact in the large intestine, the effect on intestinal structure and physiology are important endpoints for the safety evaluation of dietary cellulose. Several studies showed that large doses of cellulose from various dietary or exogenous sources and from cellulose derivatives, did not adversely alter cell morphology or cell dynamics in the gastrointestinal tract in mice and rats (Vahouny et al., 1981; Goodlard and Wright, 1983; Lupton et al., 1988).

5. Efficacy studies

Several efficacy studies testing the effect of cellulose on glycemic responses and digestibility reported no adverse effects of cellulose in cats, dogs, mice, and rats (Table 35; Delorme et al., 1981; Hasegawa et al., 1990; Nelson et al., 1991, 1998, 2000; Schwartz et al., 1980; Vahouny et al., 1987, 1988). Doses up to 20% cellulose in the diet were tested and the duration of the studies were up to 8 mo.

Table 35. Studies showing no adverse effects of cellulose in animals

Animal	% cellulose in diet	Cellulose source	Duration	Primary measurement endpoints	Reference
Carcinogenesis					
Rats treated with AOM [a]	10, 20, or 30%	Exogenous purified cellulose, source not specified.	24 wk.	Large bowel cancer incidence	Nigro et al., 1979
Rats treated with DMBA [b]	8-46%	Exogenous pure cellulose, wood and softwood	120 or 134 d	Mammary tumor promotion	Kritchevsky et al., 1984
Reproductive performance and growth					
Rat	30%	Food grade preparations of cellulose derivatives	3 generations	Reproductive performance	LSRO, 1973
Rats and mice	5%	Exogenous purified cellulose	Up to 18 wk. after weaning	Teratogenic effects and growth rates	Bieri et al., 1977

Rats, weaning males	20%	Microcrystalline cellulose [c]	4 wk.	Wt. gain, food consumption, cecal wt., cecal SCFA	Hove and King, 1979
Rat pups	4.8, 9.1, 16.7, or 28.6%	Dietary cellulose, source not specified.	4 wk.	Wt. gain, food consumption, carcass fat	Wojcik and Delorme, 1983
Rat pups	Up to 40%	Exogenous pure cellulose, wood and softwood	4 wk.	Wt. gain, food consumption	Anderson et al., 1987
Rat pups	5, 10, or 20%	Microcrystalline cellulose [c]	4 wk.	Weight gain, food consumption	Sundaravalli et al., 1971
Gastrointestinal effects					
Mice	30%	Solka Floc grade BW-100	1 wk.	Body wt., histology of the intestine	Goodlard and Wright, 1983
Rat	15%	Dietary cellulose, source not specified.	6 wk.	Body wt., histology of the intestine	Vahouny et al., 1981
Rat	10%	Avicel microcrystalline cellulose type PH-105) [c]	35 wk.	Body wt., histology of the intestine	Lupton et al., 1988
Efficacy studies showing no adverse effects of cellulose					
Cats with naturally acquired diabetes mellitus	12%	Powdered cellulose, source not specified.	24 wk.	Caloric intake, body weight, or postprandial serum glucose concentration	Nelson et al., 2000
Dogs with naturally acquired diabetes mellitus	12%	Powdered cellulose, source not specified.	8 mo.	Pre- and postprandial serum glucose concentration; urinary excretion of glucose	Nelson et al., 1998
Dogs with alloxan-induced diabetes mellitus	15%	Powdered cellulose, source not specified.	8 wk.	Postprandial serum glucose concentration	Nelson et al., 1991
Rats	10%	Dietary cellulose, source not specified.	5 wk.	Intestinal glucose absorption	Schwartz et al., 1980
Rats, weaning	5, 10, 20, or 40%	Dietary α-cellulose	4 wk.	Growth and digestibility	Delorme et al., 1981
Streptozocin- induced diabetic mice	20%	Dietary cellulose, source not specified.	8 wk.	Growth rate, insulin secretion	Hasegawa et al., 1990
Rats	10%	Purified cellulose, 10 (Solka floc)	4 wk.	Fecal mass	Vahouny et al., 1987, 1988

[a] AOM=**azoxymethane;** [b] DMBA=7,12-dimethylbenz-alpha-anthracene.

[c] MCC=microcrystalline cellulose: is a nontoxic fiber, which is used as food additive. It is indigestible, unabsorbable and well-tolerated. (Niemi et al., 1988)

6. Safety of hemicelluloses, a second major component of Parabel's LC fiber

Other fiber ingredients based on an arabinoxylan backbone, such as wheat bran (52-64% arabinoxylan; up to 70% hemicelluloses) and psyllium (85% arabinoxylan), are known for their fecal bulking effects and gastrointestinal regularity improvement without having negative effects on mineral bioavailability (Cho et al., 2001; Marteau et al., 1994; Slavin, 2008). These hemicellulose-rich fibers are fermented by intestinal microflora to produce SCFA that support colonic health. However, fermentation in the large intestine can result in the formation of gases (including hydrogen, carbon dioxide, and methane), which often is associated with flatulence and intestinal discomfort. Intestinal discomfort can be a transient symptom since the human body is able to adapt to higher intakes of dietary fiber over time. Overall, metabolites of hemicelluloses such as SCFA, CO_2, and H_2, do not raise safety concerns and no systemic risks are expected from the metabolism of the hemicellulose fraction of Parabel's LC.

7. Human clinical studies

a. Studies of cellulose, a major component of Parabel's LC fiber

Stephen's (1989) review of human studies (10-24 g cellulose) reported that edible purified cellulose significantly increased stool weights (Eastwood et al., 1973; Fleming et al, 1983; Kies et al., 1984; Slavin et al., 1985), reduced mean transit time (Slavin et al., 1985; Wrick et al., 1983; Hillman et al., 1983), and increased stool frequency (Eastwood et al., 1973; Fleming et al., 1983; Slavin et al., 1985). No adverse effects of cellulose at different forms of cellulose were reported.

Table 36 outlines additional human clinical studies showing no adverse effects of cellulose (Hillman et al., 1985; Mickelsen et al., 1979; Niemi et al., 1988; Park and Jhon, 2009; Spiller et al., 1980). Tested doses were up to 25 g/d and for up to 8 wk. In general, cellulose intake decreased intestinal transit time and increased fecal weight (relieving constipation) without having adverse effects. In observational studies with 203 consecutive appendectomized children with histologically proved appendicitis and 1922 controls, cellulose intake was correlated to lower incidence of appendicitis (Adamidis et al., 2000). In another observational study with 291 children with constipation and 1602 controls, only cellulose and pentose intakes were independently inversely correlated with chronic constipation (Roma et al., 1999). No studies reported adverse effects of cellulose.

Table 36. Human clinical studies showing no adverse effects of purified cellulose

Subject	Dosage and source of cellulose	Duration	Results/Primary measurement endpoints	Reference
10 healthy volunteers	15 g/d MCC[a]/d	8 wk	No effect on total cholesterol	Hillman et al., 1985
10 healthy volunteers	14 g powdered cellulose/d	3 wk	Decreased transit time, Increased fecal bulk	Spiller et al., 1980
6 college males	23 g powdered cellulose in bread/d	8 wk	More weight loss and more satiety	Mickelsen et al., 1979
8 constipated volunteers	25 g/d purified powdered cellulose	6 d	Lower blood triglycerides, increase in HDL, no change in serum glucose, improved bowel movement comfort, increased stool volume and quality	Park and Jhon, 2009
18 subjects with T2D	MCC [a]	12 wk	No effect on fasting serum glucose or lipid profiles	Niemi et al., 1988

[a] MCC=microcrystalline cellulose: is a nontoxic fiber, which is used as food additive. It is indigestible, unabsorbable and well-tolerated. (Niemi et al., 1988)

b. Studies showing no adverse effects of hemicelluloses, a second major component of Parabel's LC fiber

Hemicellulose (mostly arabinoxylan)-rich fiber such as psyllium (85% arabinoxylan) has been proven safe for human consumption. Based on numerous human randomized controlled trials (RCTs), FDA has approved health claims for psyllium and oat bran and risk reduction of heart disease (FDA, 2008). No safety concerns have been reported for arabinoxylan-rich fibers.

I. Safety Assessment on the use of Ethanol to produce Parabel's Degreened LENTEIN Complete

Ethanol has a long history of use in food products and in various forms of alcoholic beverages. According to NIAA's report (1987), it was proposed that moderate alcohol intake should not exceed 0.8 g/kg body weight per day, or an average of 0.7 g/kg body weight over a 3-day period.

FDA considers ethanol to be "GRAS" when added directly to food products (21 CFR 184.1293). The regulation states that "the ingredient is used as an antimicrobial agent on pizza crusts prior to final baking at levels not exceed 2.0 percent by product weight". Ethanol is also considered GRAS when used as a preservative in the filling of croissants at a concentration of 3,000 ppm (GRN 000151; FDA, 2004). Ethanol is also allowed for use as a diluent in color additives for marking food and coloring shell eggs (GRN 000470; FDA, 2013). Moreover, water and Ethanol solvent mixture is used as an extraction solvent to produce C. fimbriata powder (GRN 000500; FDA, 2014). Drinking juice can contain ethanol up to 1500 ppm (Hagenmaier, 2001).

Parabel uses ethanol as a processing aid for the extraction of the fat-soluble components and pigments within water lentils towards the production of Parabel's DGLC (refer to

Section II.D). Prior to drying and packaging, Parabel's Degreened LENTEIN Complete is desolventized.

Therefore, Parabel believes that no health and safety implications would be expected from adding ethanol during the manufacturing process of Parabel's Degreened LENTEIN Complete towards the extraction of fat and color pigments within water lentils.

J. Safety Discussion of LENTEIN Complete and Degreened LENTEIN Complete

In summary, Parabel believes that Parabel's LENTEIN Complete and its degreened version (DGLC) are Generally Recognized As Safe:

1. The literature indicates that water lentils have been well characterized, several species are a readily available and are considered a nontoxic protein source with adequate levels of all amino acids. The protein has a high nutritional quality and its amino acid composition is a good nutrient source.

2. Parabel has reviewed the estimated consumption from the proposed uses of LENTEIN Complete as discussed in Section III. The major interest is to use LC and DGLC to increase protein and fiber levels in some food products. LC and DGLC would not be intended to be used as a sole source of dietary protein but rather to supplement the levels already in the diet. The proposed use level spans a range of 1.0 – 24 g/serving.

3. Based on the data represented in two oral toxicity studies, conducted by Shriram Institute for Industrial Research (refer to Appendix O), LENTEIN™ Complete's LD_{50} ranged above 2g-5g/kg body weight and is categorized as category 5 or unclassified as per the Globally Harmonized Classification System (GHS). In addition, "under the conditions of the repeated dose oral toxicity study, the repeated oral administration of Parabel's LC in Wistar rats at the dose level of 1g/kg body weight daily for 90 days, [LC] did not induce any observable toxic effects when compared to its corresponding control group of animals. Hence, it may be considered to have a 'No Observed Adverse Effect Level' (page 6)".

4. Animal studies confirm that the use of LPC, a precursor of LC LENTEIN Complete, and whole or dried water lentils as the protein source in animal feed had no adverse effects. There is also a history of use of water lentils for human food in some Asian countries.

5. EFSA (2009, 2013) has no safety concerns with two other purified plant proteins (rapeseed protein isolate and alfalfa protein concentrate) for use in food and supplement products. There is also a long history of recognized GRAS status for several types of protein preparations, including products derived from aquatic sources which are comparable the protein profile of LC and DGLC. Data on LC and

DGLC show an amino acid composition comparable to other GRAS-notified aquatic protein sources in which the FDA responded with "no questions".

6. LC and DGLC are very well characterized and meet high chemical and biological standards. Parabel has reviewed the analytical data on its LC and DGLC and compared to levels found in common foods. The levels found in LC and DGLC do not raise concerns of safety.

7. Parabel considers that there is no evidence of allergenicity concerns with water lentils.

8. Parabel has thoroughly reviewed the occurrence of several secondary metabolites that are known to be present in water lentils and the residual levels in LC and DGLC. The levels of antinutritionals and biogenic amines are significantly lower than other food sources containing oxalic acid, tannins, phytate, trypsin and protease inhibitors and biogenic amines. As a result, no safety concern should be expected regarding the mentioned antinutritionals and secondary metabolites in Parabel's LENTEIN Complete and Degreened LENTEIN Complete.

9. Food-grade ethanol, used as a processing aid that can be potentially used to facilitate the degreening process of water lentils to produce Parabel's Degreened LENTEIN Complete as described in Section II.D is GRAS.

10. Parabel produces well-controlled manufacturing conditions and GMPs are state- of-the art to ensure no contamination is present, and eliminate pathogens and pesticides concerns raised in the literature about water lentils grown in the wild or on waste water. LENTEIN Complete will be manufactured under cGMP to meet SQF level 2 Category 19 and 21 CFR 110 Manufacture Standards for GMP, Hazard Analysis and Critical Control Point (HACCP) and food additive regulations established by the U.S. Food and Drug Administration (FDA) and the product specifications for food grade are consistent with current state-of-the-art requirements.

VII. LIST OF SUPPORTING DATA AND INFORMATION

Section IX includes the list of references with all the published literature references and information used to support the safety discussion in this GRAS Notification. All the references listed in Section IX are generally available. Additional data and information used in this GRAS Notice to support Parabel's view that Parabel's LENTEIN Complete and Degreened LENTEIN Complete are safe under the conditions of intended use as described in accordance with §170.250(a)(1) and in Section III of this notification; are provided in Appendices A through R.

VIII. CONCLUSION

Parabel Ltd. has critically evaluated the published and unpublished data and information summarized in this safety evaluation, and concludes that Parabel's LENTEIN Complete (LC) and Degreened LENTEIN Complete (DGLC) – which are produced in accordance with FDA Good Manufacturing Practices requirements and which meet the appropriate food-grade product specifications as set forth in Section II.E.2 of the provided safety evaluation and as required by FDA regulation, 21 CFR 182.1 (b)(1) - are considered to be Generally Recognized As Safe when consumed as a nutritional ingredient in commercial food products at a maximum level of 201 grams per person per day.

This declaration is made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

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CEO

(b) (6)

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Appendix A: Laboratories Certificates of Analyses

A1: Batch data from LENTEIN™ Complete and DGLC

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Eurofins Analytical Laboratories Inc.
2219 Lakeshore Drive
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Person in charge John M. Reuther
Client Support Cara Quintanilla

PARABEL USA, INC.
ATTN: Molly Sproston
14655 101ST. STREET
FELLSMERE, FL 32948

Reporting Date 04/24/2017

AR-17-QA-022894-01

REPORT OF ANALYSIS

Sample Code 468-2017-0407B101

Sample Description PROTEIN POWDER
Client Sample Code EUNFB1-CSPBWL-170207
Sample Reference

Reception Date 04/07/2017
Reception Temperature 25 (Celsius)
Sample Condition Acceptable
Purchase Order

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 04/12/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	2.86 %
QA821 - Crude Protein (Combustion)	
Completion Date: 04/12/2017 **Method:** AOAC 990.03	
* Crude Protein	43.13 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 04/12/2017 **Method:** AOAC 922.06	
* AH Fat	9.09 %
QA285 - Crude Fiber (Oilseed Meals, AOCS)	
Completion Date: 04/11/2017 **Method:** AOCS Ba 6a-05	
* Crude Fiber	13.1 %
QA13A - Ash	
Completion Date: 04/11/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	5.11 g/100 g

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

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Eurofins Sample Code: 468-2017-0407B101 **Client Sample Code: EUNFB1-CSPBWL-170207**

AR-17-QA-022894-01

REPORT OF ANALYSIS

Test Results	Result
QD161 - Fiber, Neutral Detergent	
Completion Date: 04/14/2017 **Method:** Ankom NDF	
* Fiber, Neutral Detergent	22.6 %
QD053 - Fiber, Dietary Complete	
Completion Date: 04/14/2017 **Method:** AOAC 991.43	
* Fiber, Dietary, Soluble - Calc	2.5 %
* Fiber, Dietary, Insoluble	33.9 %
* Fiber, Dietary, Total	36.4 %
QD002 - Fiber, Acid Detergent	
Completion Date: 04/14/2017 **Method:** Ankom ADF 05/03	
* Fiber, Acid Detergent	12.5 %
QQ129 - Sugar Profile (AOAC, Most Matrices)	
Completion Date: 04/14/2017 **Method:** AOAC 982.14, mod.	
* Fructose	<0.15 %
* Glucose	<0.15 %
* Lactose	<0.15 %
* Maltose	<0.15 %
* Sucrose	<0.15 %
* Total sugars	<0.35 %
UMHY9 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 04/14/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	54,000 cfu/g
KK03N - Aluminum	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Aluminium	3.9 ppm
KK03D - Arsenic	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic	0.044 ppm
KK03P - Boron	
Completion Date: 04/24/2017 **Method:**	

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Eurofins Sample Code: 468-2017-0407B101 **Client Sample Code: EUNFB1-CSPBWL-170207**

AR-17-QA-022894-01

REPORT OF ANALYSIS

Test Results	Result
* Boron	1,110 mg/kg
KK03A - Cadmium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium	0.014 ppm
KK02Y - Calcium	
Completion Date: 04/24/2017 **Method:**	
* Calcium	17,800 ppm
KK03E - Chromium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Chromium	0.888 ppm
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 04/13/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	Presumptive
KK03S - Cobalt	
Completion Date: 04/24/2017 **Method:**	
* Cobalt	<0.050 ppm
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 04/14/2017 **Method:** AOAC 991.14	
* Total Coliforms	<10 (est) cfu/g
UMVTK - Confirmation Clostridium Perfringens - AOAC 976.30	
Completion Date: 04/14/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	<10 cfu/g
UMBIP - Confirmation Escherichia coli - BAM Chapter 4	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 4	
* Escherichia coli - Confirmed	<3 MPN/g
KK03T - Copper	
Completion Date: 04/24/2017 **Method:**	
* Copper	2.79 ppm

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AR-17-QA-022894-01

REPORT OF ANALYSIS

Test Results	Result
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 4	
* E. coli	Presumptive
KK03U - Iron	
Completion Date: 04/24/2017 **Method:**	
* Iron	348 ppm
KK03B - Lead	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Lead	0.014 ppm
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 04/14/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
KK04B - Magnesium	
Completion Date: 04/24/2017 **Method:**	
* Magnesium	3,890 ppm
KK03F - Manganese	
Completion Date: 04/24/2017 **Method:**	
* Manganese	263 ppm
KK03C - Mercury	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Mercury	<0.015 ppm
KK03W - Molybdenum	
Completion Date: 04/24/2017 **Method:**	
* Molybdenum	0.076 mg/kg
UM4BV - Moulds - BAM Chapter 18	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 18	
* Mold	10 (est) cfu/g

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Eurofins Sample Code: 468-2017-0407B101 **Client Sample Code: EUNFB1-CSPBWL-170207**

AR-17-QA-022894-01

REPORT OF ANALYSIS

Test Results	Result
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 18	
* Yeast	10 (est) cfu/g
KK04A - Phosphorus	
Completion Date: 04/24/2017 **Method:**	
* Phosphorus	5,810 ppm
KK03V - Potassium	
Completion Date: 04/24/2017 **Method:**	
* Potassium	2,470 ppm
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 04/14/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g
KK03G - Selenium	
Completion Date: 04/24/2017 **Method:**	
* Selenium	<0.30 ppm
KK03Z - Sodium	
Completion Date: 04/24/2017 **Method:**	
* Sodium	1,200 ppm
QD234 - AOAC Total Starch	
Completion Date: 04/14/2017 **Method:** AOAC 996.11	
* Starch (AOAC 996.11)	5.6 %
KK03H - Tin	
Completion Date: 04/24/2017 **Method:**	
* Tin	<0.15 ppm
KK02X - Zinc	
Completion Date: 04/24/2017 **Method:**	
* Zinc	71.1 ppm

**This is not covered by our current A2LA accreditation.*

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Eurofins Sample Code: 468-2017-0407B101 **Client Sample Code: EUNFB1-CSPBWL-170207**

AR-17-QA-022894-01

REPORT OF ANALYSIS

Test Results	**Result**

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

Results shown in this report relate solely to the item submitted for analysis.
Uncertainty can be obtained upon request.

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Reporting Date 05/15/2017

AR-17-QA-022895-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-022895-01.

Sample Code 468-2017-0407B102

Sample Description	PROTEIN POWDER	**Reception Date**	04/07/2017
Client Sample Code	EUNFB2-CSPBWL-170209	**Reception Temperature**	25 (Celsius)
Sample Reference		**Sample Condition**	Acceptable
		Purchase Order	

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 04/12/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	2.47 %
QA821 - Crude Protein (Combustion)	
Completion Date: 04/12/2017 **Method:** AOAC 990.03	
* Crude Protein	46.17 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 04/12/2017 **Method:** AOAC 922.06	
* AH Fat	9.28 %
QA285 - Crude Fiber (Oilseed Meals, AOCS)	
Completion Date: 04/11/2017 **Method:** AOCS Ba 6a-05	
* Crude Fiber	9.6 %
QA13A - Ash	
Completion Date: 04/11/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	5.03 g/100 g

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Eurofins Sample Code: 468-2017-0407B102 **Client Sample Code: EUNFB2-CSPBWL-170209**

AR-17-QA-022895-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-022895-01.

Test Results	Result
QD161 - Fiber, Neutral Detergent	
Completion Date: 04/14/2017 **Method:** Ankom NDF	
* Fiber, Neutral Detergent	28.0 %
QD053 - Fiber, Dietary Complete	
Completion Date: 04/14/2017 **Method:** AOAC 991.43	
* Fiber, Dietary, Soluble - Calc	0.7 %
* Fiber, Dietary, Insoluble	33.5 %
* Fiber, Dietary, Total	34.2 %
QD002 - Fiber, Acid Detergent	
Completion Date: 04/14/2017 **Method:** Ankom ADF 05/03	
* Fiber, Acid Detergent	11.6 %
QQ129 - Sugar Profile (AOAC, Most Matrices)	
Completion Date: 04/14/2017 **Method:** AOAC 982.14, mod.	
* Fructose	<0.15 %
* Glucose	<0.15 %
* Lactose	<0.15 %
* Maltose	<0.15 %
* Sucrose	<0.15 %
* Total sugars	<0.35 %
KK03N - Aluminum	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Aluminium	3.2 ppm
KK03D - Arsenic	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic	0.047 ppm
KK03P - Boron	
Completion Date: 04/24/2017 **Method:**	
* Boron	1,080 mg/kg

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Eurofins Sample Code: 468-2017-0407B102 **Client Sample Code: EUNFB2-CSPBWL-170209**

AR-17-QA-022895-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-022895-01.

Test Results	Result
KK03A - Cadmium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium	0.011 ppm
KK02Y - Calcium	
Completion Date: 04/24/2017 **Method:**	
* Calcium	13,300 ppm
KK03E - Chromium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Chromium	0.334 ppm
KK03S - Cobalt	
Completion Date: 04/24/2017 **Method:**	
* Cobalt	<0.050 ppm
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 04/17/2017 **Method:** AOAC 991.14	
* Total Coliforms	<10 (est) cfu/g
KK03T - Copper	
Completion Date: 04/24/2017 **Method:**	
* Copper	2.27 ppm
KK03U - Iron	
Completion Date: 04/24/2017 **Method:**	
* Iron	358 ppm
KK03B - Lead	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Lead	0.011 ppm
KK04B - Magnesium	
Completion Date: 04/24/2017 **Method:**	
* Magnesium	3,430 ppm

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Eurofins Sample Code: 468-2017-0407B102 **Client Sample Code: EUNFB2-CSPBWL-170209**

AR-17-QA-022895-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-022895-01.

Test Results	Result
KK03F - Manganese	
Completion Date: 04/24/2017 **Method:**	
* Manganese	333 ppm
KK03C - Mercury	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Mercury	<0.015 ppm
KK03W - Molybdenum	
Completion Date: 04/24/2017 **Method:**	
* Molybdenum	0.100 mg/kg
KK04A - Phosphorus	
Completion Date: 04/24/2017 **Method:**	
* Phosphorus	5,780 ppm
KK03V - Potassium	
Completion Date: 04/24/2017 **Method:**	
* Potassium	4,950 ppm
KK03G - Selenium	
Completion Date: 04/24/2017 **Method:**	
* Selenium	<0.30 ppm
KK03Z - Sodium	
Completion Date: 04/24/2017 **Method:**	
* Sodium	1,680 ppm
QD234 - AOAC Total Starch	
Completion Date: 04/14/2017 **Method:** AOAC 996.11	
* Starch (AOAC 996.11)	5.2 %
KK03H - Tin	
Completion Date: 04/24/2017 **Method:**	
* Tin	<0.15 ppm

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AR-17-QA-022895-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-022895-01.

Test Results	Result
KK02X - Zinc	
Completion Date: 04/24/2017 **Method:**	
* Zinc	56.6 ppm

**This is not covered by our current A2LA accreditation.*

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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A2LA ACCREDITED TESTING CERT #2993-01

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A2LA ISO/IEC 17025:2005
Best Aquaculture Practices
International Olive Council

American Oil Chemists Society
Grain and Feed Trade Association
Federation of Oils, Seed, and Fats Associations, Ltd.

Japanese Ministry of Health and Welfare
Association of Official Analytical Chemists
United States Department of Agriculture

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Reporting Date 04/24/2017

AR-17-QA-022922-01

REPORT OF ANALYSIS

Sample Code 468-2017-0407B103

Sample Description	PROTEIN POWDER	**Reception Date**	04/07/2017
Client Sample Code	EUNFB3-CSPBWL-170213	**Reception Temperature**	25 (Celsius)
Sample Reference		**Sample Condition**	Acceptable
		Purchase Order	

Test Results — Result

QA354 - Moisture (Air Oven 130C 2 hrs)
Completion Date: 04/12/2017 **Method:** AOCS Ba 2a-38
Moisture and Volatile Matter — 5.51 %

QA821 - Crude Protein (Combustion)
Completion Date: 04/12/2017 **Method:** AOAC 990.03
* Crude Protein — 41.30 %

QA265 - Crude Fat (Acid Hydrolysis)
Completion Date: 04/12/2017 **Method:** AOAC 922.06
* AH Fat — 9.12 %

QA285 - Crude Fiber (Oilseed Meals, AOCS)
Completion Date: 04/11/2017 **Method:** AOCS Ba 6a-05
* Crude Fiber — 12.8 %

QA13A - Ash
Completion Date: 04/11/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.
* Ash — 4.52 g/100 g

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Eurofins Sample Code: 468-2017-0407B103 **Client Sample Code: EUNFB3-CSPBWL-170213**

REPORT OF ANALYSIS

AR-17-QA-022922-01

Test Results	Result
QD161 - Fiber, Neutral Detergent	
Completion Date: 04/14/2017 **Method:** Ankom NDF	
* Fiber, Neutral Detergent	21.6 %
QD053 - Fiber, Dietary Complete	
Completion Date: 04/14/2017 **Method:** AOAC 991.43	
* Fiber, Dietary, Soluble - Calc	3.5 %
* Fiber, Dietary, Insoluble	32.6 %
* Fiber, Dietary, Total	36.1 %
QD002 - Fiber, Acid Detergent	
Completion Date: 04/14/2017 **Method:** Ankom ADF 05/03	
* Fiber, Acid Detergent	13.8 %
QQ129 - Sugar Profile (AOAC, Most Matrices)	
Completion Date: 04/14/2017 **Method:** AOAC 982.14, mod.	
* Fructose	<0.15 %
* Glucose	<0.15 %
* Lactose	<0.15 %
* Maltose	<0.15 %
* Sucrose	<0.15 %
* Total sugars	<0.35 %
UMHY9 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 04/14/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	47,000 cfu/g
KK03N - Aluminum	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Aluminium	3.00 ppm
KK03D - Arsenic	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic	0.037 ppm
KK03P - Boron	
Completion Date: 04/24/2017 **Method:**	

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Eurofins Sample Code: 468-2017-0407B103 **Client Sample Code: EUNFB3-CSPBWL-170213**

AR-17-QA-022922-01

REPORT OF ANALYSIS

Test Results	Result
* Boron	947 mg/kg
KK03A - Cadmium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium	0.01 ppm
KK02Y - Calcium	
Completion Date: 04/24/2017 **Method:**	
* Calcium	15,600 ppm
KK03E - Chromium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Chromium	0.118 ppm
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 04/13/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	Presumptive
KK03S - Cobalt	
Completion Date: 04/24/2017 **Method:**	
* Cobalt	<0.050 ppm
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 04/14/2017 **Method:** AOAC 991.14	
* Total Coliforms	<10 (est) cfu/g
UMVTK - Confirmation Clostridium Perfringens - AOAC 976.30	
Completion Date: 04/14/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	<10 cfu/g
UMBIP - Confirmation Escherichia coli - BAM Chapter 4	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 4	
* Escherichia coli - Confirmed	<3 MPN/g
KK03T - Copper	
Completion Date: 04/24/2017 **Method:**	
* Copper	1.95 ppm

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Eurofins Sample Code: 468-2017-0407B103 **Client Sample Code: EUNFB3-CSPBWL-170213**

AR-17-QA-022922-01

REPORT OF ANALYSIS

Test Results	Result
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 4	
* E. coli	Presumptive
KK03U - Iron	
Completion Date: 04/24/2017 **Method:**	
* Iron	237 ppm
KK03B - Lead	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Lead	0.009 ppm
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 04/14/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
KK04B - Magnesium	
Completion Date: 04/24/2017 **Method:**	
* Magnesium	3,430 ppm
KK03F - Manganese	
Completion Date: 04/24/2017 **Method:**	
* Manganese	157 ppm
KK03C - Mercury	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Mercury	<0.015 ppm
KK03W - Molybdenum	
Completion Date: 04/24/2017 **Method:**	
* Molybdenum	0.066 mg/kg
UM4BV - Moulds - BAM Chapter 18	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 18	
* Mold	10 (est) cfu/g

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Eurofins Sample Code: 468-2017-0407B103 **Client Sample Code: EUNFB3-CSPBWL-170213**

AR-17-QA-022922-01

REPORT OF ANALYSIS

Test Results	Result
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 18	
* Yeast	40 (est) cfu/g
KK04A - Phosphorus	
Completion Date: 04/24/2017 **Method:**	
* Phosphorus	4,670 ppm
KK03V - Potassium	
Completion Date: 04/24/2017 **Method:**	
* Potassium	3,160 ppm
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 04/14/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g
KK03G - Selenium	
Completion Date: 04/24/2017 **Method:**	
* Selenium	<0.30 ppm
KK03Z - Sodium	
Completion Date: 04/24/2017 **Method:**	
* Sodium	1,340 ppm
QD234 - AOAC Total Starch	
Completion Date: 04/14/2017 **Method:** AOAC 996.11	
* Starch (AOAC 996.11)	6.4 %
KK03H - Tin	
Completion Date: 04/24/2017 **Method:**	
* Tin	<0.15 ppm
KK02X - Zinc	
Completion Date: 04/24/2017 **Method:**	
* Zinc	57.0 ppm

**This is not covered by our current A2LA accreditation.*

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Eurofins Sample Code: 468-2017-0407B103 **Client Sample Code: EUNFB3-CSPBWL-170213**

AR-17-QA-022922-01

REPORT OF ANALYSIS

Test Results	Result

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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Uncertainty can be obtained upon request.

ACCREDITED TESTING CERT #2993-01

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Reporting Date 04/24/2017

AR-17-QA-022927-01

REPORT OF ANALYSIS

Sample Code 468-2017-0407B104

Sample Description	PROTEIN POWDER	**Reception Date**	04/07/2017
Client Sample Code	EUNFB4-CSPBWL-170308	**Reception Temperature**	25 (Celsius)
Sample Reference		**Sample Condition**	Acceptable
		Purchase Order	

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 04/12/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	1.60 %
QA821 - Crude Protein (Combustion)	
Completion Date: 04/12/2017 **Method:** AOAC 990.03	
* Crude Protein	45.26 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 04/12/2017 **Method:** AOAC 922.06	
* AH Fat	9.71 %
QA285 - Crude Fiber (Oilseed Meals, AOCS)	
Completion Date: 04/11/2017 **Method:** AOCS Ba 6a-05	
* Crude Fiber	10.2 %
QA13A - Ash	
Completion Date: 04/11/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	4.34 g/100 g

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Eurofins Sample Code: 468-2017-0407B104 **Client Sample Code: EUNFB4-CSPBWL-170308**

AR-17-QA-022927-01

REPORT OF ANALYSIS

Test Results	Result
QD161 - Fiber, Neutral Detergent	
Completion Date: 04/19/2017 **Method:** Ankom NDF	
* Fiber, Neutral Detergent	23.6 %
QD053 - Fiber, Dietary Complete	
Completion Date: 04/19/2017 **Method:** AOAC 991.43	
* Fiber, Dietary, Soluble - Calc	2.3 %
* Fiber, Dietary, Insoluble	31.1 %
* Fiber, Dietary, Total	33.4 %
QD002 - Fiber, Acid Detergent	
Completion Date: 04/19/2017 **Method:** Ankom ADF 05/03	
* Fiber, Acid Detergent	10.8 %
QQ129 - Sugar Profile (AOAC, Most Matrices)	
Completion Date: 04/19/2017 **Method:** AOAC 982.14, mod.	
* Fructose	<0.15 %
* Glucose	<0.15 %
* Lactose	<0.15 %
* Maltose	0.28 %
* Sucrose	<0.15 %
* Total sugars	<0.35 %
UMHY9 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 04/14/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	26,000 (est) cfu/g
KK03N - Aluminum	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Aluminium	3.100 ppm
KK03D - Arsenic	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic	0.038 ppm
KK03P - Boron	
Completion Date: 04/24/2017 **Method:**	

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Eurofins Sample Code: 468-2017-0407B104 **Client Sample Code: EUNFB4-CSPBWL-170308**

AR-17-QA-022927-01

REPORT OF ANALYSIS

Test Results	Result
* Boron	663 mg/kg
KK03A - Cadmium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium	0.006 ppm
KK02Y - Calcium	
Completion Date: 04/24/2017 **Method:**	
* Calcium	11,500 ppm
KK03E - Chromium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Chromium	<0.200 ppm
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 04/14/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	<10 (est) cfu/g
KK03S - Cobalt	
Completion Date: 04/24/2017 **Method:**	
* Cobalt	<0.050 ppm
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 04/14/2017 **Method:** AOAC 991.14	
* Total Coliforms	<10 (est) cfu/g
UMBIP - Confirmation Escherichia coli - BAM Chapter 4	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 4	
* Escherichia coli - Confirmed	<3 MPN/g
KK03T - Copper	
Completion Date: 04/24/2017 **Method:**	
* Copper	3.63 ppm
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 4	
* E. coli	Presumptive

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Eurofins Sample Code: 468-2017-0407B104 **Client Sample Code: EUNFB4-CSPBWL-170308**

AR-17-QA-022927-01

REPORT OF ANALYSIS

Test Results	Result
KK03U - Iron	
Completion Date: 04/24/2017 **Method:**	
* Iron	525 ppm
KK03B - Lead	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Lead	0.016 ppm
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 04/14/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
KK04B - Magnesium	
Completion Date: 04/24/2017 **Method:**	
* Magnesium	3,200 ppm
KK03F - Manganese	
Completion Date: 04/24/2017 **Method:**	
* Manganese	322 ppm
KK03C - Mercury	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Mercury	<0.015 ppm
KK03W - Molybdenum	
Completion Date: 04/24/2017 **Method:**	
* Molybdenum	0.122 mg/kg
UM4BV - Moulds - BAM Chapter 18	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 18	
* Mold	<10 (est) cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 04/14/2017 **Method:** FDA BAM Chapter 18	
* Yeast	<10 (est) cfu/g

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Eurofins Sample Code: 468-2017-0407B104 **Client Sample Code: EUNFB4-CSPBWL-170308**

AR-17-QA-022927-01

REPORT OF ANALYSIS

Test Results	Result
KK04A - Phosphorus	
Completion Date: 04/24/2017 **Method:**	
* Phosphorus	5,120 ppm
KK03V - Potassium	
Completion Date: 04/24/2017 **Method:**	
* Potassium	4,860 ppm
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 04/14/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g
KK03G - Selenium	
Completion Date: 04/24/2017 **Method:**	
* Selenium	<0.30 ppm
KK03Z - Sodium	
Completion Date: 04/24/2017 **Method:**	
* Sodium	1,660 ppm
QD234 - AOAC Total Starch	
Completion Date: 04/19/2017 **Method:** AOAC 996.11	
* Starch (AOAC 996.11)	6.9 %
KK03H - Tin	
Completion Date: 04/24/2017 **Method:**	
* Tin	<0.15 ppm
KK02X - Zinc	
Completion Date: 04/24/2017 **Method:**	
* Zinc	100 ppm

**This is not covered by our current A2LA accreditation.*

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Eurofins Sample Code: 468-2017-0407B104

Client Sample Code: EUNFB4-CSPBWL-170308

AR-17-QA-022927-01

REPORT OF ANALYSIS

Test Results	Result

Respectfully Submitted,
Eurofins Central Analytical Laboratories

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Cheryl D. Stephenson, Ph.D., Laboratory Director

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Uncertainty can be obtained upon request.

TESTING CERT #2993-01

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Client Support Cara Quintanilla

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ATTN: Molly Sproston
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Reporting Date 04/24/2017

AR-17-QA-022928-01

REPORT OF ANALYSIS

Sample Code 468-2017-0407B105

Sample Description	PROTEIN POWDER	**Reception Date**	04/07/2017
Client Sample Code	EUNFB5-CSPBWL-170310	**Reception Temperature**	25 (Celsius)
Sample Reference		**Sample Condition**	Acceptable
		Purchase Order	

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 04/12/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	2.33 %
QA821 - Crude Protein (Combustion)	
Completion Date: 04/12/2017 **Method:** AOAC 990.03	
* Crude Protein	42.92 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 04/12/2017 **Method:** AOAC 922.06	
* AH Fat	8.91 %
QA285 - Crude Fiber (Oilseed Meals, AOCS)	
Completion Date: 04/11/2017 **Method:** AOCS Ba 6a-05	
* Crude Fiber	9.9 %
QA13A - Ash	
Completion Date: 04/11/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	4.11 g/100 g

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Eurofins Sample Code: 468-2017-0407B105 **Client Sample Code: EUNFB5-CSPBWL-170310**

AR-17-QA-022928-01

REPORT OF ANALYSIS

Test Results	Result
QD161 - Fiber, Neutral Detergent	
Completion Date: 04/18/2017 **Method:** Ankom NDF	
* Fiber, Neutral Detergent	19.7 %
QD053 - Fiber, Dietary Complete	
Completion Date: 04/18/2017 **Method:** AOAC 991.43	
* Fiber, Dietary, Soluble - Calc	5.6 %
* Fiber, Dietary, Insoluble	25.6 %
* Fiber, Dietary, Total	31.2 %
QD002 - Fiber, Acid Detergent	
Completion Date: 04/18/2017 **Method:** Ankom ADF 05/03	
* Fiber, Acid Detergent	11.5 %
QQ129 - Sugar Profile (AOAC, Most Matrices)	
Completion Date: 04/18/2017 **Method:** AOAC 982.14, mod.	
* Fructose	<0.15 %
* Glucose	<0.15 %
* Lactose	<0.15 %
* Maltose	<0.15 %
* Sucrose	<0.15 %
* Total sugars	<0.35 %
UMHY9 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 04/17/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	120,000 cfu/g
KK03N - Aluminum	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Aluminium	2.0 ppm
KK03D - Arsenic	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic	0.041 ppm
KK03P - Boron	
Completion Date: 04/24/2017 **Method:**	

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Eurofins Sample Code: 468-2017-0407B105 **Client Sample Code: EUNFB5-CSPBWL-170310**

AR-17-QA-022928-01

REPORT OF ANALYSIS

Test Results	Result
* Boron	779 mg/kg
KK03A - Cadmium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium	0.006 ppm
KK02Y - Calcium	
Completion Date: 04/24/2017 **Method:**	
* Calcium	13,200 ppm
KK03E - Chromium	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Chromium	0.177 ppm
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 04/17/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	<10 (est) cfu/g
KK03S - Cobalt	
Completion Date: 04/24/2017 **Method:**	
* Cobalt	<0.050 ppm
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 04/17/2017 **Method:** AOAC 991.14	
* Total Coliforms	<10 (est) cfu/g
UMBIP - Confirmation Escherichia coli - BAM Chapter 4	
Completion Date: 04/17/2017 **Method:** FDA BAM Chapter 4	
* Escherichia coli - Confirmed	<3 MPN/g
KK03T - Copper	
Completion Date: 04/24/2017 **Method:**	
* Copper	2.88 ppm
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 04/17/2017 **Method:** FDA BAM Chapter 4	
* E. coli	Presumptive

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Eurofins Sample Code: 468-2017-0407B105 **Client Sample Code: EUNFB5-CSPBWL-170310**

AR-17-QA-022928-01

REPORT OF ANALYSIS

Test Results	Result
KK03U - Iron	
Completion Date: 04/24/2017 **Method:**	
* Iron	361 ppm
KK03B - Lead	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Lead	0.01 ppm
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 04/17/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
KK04B - Magnesium	
Completion Date: 04/24/2017 **Method:**	
* Magnesium	3,090 ppm
KK03F - Manganese	
Completion Date: 04/24/2017 **Method:**	
* Manganese	223 ppm
KK03C - Mercury	
Completion Date: 04/24/2017 **Method:** AOAC 993.14 Mod.	
* Mercury	<0.015 ppm
KK03W - Molybdenum	
Completion Date: 04/24/2017 **Method:**	
* Molybdenum	0.071 mg/kg
UM4BV - Moulds - BAM Chapter 18	
Completion Date: 04/17/2017 **Method:** FDA BAM Chapter 18	
* Mold	<10 (est) cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 04/17/2017 **Method:** FDA BAM Chapter 18	
* Yeast	20 (est) cfu/g

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Eurofins Sample Code: 468-2017-0407B105 **Client Sample Code: EUNFB5-CSPBWL-170310**

AR-17-QA-022928-01

REPORT OF ANALYSIS

Test Results	Result
KK04A - Phosphorus	
Completion Date: 04/24/2017 **Method:**	
* Phosphorus	4,730 ppm
KK03V - Potassium	
Completion Date: 04/24/2017 **Method:**	
* Potassium	3,820 ppm
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 04/17/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g
KK03G - Selenium	
Completion Date: 04/24/2017 **Method:**	
* Selenium	<0.30 ppm
KK03Z - Sodium	
Completion Date: 04/24/2017 **Method:**	
* Sodium	1,340 ppm
QD234 - AOAC Total Starch	
Completion Date: 04/18/2017 **Method:** AOAC 996.11	
* Starch (AOAC 996.11)	11.4 %
KK03H - Tin	
Completion Date: 04/24/2017 **Method:**	
* Tin	<0.15 ppm
KK02X - Zinc	
Completion Date: 04/24/2017 **Method:**	
* Zinc	76.4 ppm

**This is not covered by our current A2LA accreditation.*

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Eurofins Sample Code: 468-2017-0407B105 **Client Sample Code: EUNFB5-CSPBWL-170310**

AR-17-QA-022928-01

REPORT OF ANALYSIS

Test Results	Result

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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Reporting Date 03/10/2017

AR-17-QA-007284-04

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-007284-03.

Sample Code 468-2017-0120B057

Sample Description (GREEN POWDER)
Client Sample Code GRAS 3.2
Sample Reference

Reception Date 01/20/2017
Reception Temperature 25 (Celsius)
Sample Condition Acceptable
Purchase Order

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 01/26/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	5.61 %
QA821 - Crude Protein (Combustion)	
Completion Date: 01/25/2017 **Method:** AOAC 990.03	
* Crude Protein	41.93 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 01/26/2017 **Method:** AOAC 922.06	
* AH Fat	7.70 %
QA285 - Crude Fiber (Oilseed Meals, AOCS)	
Completion Date: 01/25/2017 **Method:** AOCS Ba 6a-05	
* Crude Fiber	12.8 %
QA13A - Ash	
Completion Date: 01/23/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	4.95 g/100 g

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Eurofins Sample Code: 468-2017-0120B057 **Client Sample Code: GRAS 3.2**

AR-17-QA-007284-04

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-007284-03.

Test Results	Result
QD161 - Fiber, Neutral Detergent	
Completion Date: 02/07/2017 **Method:** Ankom NDF	
* Fiber, Neutral Detergent	29.0 %
QD053 - Fiber, Dietary Complete	
Completion Date: 02/07/2017 **Method:** AOAC 991.43	
* Fiber, Dietary, Soluble - Calc	4.5 %
* Fiber, Dietary, Insoluble	31.4 %
* Fiber, Dietary, Total	35.9 %
QD002 - Fiber, Acid Detergent	
Completion Date: 02/07/2017 **Method:** Ankom ADF 05/03	
* Fiber, Acid Detergent	16.8 %
QQ129 - Sugar Profile (AOAC, Most Matrices)	
Completion Date: 02/07/2017 **Method:** AOAC 982.14, mod.	
* Fructose	<0.15 %
* Glucose	<0.15 %
* Lactose	<0.15 %
* Maltose	<0.15 %
* Sucrose	<0.15 %
* Total sugars	<0.35 %
KK03N - Aluminum	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Aluminium	<4.0 mg/kg
KK03D - Arsenic	
Completion Date: 02/16/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic	0.08 mg/kg
KK03P - Boron	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Boron	1,000 mg/kg

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Eurofins Sample Code: 468-2017-0120B057 **Client Sample Code: GRAS 3.2**

AR-17-QA-007284-04

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-007284-03.

Test Results	Result
KK03A - Cadmium	
Completion Date: 02/16/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium	<0.01 mg/kg
KK02Y - Calcium	
Completion Date: 02/08/2017 **Method:** AOAC 993.14	
* Calcium	13,600 mg/kg
KK03E - Chromium	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Chromium	<0.200 mg/kg
KK03S - Cobalt	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Cobalt	<0.050 mg/kg
KK03T - Copper	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Copper	2.74 mg/kg
KK03U - Iron	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Iron	301 mg/kg
KK03B - Lead	
Completion Date: 02/16/2017 **Method:** AOAC 993.14 Mod.	
* Lead	<0.02 mg/kg
KK04B - Magnesium	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Magnesium	3,460 mg/kg
KK03F - Manganese	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Manganese	128 mg/kg

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Eurofins Sample Code: 468-2017-0120B057 **Client Sample Code: GRAS 3.2**

AR-17-QA-007284-04

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-007284-03.

Test Results	Result
KK03C - Mercury	
Completion Date: 02/16/2017 **Method:** AOAC 993.14 Mod.	
* Mercury	<0.01 mg/kg
KK03W - Molybdenum	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Molybdenum	0.104 mg/kg
KK03Y - Nickel	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Nickel	0.038 mg/kg
KK04A - Phosphorus	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Phosphorus	4,660 mg/kg
KK03V - Potassium	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Potassium	3,670 mg/kg
KK03G - Selenium	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Selenium	0.17 mg/kg
KK03Z - Sodium	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Sodium	2,320 mg/kg
QD234 - AOAC Total Starch	
Completion Date: 02/07/2017 **Method:** AOAC 996.11	
* Starch (AOAC 996.11)	1.1 %
KK02X - Zinc	
Completion Date: 02/08/2017 **Method:** AOAC 993.14 Mod.	
* Zinc	40.2 mg/kg

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Eurofins Sample Code: 468-2017-0120B057 **Client Sample Code: GRAS 3.2**

AR-17-QA-007284-04

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-007284-03.

Test Results	Result
Comments	The values below reference ECAL sample number 468-2016-1114B250. This sample was received in a different shipment on 11/14/2016.

REPORT OF ANALYSIS

Sample Code	468-2016-1114B250
Description	GRAS 3.2

UMHY9 - Aerobic Plate Count - AOAC 990.12

Completion Date: 11/23/2016	**Method:** AOAC 990.12
Aerobic Plate Count	58,000 cfu/g

UMDBM - Coliforms - AOAC 991.14

Completion Date: 11/23/2016	**Method:** AOAC 991.14
Total Coliforms	Presumptive

UMDP5 - Confirmation Coliforms - CMMEF Chapter 8.7

Completion Date: 11/23/2016	**Method:** CMMEF Chapter 8.7
Total Coliforms	100 cfu/g

UMMA2 - Confirmation Escherichia coli - AOAC 988.19

Completion Date: 11/23/2016	**Method:** AOAC 988.19
E. coli	<3 MPN/g

UMVUE - Escherichia coli - BAM Chapter 4

Completion Date: 11/23/2016	**Method:** FDA BAM Chapter 4
E. coli	Presumptive

UM5RF - Listeria Species - AOAC RI #050903

Completion Date: 11/23/2016	**Method:** AOAC-RI 050903
Listeria spp.	Not Detected per 25 g

UM4BV - Moulds - BAM Chapter 18

Completion Date: 11/23/2016	**Method:** FDA BAM Chapter 18

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Eurofins Sample Code: 468-2017-0120B057

Client Sample Code: GRAS 3.2

AR-17-QA-007284-04

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-007284-03.

Test Results	**Result**
Mold	<10 (est) cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 11/23/2016	**Method:** FDA BAM Chapter 18
Yeast	<10 (est) cfu/g
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 11/23/2016	**Method:** AOAC 2003.09
Salmonella	Not Detected per 25 g

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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Uncertainty can be obtained upon request.

A2LA ACCREDITED TESTING CERT #2993-01

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

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Reporting Date 01/26/2017

AR-17-QA-004737-01

REPORT OF ANALYSIS

Sample Code 468-2017-0123B110

Sample Description	PROTEIN POWDER	**Reception Date**	01/23/2017
Client Sample Code	DGLC 170116	**Reception Temperature**	25 (Celsius)
Sample Reference		**Sample Condition**	Acceptable
		Purchase Order	

Test Results	Result
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 01/26/2017 **Method:** AOAC 922.06	
AH Fat	1.42 %
QA13A - Ash	
Completion Date: 01/24/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
Ash	6.51 g/100 g

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6)
[redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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Person in charge John M. Reuther
Client Support Cara Quintanilla

PARABEL USA, INC.
ATTN: Molly Sproston
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Reporting Date 06/06/2017

AR-17-QA-032014-03

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-032014-02.

Sample Code 468-2017-0526B056

Sample Description PROTEIN POWDER
Client Sample Code EUNFB6-DGLC-170523
Sample Reference

Reception Date 05/26/2017
Reception Temperature 25 (Celsius)
Sample Condition Acceptable
Purchase Order

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 05/30/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	7.24 %
QA821 - Crude Protein (Combustion)	
Completion Date: 05/31/2017 **Method:** AOAC 990.03	
* Crude Protein	36.32 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 05/31/2017 **Method:** AOAC 922.06	
* AH Fat	1.07 %
QA13A - Ash	
Completion Date: 05/30/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	4.04 g/100 g
QD230 - Fiber, Total Dietary	
Completion Date: 06/05/2017 **Method:** AOAC 991.43	
* Total Dietary Fiber	43.9 %

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

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Eurofins Sample Code: 468-2017-0526B056 **Client Sample Code: EUNFB6-DGLC-170523**

AR-17-QA-032014-03

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-032014-02.

Test Results	Result
UMDE0 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 06/02/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	530 cfu/g
QG00N - Arsenic	
Completion Date: 06/02/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic (As)	<0.02 ppm
QG00P - Cadmium	
Completion Date: 06/02/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium (Cd)	<0.01 ppm
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 06/02/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	< 10 cfu/g
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 06/02/2017 **Method:** AOAC 991.14	
* Total Coliforms	< 10 cfu/g
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 06/02/2017 **Method:** FDA BAM Chapter 4	
* E. coli	< 3 MPN/g
QG00R - Lead	
Completion Date: 06/02/2017 **Method:** AOAC 993.14 Mod.	
* Lead (Pb)	<0.02 ppm
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 06/02/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
QG00Q - Mercury	
Completion Date: 06/02/2017 **Method:** AOAC 993.14 Mod.	
* Mercury (Hg)	<0.01 ppm

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Eurofins Sample Code: 468-2017-0526B056 **Client Sample Code: EUNFB6-DGLC-170523**

AR-17-QA-032014-03

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-032014-02.

Test Results	Result
UM4BV - Moulds - BAM Chapter 18	
Completion Date: 06/02/2017 **Method:** FDA BAM Chapter 18	
* Mold	< 10 cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 06/02/2017 **Method:** FDA BAM Chapter 18	
* Yeast	< 10 cfu/g
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 06/02/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g

**This is not covered by our current A2LA accreditation.*

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6)

Cheryl D. Stephenson, Ph.D., Laboratory Director

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A2LA ACCREDITED TESTING CERT #2993-01

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Reporting Date 08/23/2017

AR-17-QA-049475-01

REPORT OF ANALYSIS

Sample Code 468-2017-0802B030

Sample Description PROTEIN POWDER
Client Sample Code DGLC170614_B1
Sample Reference

Reception Date 08/02/2017
Reception Temperature 25 (Celsius)
Sample Condition Acceptable
Purchase Order

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 08/08/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	5.96 %
QA821 - Crude Protein (Combustion)	
Completion Date: 08/08/2017 **Method:** AOAC 990.03	
* Crude Protein	44.45 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 08/03/2017 **Method:** AOAC 922.06	
* AH Fat	0.81 %
QA13A - Ash	
Completion Date: 08/03/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	6.47 g/100 g
QD230 - Fiber, Total Dietary	
Completion Date: 08/11/2017 **Method:** AOAC 991.43	
* Total Dietary Fiber	46.4 %

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

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Eurofins Sample Code: 468-2017-0802B030 **Client Sample Code: DGLC170614_B1**

AR-17-QA-049475-01

REPORT OF ANALYSIS

Test Results	Result
UMDE0 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 08/23/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	570 cfu/g
QG00N - Arsenic	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic (As)	<0.02 mg/kg
QG00P - Cadmium	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium (Cd)	<0.01 mg/kg
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 08/23/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	<10 cfu/g
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 08/23/2017 **Method:** AOAC 991.14	
* Total Coliforms	<10 cfu/g
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 08/23/2017 **Method:** FDA BAM Chapter 4	
* E. coli	<3 MPN/g
QG00R - Lead	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Lead (Pb)	<0.02 mg/kg
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 08/23/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
QG00Q - Mercury	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Mercury (Hg)	<0.01 mg/kg
UM4BV - Moulds - BAM Chapter 18	

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Eurofins Sample Code: 468-2017-0802B030 **Client Sample Code: DGLC170614_B1**

AR-17-QA-049475-01

REPORT OF ANALYSIS

Test Results	Result
Completion Date: 08/23/2017 **Method:** FDA BAM Chapter 18	
* Mold	20 (est) cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 08/23/2017 **Method:** FDA BAM Chapter 18	
* Yeast	<10 cfu/g
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 08/23/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g

**This is not covered by our current A2LA accreditation.*

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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A2LA ACCREDITED TESTING CERT #2993-01

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

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Reporting Date 10/11/2017

AR-17-QA-046669-05

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-046669-04.

Sample Code 468-2017-0802B031

Sample Description PROTEIN POWDER
Client Sample Code DGLC170619_B2
Sample Reference

Reception Date 08/02/2017
Reception Temperature 25 (Celsius)
Sample Condition Acceptable
Purchase Order

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 08/08/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	5.90 %
QA821 - Crude Protein (Combustion)	
Completion Date: 08/08/2017 **Method:** AOAC 990.03	
* Crude Protein	44.22 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 08/03/2017 **Method:** AOAC 922.06	
* AH Fat	0.83 %
QA13A - Ash	
Completion Date: 08/03/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	6.37 g/100 g
QD230 - Fiber, Total Dietary	
Completion Date: 08/11/2017 **Method:** AOAC 991.43	
* Total Dietary Fiber	44.5 %

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

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Eurofins Sample Code: 468-2017-0802B031 **Client Sample Code: DGLC170619_B2**

AR-17-QA-046669-05

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-046669-04.

Test Results	Result
UMDE0 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 08/08/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	19,000 cfu/g
QG00N - Arsenic	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic (As)	0.02 mg/kg
QG00P - Cadmium	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium (Cd)	<0.01 mg/kg
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 08/08/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	< 10 cfu/g
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 08/08/2017 **Method:** AOAC 991.14	
* Coliforms	< 10 cfu/g
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 4	
* Escherichia coli	< 3 MPN/g
QG00R - Lead	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Lead (Pb)	0.03 mg/kg
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 08/08/2017 **Method:** AOAC-RI 050903	
* Listeria Species	Not Detected per 25 g
QG00Q - Mercury	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Mercury (Hg)	<0.01 mg/kg

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Eurofins Sample Code: 468-2017-0802B031 **Client Sample Code: DGLC170619_B2**

AR-17-QA-046669-05

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-046669-04.

Test Results	Result
UM4BV - Moulds - BAM Chapter 18	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 18	
* Moulds	< 10 cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 18	
* Yeast	< 10 cfu/g
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 08/08/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g

**This is not covered by our current A2LA accreditation.*

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6)

Cheryl D. Stephenson, Ph.D., Laboratory Director

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A2LA ACCREDITED TESTING CERT #2993-01

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Person in charge John M. Reuther
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PARABEL USA, INC.
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Reporting Date 08/11/2017

AR-17-QA-046670-01

REPORT OF ANALYSIS

Sample Code 468-2017-0802B032

Sample Description PROTEIN POWDER
Client Sample Code DGLC170621_B3
Sample Reference

Reception Date 08/02/2017
Reception Temperature 25 (Celsius)
Sample Condition Acceptable
Purchase Order

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 08/08/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	5.94 %
QA821 - Crude Protein (Combustion)	
Completion Date: 08/08/2017 **Method:** AOAC 990.03	
* Crude Protein	43.68 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 08/03/2017 **Method:** AOAC 922.06	
* AH Fat	0.63 %
QA13A - Ash	
Completion Date: 08/03/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	6.21 g/100 g
QD230 - Fiber, Total Dietary	
Completion Date: 08/11/2017 **Method:** AOAC 991.43	
* Total Dietary Fiber	43.4 %

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Eurofins Sample Code: 468-2017-0802B032 **Client Sample Code: DGLC170621_B3**

AR-17-QA-046670-01

REPORT OF ANALYSIS

Test Results	Result
UMDE0 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 08/08/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	2,600 (est) cfu/g
QG00N - Arsenic	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic (As)	0.02 mg/kg
QG00P - Cadmium	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium (Cd)	<0.01 mg/kg
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 08/08/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	< 10 cfu/g
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 08/08/2017 **Method:** AOAC 991.14	
* Total Coliforms	< 10 cfu/g
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 4	
* E. coli	< 3 MPN/g
QG00R - Lead	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Lead (Pb)	<0.02 mg/kg
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 08/08/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
QG00Q - Mercury	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Mercury (Hg)	<0.01 mg/kg
UM4BV - Moulds - BAM Chapter 18	

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Eurofins Sample Code: 468-2017-0802B032 **Client Sample Code: DGLC170621_B3**

AR-17-QA-046670-01

REPORT OF ANALYSIS

Test Results	Result
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 18	
* Mold	10 (est) cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 18	
* Yeast	< 10 cfu/g
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 08/08/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g

**This is not covered by our current A2LA accreditation.*

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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A2LA ACCREDITED TESTING CERT #2993-01

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Reporting Date 10/11/2017

AR-17-QA-046671-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-046671-01.

Sample Code 468-2017-0802B033

Sample Description	PROTEIN POWDER	**Reception Date**	08/02/2017
Client Sample Code	DGLC170710_B4	**Reception Temperature**	25 (Celsius)
Sample Reference		**Sample Condition**	Acceptable
		Purchase Order	

Test Results	Result
QA354 - Moisture (Air Oven 130C 2 hrs)	
Completion Date: 08/08/2017 **Method:** AOCS Ba 2a-38	
Moisture and Volatile Matter	6.53 %
QA821 - Crude Protein (Combustion)	
Completion Date: 08/08/2017 **Method:** AOAC 990.03	
* Crude Protein	42.03 %
QA265 - Crude Fat (Acid Hydrolysis)	
Completion Date: 08/03/2017 **Method:** AOAC 922.06	
* AH Fat	0.97 %
QA13A - Ash	
Completion Date: 08/03/2017 **Method:** AOAC 923.03 / 32.1.05 16th Ed.	
* Ash	7.11 g/100 g
QD230 - Fiber, Total Dietary	
Completion Date: 08/11/2017 **Method:** AOAC 991.43	
* Total Dietary Fiber	44.7 %

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Eurofins Sample Code: 468-2017-0802B033 **Client Sample Code: DGLC170710_B4**

AR-17-QA-046671-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-046671-01.

Test Results	Result
UMDE0 - Aerobic Plate Count - AOAC 990.12	
Completion Date: 08/08/2017 **Method:** AOAC 990.12	
* Aerobic Plate Count	6,700 cfu/g
QG00N - Arsenic	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Arsenic (As)	0.02 mg/kg
QG00P - Cadmium	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Cadmium (Cd)	<0.01 mg/kg
UMGVG - Clostridium Perfringens - AOAC 976.30	
Completion Date: 08/08/2017 **Method:** AOAC 976.30	
* Clostridium Perfringens	< 10 cfu/g
UMDBM - Coliforms - AOAC 991.14	
Completion Date: 08/08/2017 **Method:** AOAC 991.14	
* Total Coliforms	< 10 cfu/g
UMVUE - Escherichia coli - BAM Chapter 4	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 4	
* E. coli	< 3 MPN/g
QG00R - Lead	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Lead (Pb)	<0.02 mg/kg
UM5RF - Listeria Species - AOAC RI #050903	
Completion Date: 08/08/2017 **Method:** AOAC-RI 050903	
* Listeria spp.	Not Detected per 25 g
QG00Q - Mercury	
Completion Date: 08/08/2017 **Method:** AOAC 993.14 Mod.	
* Mercury (Hg)	<0.01 mg/kg

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Eurofins Sample Code: 468-2017-0802B033 **Client Sample Code: DGLC170710_B4**

AR-17-QA-046671-02

REPORT OF ANALYSIS

This analytical report supersedes AR-17-QA-046671-01.

Test Results	Result
UM4BV - Moulds - BAM Chapter 18	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 18	
* Mold	40 (est) cfu/g
UM4BV - Yeast - BAM Chapter 18	
Completion Date: 08/08/2017 **Method:** FDA BAM Chapter 18	
* Yeast	< 10 cfu/g
UMAEK - Salmonella - AOAC 2003.09	
Completion Date: 08/08/2017 **Method:** AOAC 2003.09	
* Salmonella	Not Detected per 25 g

**This is not covered by our current A2LA accreditation.*

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

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A2LA ACCREDITED TESTING CERT #2993-01

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A2LA ISO/IEC 17025:2005
Best Aquaculture Practices
International Olive Council
American Oil Chemists Society
Grain and Feed Trade Association
Federation of Oils, Seed, and Fats Associations, Ltd.
Japanese Ministry of Health and Welfare
Association of Official Analytical Chemists
United States Department of Agriculture

Appendix A2: Amino Acid and Oxalic Acid Data of LENTEIN™ Complete and DGLC

Final Report

Medallion Labs

www.medallionlabs.com
1-800-245-5615 info@medlabs.com

Completion Date: April 26, 2017
Date Submitted: April 10, 2017
Medallion Company ID: PARABEL01
Company Code: 15223

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Email: msproston@parabel.com

Library Number: 2017-MED-4645
PO Number: Sproston10
Fax:

Medallion Labs Sample ID: 2017-MED-4645-01 Protein Powder
Customer Sample ID: EUNFb1-CSPBWL-170207

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	04/10/17
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	0.246 %	04/15/17
	Citric Acid	0.011 %	
	Malic Acid	<0.001 %	
	Lactic Acid	0.127 %	
	Tartaric Acid	0.002 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.01 %	
	Gluconic Acid	0.859 %	
	Oxalic Acid	0.032 %	
	Propionic Acid	0.007 %	
	Quinic Acid	0.023 %	
	Succinic Acid	0.008 %	
Fats, by Gas Chromatography	Total Fat	6.19 %	04/20/17
	Saturated Fat	1.17 %	
	Monounsaturated Fat	0.13 %	
	cis-cis Polyunsaturated Fat	4.48 %	
	trans Fat	0.13 %	
[1] [2] Amino Acids	HydroxyProline	ND %	04/25/17
	Aspartic Acid	3.203 %	
	Threonine	1.549 %	
	Serine	1.466 %	
	Glutamic Acid	3.838 %	
	Proline	1.466 %	
	Glycine	1.630 %	
	Alanine	1.972 %	
	Valine	2.093 %	
	Isoleucine	1.710 %	
	Leucine	3.099 %	
	Tyrosine	1.419 %	

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Unless otherwise noted above, samples were received in acceptable condition and analyzed as received.

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Final Report

Medallion Labs

www.medallionlabs.com
1-800-245-5615 info@medlabs.com

Completion Date: April 26, 2017
Date Submitted: April 10, 2017
Medallion Company ID: PARABEL01
Company Code: 15223

Molly Sproston
Parabel, Inc.
14655 101st Street
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Email: msproston@parabel.com

Library Number: 2017-MED-4645
PO Number: Sproston10
Fax:

Medallion Labs Sample ID: 2017-MED-4645-01 Protein Powder
Customer Sample ID: EUNFb1-CSPBWL-170207

Assay Group	Test	Results	Test Date
	Phenylalanine	2.041 %	
	Lysine	2.405 %	
	Histidine	0.785 %	
	Arginine	2.354 %	
	Total Hydrolyzed Amino Acids	31.030 %	
[1] [2] Cysteine and Methionine	Cysteine	0.423 %	04/25/17
	Methionine	0.763 %	
[1] [2] Tryptophan	Tryptophan	0.99 %	04/17/17

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Completion Date: April 26, 2017
Date Submitted: April 10, 2017
Medallion Company ID: PARABEL01
Company Code: 15223

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Library Number: 2017-MED-4645
PO Number: Sproston10
Fax:

Medallion Labs Sample ID: 2017-MED-4645-02 Protein Powder
Customer Sample ID: EUNFb2-CSPBWL-170209

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	04/10/17
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	0.156 %	04/15/17
	Citric Acid	0.009 %	
	Malic Acid	0.009 %	
	Lactic Acid	0.108 %	
	Tartaric Acid	<0.001 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.01 %	
	Gluconic Acid	1.282 %	
	Oxalic Acid	0.037 %	
	Propionic Acid	0.003 %	
	Quinic Acid	0.019 %	
	Succinic Acid	0.006 %	
Fats, by Gas Chromatography	Total Fat	6.21 %	04/20/17
	Saturated Fat	1.28 %	
	Monounsaturated Fat	0.16 %	
	cis-cis Polyunsaturated Fat	4.36 %	
	trans Fat	0.14 %	
[1] [2] Amino Acids	HydroxyProline	ND %	04/25/17
	Aspartic Acid	3.408 %	
	Threonine	1.660 %	
	Serine	1.583 %	
	Glutamic Acid	4.075 %	
	Proline	1.565 %	
	Glycine	1.748 %	
	Alanine	2.104 %	
	Valine	2.232 %	
	Isoleucine	1.823 %	
	Leucine	3.319 %	
	Tyrosine	1.534 %	

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Completion Date: April 26, 2017
Date Submitted: April 10, 2017
Medallion Company ID: PARABEL01
Company Code: 15223

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Library Number: 2017-MED-4645
PO Number: Sproston10
Fax:

Medallion Labs Sample ID: 2017-MED-4645-02 Protein Powder
Customer Sample ID: EUNFb2-CSPBWL-170209

Assay Group	Test	Results	Test Date
	Phenylalanine	2.188 %	
	Lysine	2.569 %	
	Histidine	0.839 %	
	Arginine	2.518 %	
	Total Hydrolyzed Amino Acids	33.165 %	
[1] [2] Cysteine and Methionine	Cysteine	0.452 %	04/25/17
	Methionine	0.795 %	
[1] [2] Tryptophan	Tryptophan	1.05 %	04/17/17

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Final Report

Medallion Labs

Completion Date:	April 26, 2017
Date Submitted:	April 10, 2017
Medallion Company ID:	PARABEL01
Company Code:	15223

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Library Number: 2017-MED-4645
PO Number: Sproston10
Fax:

Medallion Labs Sample ID: 2017-MED-4645-03 Protein Powder
Customer Sample ID: EUNFb5-CSPBWL-170310

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	04/10/17
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	0.143 %	04/15/17
	Citric Acid	0.007 %	
	Malic Acid	<0.001 %	
	Lactic Acid	0.082 %	
	Tartaric Acid	0.002 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.01 %	
	Gluconic Acid	0.107 %	
	Oxalic Acid	0.026 %	
	Propionic Acid	0.002 %	
	Quinic Acid	0.020 %	
	Succinic Acid	0.002 %	
Fats, by Gas Chromatography	Total Fat	6.22 %	04/20/17
	Saturated Fat	1.19 %	
	Monounsaturated Fat	0.15 %	
	cis-cis Polyunsaturated Fat	4.47 %	
	trans Fat	0.13 %	
[1] [2] Amino Acids	HydroxyProline	ND %	04/25/17
	Aspartic Acid	3.230 %	
	Threonine	1.583 %	
	Serine	1.496 %	
	Glutamic Acid	3.861 %	
	Proline	1.500 %	
	Glycine	1.663 %	
	Alanine	1.997 %	
	Valine	2.111 %	
	Isoleucine	1.725 %	
	Leucine	3.168 %	
	Tyrosine	1.442 %	

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Date Issued: April 26, 2017 Medallion Labs 9000 Plymouth Ave. N., Minneapolis, MN 55427 Page 5 of 6

Final Report

Medallion Labs

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Completion Date: April 26, 2017
Date Submitted: April 10, 2017
Medallion Company ID: PARABEL01
Company Code: 15223

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Library Number: 2017-MED-4645
PO Number: Sproston10
Fax:

Medallion Labs Sample ID: 2017-MED-4645-03 Protein Powder
Customer Sample ID: EUNFb5-CSPBWL-170310

Assay Group	Test	Results	Test Date
	Phenylalanine	2.097 %	
	Lysine	2.430 %	
	Histidine	0.802 %	
	Arginine	2.342 %	
	Total Hydrolyzed Amino Acids	31.447 %	
[1] [2] Cysteine and Methionine	Cysteine	0.427 %	04/25/17
	Methionine	0.775 %	
[1] [2] Tryptophan	Tryptophan	1.03 %	04/17/17

Results Approved By: Michael Aufderhar (Authorized Reviewer)

Method References:

Assay Group	Method Reference
Amino Acids	Please contact for method details
Cysteine and Methionine	Please contact for method details
Fats, by Gas Chromatography	AOAC: 996.06*
ORGAN_ACID_PROFILE	Please contact for method details
Tryptophan	Please contact for method details

* This method has been modified.

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Final Report

Medallion Labs

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Completion Date: February 10, 2017
Date Submitted: January 23, 2017
Medallion Company ID: PARABEL01
Company Code: 15223

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Email: gkasat@parabel.com

Library Number: 2017-MED-0879
PO Number: Kasat23

Fax:

Medallion Labs Sample ID: 2017-MED-0879-02 Lentein Complete
Customer Sample ID: Gras 3.2

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	01/23/17
[1] [2] Amino Acids	HydroxyProline	ND %	02/10/17
	Aspartic Acid	3.151 %	
	Threonine	1.535 %	
	Serine	1.431 %	
	Glutamic Acid	3.781 %	
	Proline	1.490 %	
	Glycine	1.637 %	
	Alanine	1.972 %	
	Valine	2.069 %	
	Isoleucine	1.701 %	
	Leucine	3.101 %	
	Tyrosine	1.415 %	
	Phenylalanine	2.047 %	
	Lysine	2.317 %	
	Histidine	0.805 %	
	Arginine	2.218 %	
	Total Hydrolyzed Amino Acids	30.670 %	
[1] [2] Cysteine and Methionine	Cysteine	0.429 %	02/10/17
	Methionine	0.757 %	
[1] [2] Tryptophan	Tryptophan	1.00 %	02/01/17

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Final Report

Medallion Labs

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Completion Date: December 28, 2016
Date Submitted: December 16, 2016
Medallion Company ID: PARABEL01
Company Code: 15223

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Library Number: 2016-MED-17359
PO Number: Kasat16
Fax:

Medallion Labs Sample ID: 2016-MED-17359-02 Lentein Complete
Customer Sample ID: Gras 3.2

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	12/16/16
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	0.092 %	12/27/16
	Citric Acid	0.030 %	
	Malic Acid	<0.001 %	
	Lactic Acid	<0.001 %	
	Tartaric Acid	<0.001 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.001 %	
	Gluconic Acid	0.881 %	
	Oxalic Acid	0.057 %	
	Propionic Acid	<0.001 %	
	Quinic Acid	<0.001 %	
	Succinic Acid	1.665 %	

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2219 Lakeshore Drive
New Orleans, LA 70122
www.centralanalytical.com
Tel.+1 504 297 3400
Fax:+1 504 297 3410

Person in charge John M. Reuther
Client Support Cara Quintanilla

PARABEL USA, INC.
ATTN: Molly Sproston
14655 101ST. STREET
FELLSMERE, FL 32948

Reporting Date 10/04/2017

AR-17-QA-058833-01

REPORT OF ANALYSIS

Sample Code 468-2017-0927B073

Sample Description	PROTEIN POWDER	**Reception Date**	09/27/2017
Client Sample Code	DGLC- 170920--RL-30	**Reception Temperature**	25 (Celsius)
Sample Reference		**Sample Condition**	Acceptable
		Purchase Order	2016-000823

Test Results	Result
QQ141 - Tryptophan (AOAC, Most Matrices)	
Completion Date: 10/04/2017 **Method:** AOAC 988.15	
Tryptophan	1.01 %
QQ176 - Amino Acids by AH (AOAC, Most Matrices)	
Completion Date: 10/04/2017 **Method:** AOAC 982.30 mod.	
Alanine	2.88 %
Arginine	2.80 %
Aspartic Acid	4.23 %
Glutamic Acid	4.88 %
Glycine	2.58 %
Histidine	1.02 %
Isoleucine	2.29 %
Leucine	4.09 %
Phenylalanine	2.51 %
Proline	2.08 %
Serine	2.12 %
Threonine	2.09 %
Total Lysine	3.42 %
Tyrosine	1.56 %

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Eurofins Sample Code: 468-2017-0927B073 **Client Sample Code: DGLC- 170920--RL-30**

AR-17-QA-058833-01

REPORT OF ANALYSIS

Test Results	Result
Valine	2.83 %

QQ177 - Cystine & Methionine (AOAC, Most Matrices)
Completion Date: 10/04/2017 **Method:** AOAC 994.12 mod.

Cystine	0.52 %
Methionine	0.94 %

Respectfully Submitted,
Eurofins Central Analytical Laboratories

(b) (6) [redacted]

Cheryl D. Stephenson, Ph.D., Laboratory Director

Results shown in this report relate solely to the item submitted for analysis.
Uncertainty can be obtained upon request.

Final Report

Medallion Labs

www.medallionlabs.com
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Completion Date: August 18, 2017
Date Submitted: August 03, 2017
Medallion Company ID: PARABEL01
Company Code: 15223

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Library Number: 2017-MED-9760
PO Number: 2016-000642

Medallion Labs Sample ID: 2017-MED-9760-01 Protein Powder
Customer Sample ID: DGLC170614_b1

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	08/03/17
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	0.040 %	08/18/17
	Citric Acid	0.020 %	
	Malic Acid	<0.001 %	
	Lactic Acid	0.020 %	
	Tartaric Acid	<0.001 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.001 %	
	Gluconic Acid	0.097 %	
	Oxalic Acid	<0.001 %	
	Propionic Acid	<0.001 %	
	Quinic Acid	<0.001 %	
	Succinic Acid	<0.001 %	

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Completion Date: August 18, 2017
Date Submitted: August 03, 2017
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Library Number: 2017-MED-9760
PO Number: 2016-000642
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Medallion Labs Sample ID: 2017-MED-9760-02 Protein Powder
Customer Sample ID: DGLC170619_b2

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	08/03/17
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	0.030 %	08/18/17
	Citric Acid	0.010 %	
	Malic Acid	<0.001 %	
	Lactic Acid	0.020 %	
	Tartaric Acid	<0.001 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.001 %	
	Gluconic Acid	0.150 %	
	Oxalic Acid	<0.001 %	
	Propionic Acid	0.003 %	
	Quinic Acid	<0.001 %	
	Succinic Acid	<0.001 %	

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Completion Date: August 18, 2017
Date Submitted: August 03, 2017
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Library Number: 2017-MED-9760
PO Number: 2016-000642

Fax:

Medallion Labs Sample ID: 2017-MED-9760-03 Protein Powder
Customer Sample ID: DGLC170621_b3

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	08/03/17
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	<0.001 %	08/18/17
	Citric Acid	<0.001 %	
	Malic Acid	<0.001 %	
	Lactic Acid	0.018 %	
	Tartaric Acid	0.005 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.001 %	
	Gluconic Acid	0.143 %	
	Oxalic Acid	<0.001 %	
	Propionic Acid	0.008 %	
	Quinic Acid	<0.001 %	
	Succinic Acid	<0.001 %	

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Completion Date:	August 18, 2017
Date Submitted:	August 03, 2017
Medallion Company ID:	PARABEL01
Company Code:	15223

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Library Number: 2017-MED-9760
PO Number: 2016-000642
Fax:

Medallion Labs Sample ID: 2017-MED-9760-04 Protein Powder
Customer Sample ID: DGLC170710_b4

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	08/03/17
[1] [2] ORGAN_ACID_PROFILE	Acetic Acid	0.030 %	08/18/17
	Citric Acid	0.020 %	
	Malic Acid	<0.001 %	
	Lactic Acid	0.030 %	
	Tartaric Acid	<0.001 %	
	Fumaric Acid	<0.001 %	
	Butyric Acid	<0.001 %	
	Gluconic Acid	0.234 %	
	Oxalic Acid	<0.001 %	
	Propionic Acid	0.010 %	
	Quinic Acid	<0.001 %	
	Succinic Acid	<0.001 %	

Results Approved By: Alyssa Ofsthun (Authorized Reviewer)

Method References:

Assay Group	Method Reference
ORGAN_ACID_PROFILE	Please contact for method details

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