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February 8, 2021


Via Fed.Ex

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5100 Campus Drive
College Park, Maryland 20740

**Re: GRAS Notice for f3-Lactoglobulin (Lacprodan® BLG);**
**Our File No.: AR14104-04**

Dear Sir or Madam:

We respectfully submit the enclosed GRAS Notice as both a hard copy and in electronic format *(i.e.,* compact disk) on behalf of our client, Arla Foods Ingredients Group P/S in support of this notice determining that beta-lactoglobulin (BLG) from cow's milk is generally recognized as safe (GRAS) for use in foods and medical foods that currently use a source of dietary protein from milk. The enclosed GRAS notice provides detailed information related to the intended uses, manufacturing, and safety of BLG.

We look forward to FDA's review of this submission and would be happy to answer any questions. Thank you for your consideration of the enclosed submission.

Cordially yours,

Natalie E. Rainer

Enclosures

**GRAS Notice for β-Lactoglobulin (Lacprodan® BLG)**

U.S. Food and Drug Administration
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
4300 River Road
College Park, MD 20740

Prepared by: Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20001

Date: February 8, 2021

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## Part 1. Signed Statements and Certification

### 1. Applicability of 21 C.F.R. part 170, subpart E

In accordance with Subpart E ("Generally Recognized as Safe (GRAS) Notice") of 21 C.F.R. Part 170 ("Food additives"), Keller and Heckman LLP submits the enclosed information on behalf of our client, Arla Foods Ingredients Group P/S ("Arla").

The analytical data, published studies, and information that are the basis for this GRAS determination are available for FDA review and copying at reasonable times at Keller and Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001, or will be sent to FDA upon request.

### 2. Name and Address of the Notifier

Arla Foods Ingredients Group P/S
Sønderhøj 10-12, 8260 Viby J
Denmark

All communications on this matter are to be sent to Counsel for the Notifier:

Natalie E. Rainer
Keller and Heckman LLP
Three Embarcadero Center
Suite 1420
San Francisco, CA 94111
Telephone: (415) 948-2821
Facsimile: (415) 948-2808
Email: rainer@khlaw.com

### 3. Names of the notified substance

β-Lactoglobulin
Beta-lactoglobulin
Lacprodan® BLG
BLG

### 4. Applicable conditions of use of the notified substance

Arla intends to market Lacprodan® BLG as a source of protein for general use in foods and medical foods that currently use protein from milk as a source of dietary protein.

### 5. Basis for the GRAS determination

Keller and Heckman LLP, on behalf of Arla Foods, hereby notifies the Agency of its determination that its β-lactoglobulin isolate from cow's milk is GRAS for its intended use, consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). This GRAS conclusion is based on scientific procedures in accordance with 21 C.F.R. §170.30(a) and

(b) and conforms to the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. §170.36, 81 Fed. Reg. 54,960 (Aug. 17, 2016). The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with proposed 21 C.F.R. § 170.36. The GRAS status of β-lactoglobulin is based on data generally available in the public domain and on the long history of milk and milk derived protein consumption in human foods.

**6. Exclusion from premarket approval**

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

**7. Availability of data and information**

The information for this GRAS conclusion including analytical data, published studies, and information that are the basis for this GRAS determination are available to FDA upon request as required by 21 C.F.R. § 170.225(c)(7)(ii)(A) or (B) by contacting Keller and Heckman LLP at the below address.

Natalie E. Rainer
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Three Embarcadero Center
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**8. Applicability of FOIA exemptions**

Arla Foods is not claiming any information in Parts 2 through 7 of this document as trade secret, confidential or financial information that is privileged or confidential. Thus, all information and data in this submission are not exempt from the Freedom of Information Act (FOIA), 5 U.S.C. Section 552.

**9. Certification**

I hereby certify that, to the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance

Signature: [redacted] Date: February 8, 2021

Natalie E. Rainer
Counsel to Arla

**Part 2. Identity, method of manufacture, specifications, and physical or technical effect**

**1. Scientific data and information that identifies the notified substance**

**a. Common or usual name:**

β-Lactoglobulin
Beta-lactoglobulin
BLG

**2. Identity**

Lacprodan® BLG is a product extracted from bovine whey via crystallization and further processed by industry standard ultrafiltration processes.

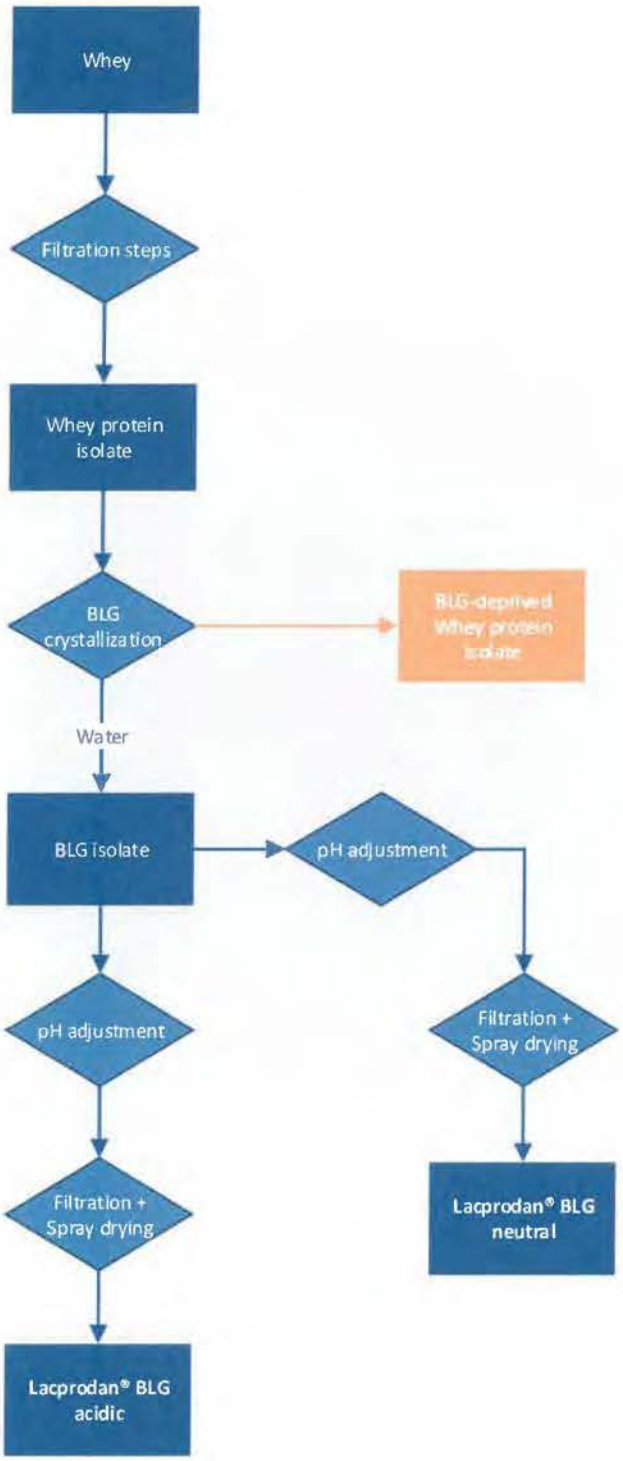
β-Lactoglobulin (BLG) is present in whey at a level of about 50 - 58% (w/w) of the total protein content (Jovanovic *et al.* 2007; Madureira *et al.* 2007). There are two predominant forms of BLG named A and B. Not only is BLG the predominant protein in whey but it is also substantially equivalent to normal whey protein concentrate/isolate in terms of its nutritional value, intended use and levels of undesirable substances. In Lacprodan® BLG, BLG constitutes more than 90% of total protein.

**a. Manufacturing Process**

Lacprodan® BLG is produced from whey which is kept in storage tanks at 5°C until it undergoes pasteurization and ultrafiltration. The whey is further filtrated to remove lactose, minerals, and fat. The resulting product is pH adjusted with suitable pH adjusting ingredients, followed by additional ultrafiltration and crystallization. The crystal solution is decanted to isolate the BLG crystals from the soluble components. The BLG crystals are washed and separated from the mother liquor (ML) and the BLG stream is collected in a tank. The product can go through additional pH adjustment and filtration steps to produce acidic or neutral BLG prior to spray drying.[1] **Figure 1** summarizes the Lacprodan® BLG production process.

[1] Arla offers acidic and neutral flavors based on the customer's preference. Acidic BLG is used in products with acidic flavors (*e.g.*, lemon, lime, orange, pineapple). Neutral pH is used in flavors that are less acidic (*e.g.*, mango, watermelon, strawberry).

**Figure 1: Lacprodan® BLG Production Process Flow Chart**



## 3. Product Specifications

Arla provides compositional and microbiological specifications for its Lacprodan® BLG, as summarized in **Tables 1** and **2** below.

**Table 1. Acidic Lacprodan® BLG Compositional Specifications**

| Parameter | Limit | Method |
|---|---|---|
| **Chemical** | | |
| Protein as is (Nx6.38) [%] | ≥ 86.0 | ISO 8968-3:2004 / IDF 20-3:2004 |
| Protein in dry matter (Nx6.38) [%] | ≥ 90.0 | Calculation |
| BLG % of protein [%] | ≥ 90.0 | AFI RP-HPLC |
| Lactose [%] | ≤ 0.2 | ISO 5765-2/ IDF 79-2:2002 |
| Fat [%] | ≤ 0.5 | ISO 1736/IDF9:2008 |
| Ash [%] | ≤ 4.5 | INMKL 173:2005 |
| Moisture [%] | ≤ 5.5 | ISO 6731:2010/IDF 21:2010 |
| **Physical** | | |
| Solubility index [mL] | ≤ 0.3 | ISO 8156:2005 / IDF 129:2005 |
| Scorched particles | Disc A | ADPI Bulletin 916 |
| Appearance | White to cream powder | Visual inspection. AFI[1] sensoric analysis |
| Flavor/odor | No off Flavor/ odor | AFI sensoric analysis |
| Texture | Homogenous | AFI sensoric analysis |
| pH (10% solution) | 3.5 – 4.0 | ISO 5546 / IDF 115 |
| **Minerals** | | |
| Sodium [%] | ≤ 0.1 | AFI ICP analysis |
| Magnesium [%] | ≤ 0.1 | AFI ICP analysis |
| Phosphorus [%] | ≤ 0.03 | AFI ICP analysis |
| Chloride [%] | ≤ 1.9 | ISO 5943:2006 / IDF 88:2006 |
| Potassium [%] | ≤ 0.1 | AFI ICP analysis |
| Calcium [%] | ≤ 0.05 | AFI ICP analysis |
| **Microbiological** | | |
| Total plate count (30°C) [CFU/g] | ≤ 10000 | ISO 4833-1:2013 |
| *Enterobacteriaceae* [CFU/g] | <10 | ISO 21528-2-2:2017 |
| Yeast/mould [CFU/g] | <10 | ISO 6611:2004/IDF 94:2004 |
| *Bacillus cereus* [CFU/g] | <100 | ISO 7932:2004 |
| Sulphite reducing *Clostridia* [CFU/g] | <100 | ISO 15213:2003 |
| *Staphylococcus aureus* (in 1g) | Absent | ISO 6888-1:1999/Amd.1:2003 |
| *Salmonella* (in 125g) | Absent | ISO 6579 |
| *Listeria* (in 25g) | Absent | ISO 11290 |

**Table 2. Neutral Lacprodan® BLG Compositional Specifications**

| Parameter | Limit | Method |
|---|---|---|
| **Chemical** | | |
| Protein as is (Nx6.38) [%] | ≥ 86.0 | ISO 8968-3:2004 / IDF 20-3:2004 |
| Protein in dry matter (Nx6.38) [%] | ≥ 90.0 | Calculation |
| BLG % of protein [%] | ≥ 90.0 | AFI RP-HPLC |
| Lactose [%] | ≤ 1.0 | ISO 5765-2/ IDF 79-2:2002 |
| Fat [%] | ≤ 1.0 | ISO 1736/IDF9:2008 |
| Ash [%] | ≤ 5.0 | INMKL 173:2005 |
| Moisture [%] | ≤ 5.5 | ISO 6731:2010/IDF 21:2010 |
| **Physical properties** | | |
| Solubility index [mL] | ≤ 0.3 | ISO 8156:2005 / IDF 129:2005 |
| Scorched particles | Disc A | ADPI Bulletin 916 |
| Appearance | White to cream powder | Visual inspection. AFI[1] sensoric analysis |
| Flavor/odor | No off Flavor/odor | AFI sensoric analysis |
| Texture | Homogenous | AFI sensoric analysis |
| pH (10% solution) | 6 – 8 | AFI analysis |
| **Minerals** | | |
| Sodium [%] | ≤ 0.5 | AFI ICP analysis |
| Magnesium [%] | ≤ 0.1 | AFI ICP analysis |
| Phosphorus [%] | ≤ 0.03 | AFI ICP analysis |
| Chloride [%] | ≤ 1.0 | ISO 5943:2006 / IDF 88:2006 |
| Potassium [%] | ≤ 1.8 | AFI ICP analysis |
| Calcium [%] | ≤ 0.15 | AFI ICP analysis |
| **Microbiology** | | |
| Total plate count (30°C) [CFU/g] | ≤ 10000 | ISO 4833-1:2013 |
| *Enterobacteriaceae* [CFU/g] | <10 | ISO 21528-2-2:2017 |
| Yeast/mould [CFU/g] | <10 | ISO 6611:2004/IDF 94:2004 |
| *Bacillus cereus* [CFU/g] | <100 | ISO 7932:2004 |
| Sulphite reducing *Clostridia* [CFU/g] | <100 | ISO 15213:2003 |
| *Staphylococcus aureus* (in 1g) | Absent | ISO 6888-1:1999/Amd.1:2003 |
| *Salmonella* (in 125g) | Absent | ISO 6579 |
| *Listeria monocytogenes* (in 25g) | Absent | ISO 11290 |

Batch analyses demonstrating compliance with specifications are provided below.

**Table 3. Acidic Lacprodan® BLG Product Analysis**

| Parameter | Limit | J41042 | J47013 | J50114 | J54095 |
|---|---|---|---|---|---|
| **Chemical** | | | | | |
| Protein as is (Nx6.38) [%] | ≥ 86.0 | 91.14 | 92.30 | 91.67 | 92.94 |
| Protein in dry matter (Nx6.38) [%] | ≥ 90.0 | 94.63 | 95.85 | 95.21 | 96.23 |

| Parameter | Limit | J41042 | J47013 | J50114 | J54095 |
|---|---|---|---|---|---|
| BLG % of protein [%] | ≥ 90.0 | 103.00 | 104.69 | 106.29 | 102.65 |
| Lactose [%] | ≤ 0.2 | 0.09 | 0.09 | 0.09 | 0.09 |
| Fat [%] | ≤ 0.5 | 0.06 | 0.11 | 0.22 | 0.09 |
| Ash [%] | ≤ 4.5 | 0.09 | 0.09 | 0.09 | 0.09 |
| Moisture [%] | ≤ 5.5 | 3.69 | 3.70 | 3.72 | 3.42 |
| **Physical** | | | | | |
| Solubility index [mL] | ≤ 0.3 | 0.09 | 0.09 | 0.09 | 0.09 |
| Scorched particles | Disc A | A | A | A | A |
| Appearance | White to cream powder | White to cream powder | White to cream powder | White to cream powder | White to cream powder |
| Flavor/odor | No off flavor/odor | No off-flavor/odor | No off-flavor/odor | No off-flavor/odor | No off-flavor/odor |
| Texture | Homogenous | Homogenous | Homogenous | Homogenous | Homogenous |
| pH (10% solution) | 3.5 – 4.0 | 3.79 | 3.69 | 3.77 | 3.78 |
| **Minerals** | | | | | |
| Sodium [%] | ≤ 0.1 | 0.025 | 0.025 | 0.025 | 0.025 |
| Magnesium [%] | ≤ 0.1 | 0.003 | 0.003 | 0.003 | 0.003 |
| Phosphorus [%] | ≤ 0.03 | 0.025 | 0.025 | 0.025 | 0.025 |
| Chloride [%] | ≤ 1.9 | 1.45 | 1.61 | 1.52 | 1.52 |
| Potassium [%] | ≤ 0.1 | 0.025 | 0.025 | 0.025 | 0.025 |
| Calcium [%] | ≤ 0.05 | 0.025 | 0.025 | 0.025 | 0.025 |
| **Microbiological** | | | | | |
| Total plate count (30°C) [CFU/g] | ≤ 10000 | <1000 | <1000 | <1000 | <1000 |
| *Enterobacteriaceae* [CFU/g] | <10 | <10 | <1 | <1 | <10 |
| Yeast/mould [CFU/g] | <10 | <10 | <10 | <10 | <10 |
| *Bacillus cereus* [CFU/g] | <100 | 80 | 10 | <10 | 50 |
| Sulphite reducing *Clostridia* [CFU/g] | <100 | <10 | <10 | <10 | <10 |
| *Staphylococcus aureus* (in 1g) | Absent | Absent | Absent | Absent | Absent |
| *Salmonella* (in 125g) | Absent | Absent | Absent | Absent | Absent |
| *Listeria* (in 25g) | Absent | Absent | Absent | Absent | Absent |

**Table 4. Neutral Lacprodan® BLG Product Analysis**

| Parameter | Limit | J26017 | J30020 | J35024 | J42027 | J47031 |
|---|---|---|---|---|---|---|
| **Chemical** | | | | | | |
| Protein as is (Nx6.38) [%] | ≥ 86.0 | 94.39 | 93.67 | 93.87 | 92.24 | 93.83 |

| Parameter | Limit | J26017 | J30020 | J35024 | J42027 | J47031 |
|---|---|---|---|---|---|---|
| Protein in dry matter (Nx6.38) [%] | ≥ 90.0 | 98.30 | 97.73 | 97.54 | 95.90 | 97.41 |
| BLG % of protein [%] | ≥ 90.0 | 97.33 | 98.50 | 100.04 | 102.48 | 97.58 |
| Lactose [%] | ≤ 1.0 | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 |
| Fat [%] | ≤ 1.0 | 0.09 | 0.09 | 0.04 | 0.04 | 0.04 |
| Ash [%] | ≤ 5.0 | 1.86 | 1.87 | 1.80 | 1.76 | 1.82 |
| Moisture [%] | ≤ 5.5 | 3.98 | 4.16 | 3.76 | 3.82 | 3.68 |
| **Physical** | | | | | | |
| Solubility index [mL] | ≤ 0.3 | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 |
| Scorched particles | Disc A | A | A | A | A | A |
| Appearance | White to cream powder | White to cream powder | White to cream powder | White to cream powder | White to cream powder | White to cream powder |
| Flavor/odor | No off Flavor/odor | No off Flavor/odor | No off Flavor/odor | No off Flavor/odor | No off Flavor/odor | No off Flavor/odor |
| Texture | Homogenous | Homogenous | Homogenous | Homogenous | Homogenous | Homogenous |
| pH (10% solution) | 6 – 8 | 7.07 | 7.08 | 7.08 | 7.08 | 7.08 |
| **Minerals** | | | | | | |
| Sodium [%] | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 |
| Magnesium [%] | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 |
| Phosphorus [%] | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Chloride [%] | 0.670 | 0.679 | 0.677 | 0.662 | 0.678 | 0.670 |
| Potassium [%] | 0.013 | 0.012 | 0.012 | 0.011 | 0.011 | 0.013 |
| Calcium [%] | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 |
| **Microbiological** | | | | | | |
| Total plate count (30°C) [CFU/g] | ≤ 10000 | <10 | 70 | <10 | <10 | <10 |
| *Enterobacteriaceae* [CFU/g] | <10 | <10 | <10 | <10 | <10 | <10 |
| Yeast/mould [CFU/g] | <10 | <10 | <10 | <10 | <10 | <10 |
| *Bacillus cereus* [CFU/g] | <100 | <10 | <10 | <10 | <10 | <10 |
| Sulphite reducing *Clostridia* [CFU/g] | <100 | <10 | <10 | <10 | <10 | <10 |
| *Staphylococcus aureus* (in 1g) | Absent | Absent | Absent | Absent | Absent | Absent |
| *Salmonella* (in 125g) | Absent | Absent | Absent | Absent | Absent | Absent |
| *Listeria* (in 25g) | Absent | Absent | Absent | Absent | Absent | Absent |

## 4. Physical and Technical Effect

Lacprodan® BLG is intended for use in sports nutrition, health foods and for use in medical foods, targeting the general adult population and children 3 years of age or older. Lacprodan® BLG serves as a source of protein in these foods, as a replacement for other protein sources. Use of Lacprodan® BLG is therefore not expected to increase overall protein

consumption. As with all medical foods, such products which contain Lacprodan® BLG are provided under medical supervision.

## 5. Consideration of Potential Contaminating Materials

Arla has monitoring programs in place for pesticide residues, melamine, and drug residues.

## Part 3. Estimated Consumption of BLG

Lacprodan® BLG is intended to be used as an ingredient in products for medical nutrition, health food and sports nutrition, as summarized in **Table 5**.

**Table 5. Proposed Uses and Use levels for Lacprodan® BLG.**

| Use | Use Population | Use Level (g/ 100 mL) |
|---|---|---|
| **Use in foods for general consumption** | | |
| Ready to drink sports drinks | Adults | 25 |
| Nutritional shakes | | 12 |
| Ready to drink milk drinks | | 12 |
| Protein-enriched fruit juice | | 6 |
| Yogurt | | 12 |
| **Medical Foods** | | |
| Ready to drink high protein drink | Adults other than pregnant and lactating women | 25 |
| Ready to drink high energy drink | Adults other than pregnant and lactating women | 7 |
| | Children over 3 years old | 4 |
| Foods for dietary management of chronic kidney disease | Adults other than pregnant and lactating women | 7 |
| | Children over 3 years old | 4 |

Lacprodan® BLG is intended for use in sports nutrition, health foods and for use in medical foods, targeting the general adult population and children 3 years of age or older. Lacprodan® BLG serves as a source of protein in these foods, as a replacement for other protein sources. Use of Lacprodan® BLG is therefore not expected to increase overall protein consumption.

With regard to medical food applications, in patients where oral dietary intake from regular meals cannot maintain adequate nutritional status, nutritional supplementation is shown to be effective in replenishing protein and energy stores (Ikizler *et al.* 2013). Lacprodan® BLG is therefore intended for use in general medical foods and in disease-specific medical foods for patients with chronic kidney disease (CKD). Lacprodan® BLG is well suited for use in patients with CKD due to its low mineral profile – especially due to its low phosphorus content. CKD patients often experience protein-energy wasting (PEW) and hyperphosphatemia. PEW is characterized by abnormally low levels or excessive losses of body protein mass and energy reserves and is associated with adverse clinical outcomes, especially in individuals receiving maintenance dialysis therapy. Hyperphosphatemia occurs as a result of impaired excretion by the

damaged kidney which challenges the intake of naturally occurring proteins of high biological value (generally consumed in the normal diet from *e.g.* eggs, meat, milk, yogurt) which have a high phosphorous content. The low mineral profile of Lacprodan® BLG provides a unique possibility to provide high-quality proteins without exceeding the recommended intakes of phosphorus for this patient group. As with all medical foods, such products which contain Lacprodan® BLG are provided under medical supervision.

## Part 4. Self-Limiting Levels of Use (21 C.F.R. § 170.240)

The use of BLG is not self-limiting. The maximum use levels in food are described above.

## Part 5. Experience based on common use in food before 1958

While the basis for this GRAS Notice is scientific procedures, rather than common use in food, we note that BLG is a component of milk. Milk and products derived from milk, such as whey, have a long history of safe consumption by humans at all ages in the form of fluid milk, in dried form (*i.e.,* milk powder), or as milk-derived ingredients. Therefore, the history of milk consumption provides supplemental support for the safety of BLG's intended use.

## Part 6. GRAS Notice Narrative

### 1. Overview

The protein in cow's milk is 80% casein protein and 20% whey protein, where the whey protein is a collection of soluble, globular proteins comprised primarily of β-lactoglobulin (~50-65%), and α-lactalbumin (~25%) (Haug *et al.* 2007). While high purity β-lactoglobulin products are relatively novel, they are equivalent to traditional whey protein and other purified milk protein products from the standpoint of nutritional properties and safety. Given the long history of human consumption of milk, milk and milk proteins are of little toxicological concern to humans or animals. FDA has issued "no questions" letters for several GRAS notices which contain significant amounts of BLG and one notice for BLG itself. These notices provide further evidence for the safety of Arla's BLG product. These notices are summarized in **Table 6.**

**Table 6: Summary of GRAS Notices for products containing BLG**

| GRN | Product | Use |
|---|---|---|
| 37 | Whey protein isolate | General use in foods |
| 504 | Milk protein isolate | General use in foods |
| 633 | Concentrated milk protein | Emulsifier, protein source |
| 809 | Fractionated whey protein | Infant formula |
| 863 | β-lactoglobulin | Protein ingredient |

With the exception of sensitive populations who are allergic to milk proteins or who are lactose-intolerant, we are not aware of adverse effects associated with consumption of milk or milk derived products in general or β-lactoglobulin specifically. We note that Lacprodan® BLG is reduced in lactose content (<1%) from typical levels in cow's milk (4-5%), but it is not lactose "free". A literature search (October 2020) did not yield any reported adverse effects other than allergy issues, discussed below.

### 2. ADME

The *in vitro* digestion of BLG was assessed and the authors concluded that BLG is pepsin-resistant throughout the gastric phase, however significant hydrolysis is observed during *in vitro* intestinal digestion of BLG (Mackie *et al.* 2019).

Whey proteins, and ß-Lactoglobulin in particular, are rapidly digested due to ß-Lactoglobulin retaining its solubility within the stomach, leading to fast gastric emptying and rapid hydrolysis and absorption in the proximal intestine (Mahé *et al.*, 1995, 1996). Farnfield *et al.* (2009) evaluated the absorption of ß-Lactoglobulin enriched whey protein isolate in comparison to a standard whey protein isolate and a hydrolysed version *via* blood plasma amino acid kinetics following acute consumption of a single dose (25 g/500 ml) in healthy adults. The absorption kinetics of all proteins demonstrated parabolic-like curves over the testing period compared to protein-free control.

## 3. Human Studies

While no published studies on BLG *per se* were identified in the public literature, a series of studies which fed participants whey protein were found. As BLG constitutes a significant portion of whey protein, these studies can be taken as significant evidence for the safety of Lacprodan® BLG.

Chungchunlam *et al.*, 2017 reported on acute BLG ingestion (54 g per serving) by healthy young women. The results showed no adverse reactions at an average intake of 0.9 g/kg bw per serving for a 60kg women. In healthy adult males with a weight of 70 kg, acute intakes of BLG ranging from 0.7-0.9 g/kg bw per serving, resulted in no adverse reactions (Areta *et al.*, 2013, Mahé *et al.*, 1996). In a 9 month-long, double-blind randomised community based study, overweight and obese adult males and females that ingested approximately 36g BLG daily (0.5g BLG/kg bw/day for a 70kg person), no adverse reactions to the protein intake were reported (Weinheimer *et al.*, 2012).

## 4. Unpublished Studies

In addition to the published studies described above, Arla Foods has commissioned a series of genotoxicity, rodent, and human studies on Lacprodan® BLG. BLG was negative for *in vitro* genotoxicity in bacterial reverse mutation (Ames) and micronucleus (human lymphocyte) assays, both with and without metabolic activation (OECD 471 and 487).

Oral administration of BLG to Wistar rats for 13 weeks (OECD 408) showed no adverse effects at the maximum dose tested (NOAEL 1,000 mg/kg bw/day). Finally, in human trials conducted by Arla, administering up to 48 g pure Lacprodan® BLG (single dose) and repeated dosages for up to a year (~22 g BLG daily), no adverse effects of toxicological relevance were observed. As previously described, human studies found in the published literature support this conclusion as single ingestion of 54g isolated BLG and repeated intakes for 9 months (~36 g BLG daily) did not elicit any adverse reactions.

## 5. Allergenicity of Milk Protein

An allergy to milk is among the eight most common food allergies. Because the substances are chemically identical, milk and isolated milk proteins will produce a milk protein allergy when consumed. All concentrated milk protein ingredients will clearly indicate that the product is derived from milk protein and will inform those consumers who are allergic to milk and satisfy food allergen labeling requirements.

For any food containing BLG, the label will bear a statement indicating that the product has been derived from a milk source to satisfy allergen labeling requirements.

## 6. Summary of Basis for GRAS Determination

Arla Foods has determined that BLG is Generally Recognized as Safe (GRAS) based on the following:

- The fact that β-lactoglobulin is manufactured under cGMP for food (21 C.F.R. Part 117) and meets appropriate food grade specifications;
- That potential contaminants, such as heavy metals, mycotoxins, and pathogenic microbes, are either absent (not detected) or below toxicological and regulatory limits;
- The intended uses and the estimated consumption of β-lactoglobulin;
- The proper labeling of the products; and
- The long history of safe use of milk and milk protein as food.

* * *

Based on the documentation provided in this GRAS Notice, and as discussed above, Arla Inc. has concluded that Lacprodan® BLG is GRAS based on scientific procedures for use in nutritional products and dairy-based products.

## Part 7. List of supporting data and information


Areta J.L., *et al.* (2013) Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis, *Journal of Physiology*, 591(9):2319–2331. doi: 10.1113/jphysiol.2012.244897

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GRN 633, US FDA 2016. Concentrated milk protine with a ≥ 60:40 whey:casein ratio. *Available at*: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=633&sort=GRN_No&order=DESC&startrow=1&type=basic&search=milk%20protein.

GRN 809, US FDA 2019. Fractionated whey protein concentrate containing 41% alpha-lactalbumin. *Available at*: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=809.

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November 24, 2021

***Via Electronic Mail***

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**Re: Arla Foods Ingredients Group P/S's GRAS Notice No. 001005; Our File No. AR14104.4**

Dear Ms. Santos:

We are writing to respond to the questions posed in your November 1, 2021 correspondence, regarding GRAS Notice (GRN) 001005 for β-lactoglobulin (BLG) from cow's milk. For ease of reference, we reproduce each question below in italics, followed by the relevant response.

***Chemistry***

1. *The notice provides insufficient information regarding the identity of the ingredient. Please provide the following information about the β-lactoglobulin ingredient:*
    a. *CAS Reg. No.*
        i. The CAS Reg. No. of BLG is 9045-23-2.
    b. *molecular weight*
        i. The molecular weight of BLG is 18,300 Da.
    c. *isoelectric point*

*i.* The isoelectric point of BLG is pH 5.13.[1]

*d. A summary of the amino acid profile of β-lactoglobulin*

We have provided as **Attachment 1** an amino acid analysis of acidic BLG and of neutral BLG.

*e. Clarification of the isoforms present in the β-lactoglobulin ingredient*

Bovine BLG is a protein that contains 162 amino acid residues and has a molecular weight of 18,300 Da. Bovine BLG is found in 13 genetic variants;[2] however, the A and B variants are the most abundant forms in milk. The existence of two abundant genetic variants is the reason why bovine BLG elutes in two separate peaks when analyzed by RP-HPLC. The A and B variants differ in amino acid sequence at two locations; specifically, in the B variant Asp64 is replaced by a glycine residue, while Val118 is replaced by alanine.[3] Both contain two disulfide bonds at (Cys66-Cys160) and (Cys106-Cys119), and one sulfhydryl group on Cys121 is buried within the protein structure.[4, 5] The structure of BLG has been studied by several techniques, such as infrared spectroscopy, circular dichroism, nuclear magnetic resonance spectroscopy, and X-ray. The structure consists of 45% β-sheets, 8% α-helices, and 47% random coils [6]. Under physiological conditions (pH, temperature, etc.) bovine BLG exists as a dimer. The association/dissociation behavior of BLG, which has been a topic of extensive research, is

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[1] Wang, Wen (2010) Examining the influence of ultraviolet C irradiation on recombinant human γD-crystallin. Molecular Vision 2010;16:2777-2790.

[2] Ng-Kwai-Hang KF, and Grosclaude F. Genetic Polymorphism of Milk Proteins. In: Fox P.F., McSweeney P.L.H. (eds) Advanced Dairy Chemistry—Volume 1: Proteins 3rd Edition. Springer, Boston, MA. https://doi.org/10.1007/978-1-4419-8602-3_22

[3] Botelho MM, *et al.* (2000) Pressure denaturation of beta-lactoglobulin. Different stabilities of isoforms A and B, and an investigation of the Tanford transition. *Eur J Biochem*. 267 (8):2235-41.

[4] McKenzie HA, Ralston GB, Shaw DC. Location of sulfhydryl and disulfide groups in bovine betalactoglobulins and effects of urea. Biochemistry. 1972;11(24):4539-4547. doi:10.1021/bi00774a017

[5] Papiz MZ, Sawyer L, Eliopoulos EE, et al. The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein. Nature. 1986;324(6095):383-385. doi:10.1038/324383a0

[6] Sawyer L. β-Lactoglobulin. In: McSweeney PLH, Fox PF, eds. Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects, 4th Edition. Springer US; 2013:211-259. doi:10.1007/978-1-4614- 4714-6_7

dependent on several factors, such as pH, temperature, ionic strength, and protein concentration. [5, 7] Native BLG dissociates into monomers at a pH below 3.5 and above pH 7.5. However, the dissociation is also observed at low ionic strength [8].

Overall, it is very difficult to differentiate analytically between the A and B isoforms as the physical and chemical properties of the two isoforms are essentially identical. Further, since there are only two amino acid differences between the two isoforms, it is not necessary or practical to perform analytical tests to determine the amount of each isoform present in the final product. Further, as the only practical difference is two amino acid changes between the isoforms, there would be no impact on safety or protein quality.

2. *Please note that standards and regulations for environmental contaminants, animal drugs, and pesticides in foods such as milk are outlined in 21 CFR 109.30 (tolerances for PCBs), 21 CFR Part 556 (tolerances for residues of new animal drugs in food), and 40 CFR Part 180 (tolerances for pesticides in food and feed). FDA also has action levels for several pesticides (listed in Compliance Policy Guide (CPG) 575.100)[9] and for aflatoxin M1 (FDA's CPG Section 555.400),[10] and derived intervention levels (DILs) for radionuclides (CPG 555.880).[11] In addition to tolerances and action levels, FDA also may use "target testing levels" as guidelines for certain drug residues, including those with a tolerance of zero in milk (e.g., erythromycin, penicillin). In accordance with Appendix N of the Pasteurized Milk Ordinance (PMO), target testing levels have been communicated via Memoranda of Information (M-I) from FDA, most recently M-I-18-9,*

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[7] Brownlow S, Cabral JHM, Cooper R, et al. Bovine β-lactoglobulin at 1.8 Å resolution — still an enigmatic lipocalin. Structure. 1997;5(4):481-495. doi:https://doi.org/10.1016/S0969-2126(97)00205-0

[8] Renard D, Lefebvre J, Griffin MCA, Griffin WG. Effects of pH and salt environment on the association of β-lactoglobulin revealed by intrinsic fluorescence studies. Int J Biol Macromol. 1998;22(1):41-49.

[9] FDA, Compliance Policy Guide Sec 575.100 Pesticide Residues in Food and Feed - Enforcement Criteria (March 1995), available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-575100-pesticide-residues-food-and-feed-enforcement-criteria.

[10] FDA, Compliance Policy Guide Sec. 555.400 Aflatoxins in Human Food (June 2021), available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/compliance-policy-guide-sec-555400-aflatoxins-human-food.

[11] FDA, Compliance Policy Guide Sec 555.880 Radionuclides in Imported Foods - Levels of Concern (Nov 2005), available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-555880-radionuclides-imported-foods-levels-concern.

*issued February 12, 2018.*[12] *Please provide a statement that the starting material for β-lactoglobulin (whey) is from food-grade milk produced in accordance with good agricultural practices and applicable US regulations.*

We can confirm that the starting material for the notified substance is food-grade milk produced in accordance with good agricultural practices and applicable US regulations.

3. *Food-grade milk products are produced in compliance with 21 CFR 1240.61 (*mandatory *pasteurization for all milk and milk products in final package form intended for direct human consumption. Please confirm that the starting material for β-lactoglobulin (whey) is made from fluid milk pasteurized in accordance with the provisions of the PMO*[13]*.*

Arla confirms that the starting material for the notified substance is made from fluid milk pasteurized in accordance with the provisions of the Pasteurized Milk Ordinance.

4. *The method of manufacture does not clearly describe how the neutral and acidic forms of the β-lactoglobulin ingredient are produced. We request that you cite a reference supporting the purification method and provide the following information:*

    a. *Clarification if the pH adjustment step results in an increase or decrease in pH and/or if the pH adjustment is different for the acidic or neutral forms of β-lactoglobulin.*

Figure 1 in GRN 001005 consists of a flowchart, showing two processing streams: one for Lacprodan® BLG acidic and one for Lacprodan® BLG neutral. Arla employs food-grade hydrochloric acid, which is permitted for this use under 21 C.F.R. § 182.1057, to produce acidic BLG and employs potassium hydroxide or sodium hydroxide, which are affirmed as GRAS at 21 C.F.R. §§ 184.1631 and 184.1763, respectively. Consistent with Good Manufacturing Practice (GMP), the minimum amount of hydrochloric acid, potassium hydroxide, and sodium hydroxide will be used to adjust the pH of BLG. We do not have a method to cite for the purification method. We note that FDA has considered a number of GRAS Notices for other dairy filtration products (*e.g.*, whey protein concentrate, alpha lactalbumin, etc.) that do not cite to a method to support the filtration steps.

    b. *The type of filtration membranes used (e.g., ultrafiltration, microfiltration, nanofiltration) and the major components removed at each filtration step.*

BLG is produced using ultrafiltration prior to the production of BLG isolate. Microfiltration removes microorganisms and large molecules (*e.g.*, fat, carbohydrates, non-

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[12] Target testing levels (formerly "safe levels") are indicated in M-I-18-9. Available from: https://gams.fda.gov/active/M-I-18-9_FINAL.pdf. Tolerances for new animal drugs are stated in 21 CFR 556.

[13] https://www.fda.gov/media/140394/download

soluble proteins). Ultrafiltration using size exclusion membranes is then used to remove small molecules (*e.g.*, minerals and small organic molecules).

*c. A statement that pH adjusting agents are food-grade.*

As noted above in response to Question 4a, the pH adjustors are food grade.

*d. A statement that filtration membranes are used in accordance with appropriate U.S. regulations or food contact notifications for their intended use.*

All filtration membranes are used in accordance with appropriate U.S. regulations or food contact notifications for their intended use.

5. *For analytical methods used to analyze batches and demonstrate compliance with specifications, please provide a statement that the methods are validated and fit for purpose.*

All listed analytical methods are validated for detection of the listed analyte(s) and fit for purpose of detection of said analytes in the food matrix.

6. *According to Tables 1 and 2, AFI RP-HPLC method is used to determine the content of β-lactoglobulin. We note that the results of batch analyses (Table 3, p. 10) indicate that the percent of protein as β-lactoglobulin ranges from 102.65 to 106.29%, while the specification is ≥90%.*

*a. Please provide the detection method associated with AFI RP-HPLC.*

Detection is accomplished via ultraviolet/visible light detector.

*b. A specification of ≥90% suggests that proteins other than beta-lactoglobulin may be present. Please clarify if other whey proteins are expected to be present in the ingredient. If so, please identify which whey proteins remain in the ingredient.*

Alpha-lactalbumin (ALA) is the only detectable whey protein other than beta-lactoglobulin known to be present in the notified substance. ALA is present at <1% of total protein.

*c. Please briefly discuss the analytical results (~103-106%) in the context of the extent of purification of β-lactoglobulin expected using the method of manufacture.*

The reason for the analysis yielding a BLG content of more than 100% is a combined effect of the problems with determining exact protein amounts with the Kjeldahl method, combined with quantification of BLG from a standard curve, where the purest analysis standard of BLG available on the market is only up to 90% pure. However, assessing from RP-HPLC analysis of the powder from batches, the BLG is more than 99% pure, with less than 1% of alpha-lactalbumin (ALA) as the only detectable impurity.

7. *Please address the levels of minerals in the β-lactoglobulin ingredient in relation to the target limits for these minerals in the medical foods that are expected to contain β-lactoglobulin.*

The levels of minerals in BLG are not relevant to the intended use of BLG in medical foods other than for patients with chronic kidney disease (CKD). In non-CKD patients using BLG in medical foods, a medical professional is determining that oral dietary intake from regular meals is inadequate and that nutritional supplementation is necessary to replenish protein and energy stores.[14] Thus, when Lacprodan® BLG is used in medical foods administered in a healthcare setting without regard to specific medical conditions (*e.g.*, in individuals requiring nutritional supplementation after surgery), the mineral content of BLG is irrelevant to its safety or utility.

With respect to CKD, Lacprodan® BLG is well-suited for use in medical foods for patients with CKD due to its low mineral content, specifically its low phosphate content. As stated in GRN 001005 (emphasis added),

> Lacprodan® BLG is well suited for use in patients with CKD due to its low mineral profile – especially due to its low phosphorus content. CKD patients often experience protein-energy wasting (PEW) and hyperphosphatemia. PEW is characterized by abnormally low levels or excessive losses of body protein mass and energy reserves and is associated with adverse clinical outcomes, especially in individuals receiving maintenance dialysis therapy. Hyperphosphatemia occurs as a result of impaired excretion by the damaged kidney which challenges the intake of naturally occurring proteins of high biological value (generally consumed in the normal diet from *e.g.*, eggs, meat, milk, yogurt) which have a high phosphorous content. The low mineral profile of Lacprodan® BLG provides a unique possibility to provide high-quality proteins without exceeding the recommended intakes of phosphorus for this patient group. As with all medical foods, such products which contain Lacprodan® BLG are provided under medical supervision.

To add to this discussion, in guidelines issued in 2006, the European Society for Clinical Nutrition and Metabolism (ESPEN) discussed clinical recommendations for protein intakes in patients with chronic renal failure (not on dialysis) as being 0.55-0.50 g/kg/d; for patients on dialysis, the clinical protein intake recommendation is 0.55-0.60 g/kg/d.[15] Likewise, the Kidney Disease Outcomes Quality Initiative (KDOQI) recommended protein intakes in patients with chronic renal

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[14] Ikizler, T. A., et al. (2013), *Prevention and treatment of protein energy wasting in chronic kidney disease patients: a consensus statement by the International Society of Renal Nutrition and Metabolism*, Kidney Int, 84 (6), 1096-107.

[15] ESPEN (2006), ESPEN Guidelines on Enteral Nutrition: Adult renal failure, Clin Nutr, 25 (2006), 295-310.

failure (not on dialysis) as being 0.55-0.50 g/kg/d and 1.00-1.20 g/kg/d for patients on dialysis.[16] In patients with CKD, it is desirable to minimize the level of phosphorous in dietary protein. The specification of less than or equal to 0.03% phosphorous in BLG is sufficiently low to address the concern regarding phosphorous in CKD patients' dietary protein.

8. *Please clarify the intended uses of β-lactoglobulin listed in Table 5 (p. 12).*

   a. *The intended use in ready to drink (RTD) sports drinks, nutritional shakes, RTD milk drinks, protein-enriched fruit juice, and yogurt is described as the use in foods for the adult population. However, we note that these foods may also be consumed by children, including young children aged 1 to 3 years. Please confirm that you considered all population groups that may consume these foods, including young children, in your safety determination.*

Yes, all of the relevant population groups, including children aged 1 to 3 years, were considered in the safety determination.

   b. *You have included RTD high protein drinks and high energy drinks for adults other than pregnant or lactating women under the category of "medical foods". We note that we would not consider these foods to be "medical foods"; rather we would consider them to be meal replacement drinks, shakes, and sports/energy drinks. We would not consider these foods to be limited to non-pregnant, non-lactating adults. Please confirm that you considered all population groups that may consume these foods, including young children and pregnant or lactating women, in your safety determination.*

Yes, all of the relevant population groups, including young children and women who are pregnant or lactating have been considered in our safety determination.

   c. *You have included foods for dietary management of chronic kidney disease and list children aged 3 years and older among the potential consumers. Please clarify that you considered children aged 2 years and older in your safety determination.*

Yes, we have considered children aged 2 years and older in our safety determination.

   d. *The intended uses are limited to drinks and yogurt. Please confirm that there are no uses in bars or other solid foods or revise the intended uses to include foods such as snack bars, meal replacement bars, etc.*

[16] KDOQI (2019), Clinical practice guideline for nutrition in chronic kidney disease: 2019 Update.

We can confirm that the intended uses of the BLG is limited to drinks and yogurt and does not include solid foods such as snack bars, meal replacements, etc.

***9.*** *In "Part 3. Estimated Consumption of BLG"( p. 12), you provide intended use information in lieu of estimating dietary exposure to β-lactoglobulin. The notice is therefore missing estimates of exposure. While you indicate that β-lactoglobulin will replace other protein sources, you do not indicate if this would be a partial or total replacement of other protein sources and you do not provide estimates (either published or calculated for purposes of the notice) of dietary exposure to β-lactoglobulin resulting from its intended use. Please provide mean and 90th percentile estimates of dietary exposure to β-lactoglobulin for children aged 2 to5 years and the total population aged 2 years and older. Separately, please provide estimates of dietary exposures to β-lactoglobulin for children aged 2 to 5 years and the total population aged 2 years and older consuming foods for dietary management of chronic kidney disease.*

The per capita dry whey and whey protein concentrates consumption is estimated to be approximately 2.9 g/person/day with intakes for high-end users (typically considered to be 90$^{th}$ percentile users) could be twice this number – 5.8 g/person/day based on an FDA approximation for widely used additives.[17] BLG is the major protein component of whey, constituting 50-60% of the total protein in whey. Therefore, the expected exposure to BLG from dry whey and whey protein concentrates is approximately 2.9-3.5 g/person/day. According to the USDA's Economic Research Service (ERS), per capita fluid milk consumption is estimated to be 141 lbs/person/year, which corresponds to 175 g/person/day.[18] The 90$^{th}$ percentile consumer of milk is expected to consume twice this amount, or 350 g/person/day. As BLG comprises approximately 0.3% of liquid milk, the 90$^{th}$ percentile consumption of BLG proteins as a component of liquid milk is estimated to be 1.6 g/person/day. The total consumption (90$^{th}$ percentile) of BLG from both dry whey/whey protein concentrates and liquid milk is 5.1 g/person/day. As the subject of this notification, BLG, will simply serve as a replacement for dry whey and whey protein concentrates, it is not expected to significantly increase consumer

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[17] *See* Guidance for Industry: Estimating Dietary Intake of Substances in Food (Aug. 2006), available at http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/foodingredientsandpackaging/ucm074725.htm (last accessed July 27, 2018).

[18] *See* USDA, ERS Dairy Products: Per capita consumption, United States (Annual), at https://www.ers.usda.gov/data-products/dairy-data/ (last accessed November 2021). Our calculation is as follows:

141 lbs/person/year x 453.59 g/lb ÷ 365 days/year = 175 g/person/day

exposures to such substances. For children 2-3 USDA recommends 2 cup equivalents of milk per day, and 2.5 cups per day for ages 4-8[19]. One cup of milk is approximately 243 g, which contains 0.3% BLG. This suggested consumption is equivalent to 1.5 g/person/day BLG exposure for 2-3 year olds and 3.6 g/person/day BLG exposure for 4-8 year olds. When used as a replacement for dry whey and whey protein concentrates, the 90$^{th}$ percentile consumer of BLG is expected to consume approximately 3.5 g/person/day; although this represents an increase compared to background levels of exposure to BLG, the overall exposure to whey proteins, generally, is expected to remain the same.

In terms of estimates of dietary exposures to β-lactoglobulin for children aged 2 to 5 years and the total population aged 2 years and older consuming foods for dietary management of chronic kidney disease, the determination as to the protein intake from medical foods—and, therefore, exposure to Arla's BLG product—would be appropriately determined by a medical professional.

***Toxicology***

1. *On p. 13, the notifier states, "The low mineral profile of Lacprodan® BLG provides a unique possibility to provide high-quality proteins without exceeding the recommended intakes of phosphorus for this patient group." Because Chronic Kidney Disease (CKD) patients should be on restricted protein diet, it is difficult to envision how β-lactoglobulin is good/appropriate for CKD patients consuming proteins just like normal subjects. There are 5 clinical stages of CKD that have varying dietary management needs, and patients on dialysis would have additional dietary management requirements.*

    a. *How is β-lactoglobulin considered a unique and/or safer protein source for CKD patients?*

    b. *Please provide a citation or reputable source, which describes the recommended protein and mineral/phosphorus intakes for CKD patients.*

    c. *Please provide the estimated dietary exposure to β-lactoglobulin based on its intended use in medical foods for this patient group and demonstrate that the estimated dietary exposure to protein and mineral/phosphorus would not present a safety concern for CKD patients.*

    d. *Please clarify how intended uses of β-lactoglobulin will address dietary management needs of the five different clinical stages of CKD and patients undergoing dialysis.*

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[19] *See* USDA, ERS Per capita U.S. milk consumption fell for all ages during 1977-2008 https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=76937.

As discussed above and in GRN 1005, Arla's BLG is a safe and appropriate source of protein for CKD patients primarily due to its high protein quality and low mineral (*i.e.*, phosphorous) content. Due to the nature of CKD disease progression and need for medical supervision of related diets, Arla believes that establishing the general safety of the product is sufficient to establish safety for medical food use in CKD patients. Each patients' individual needs will be assessed by their physician, who can make the relevant decisions related to dietary needs based on clinical stage. Arla believes the description of BLG provided on the label (*i.e.*, Nutrition Facts information), along with a general determination of safety, provides physicians with the required information needed to make recommendations to individual patients.

2. *The notifier states that β-lactoglobulin will be substitutional for other protein sources in the diet. Replacement of diverse dietary protein sources with a single peptide could impact nutritional status of essential amino acids if the protein quality of β-lactoglobulin is not equal to or greater than the quality of the proteins it is intended to replace. Please discuss the protein quality of β-lactoglobulin.*

The protein quality is not calculated in the amino acid Certificate of Analysis (**Attachment 1**), but we have done so below in **Table 1** (average of 5 batches)**.** The protein Digestibility Corrected Amino Acid Score (PDCAAS) and Digestible Indispensable Amino Acid Score (DIAAS) indicate that Arla's acidic BLG has PDCAAS and DIAAS scores of 0.0.88 and 0.988, respectively, with histidine being the limiting amino acid, while neutral BLG has PDCAAS and DIAAS scores of 0.92 and 1, respectively. Protein quality scores were determined by comparing the amount of essential amino acids present in a reference protein (PDCAAS) or dietary requirements (DIAAS) and those present in the notified substance. Further, as Arla's BLG is intended as a replacement for other whey protein isolate (WPI) or whey protein concentrate (WPC) products, we have included a comparison of BLG to the amino acid composition of two such products (**Attachment 2**)(average of 3 batches). These scores support that BLG is a high-quality protein.

**Table 1: Protein Quality Calculations**

| Amino Acid | PDCAAS Reference Protein (mg/g)[20] | DIAAS Requirements- Over 3 years (mg/g)[21] | Acidic BLG (PDCAAS) [DIAAS] | Neutral BLG (PDCAAS) [DIAAS] | WPI (PDCAAS) [DIAAS] | WPC (PDCAAS) [DIAAS] |
|---|---|---|---|---|---|---|
| Isoleucine | 25 | 30 | 61.1 | 63.3 | 70.6 | 68 |
| Leucine | 55 | 61 | 153 | 162 | 120 | 113 |
| Lysine | 51 | 48 | 120 | 124 | 106 | 101 |
| Methionine +Cysteine | 25 | 23 | 57.9 | 58 | 47 | 42.6 |
| Phenylalanine +Tyrosine | 47 | 41 | 70 | 74.1 | 61 | 63.3 |
| Threonine | 27 | 25 | 52 | 52.7 | 79.3 | 77.6 |
| Tryptophan | 7 | 7 | 22 | 22.8 | 19.3 | 20 |
| Valine | 32 | 40 | 58.5 | 60.4 | 65.3 | 62 |
| Histidine | 18 | 16 | 15.8 (0.88) [0.988] | 16.6 (0.92) [1] | 16.7 (0.93) [1] | 19 (1) [1] |

3. *On p. 17, the notifier describes a clinical study by Weinheimer et al., 2012 and OECD guidelines. However, full citations of these publication are not available in the notice.*

   a. *Please provide complete citations for all cited information in the notice.*

   i. Weinheimer EM, Conley TB, Kobza VM, Sands LP, Lim E, Janle EM, Campbell WW. Whey protein supplementation does not affect exercise training-induced changes in body composition and indices of metabolic syndrome in middle-aged overweight and obese adults. J Nutr. 2012 Aug;142(8):1532-9.

   ii. OECD (2016), *Test No. 487: In Vitro Mammalian Cell Micronucleus Test*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

[20] NASEM (2005) Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. *National Academies Press*. *Available at*: 10 Protein and Amino Acids | Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids | The National Academies Press (nap.edu)

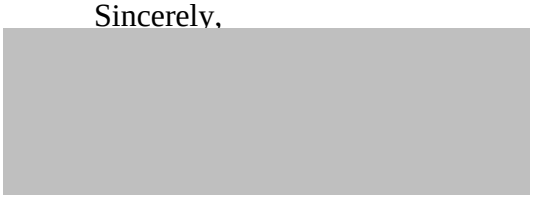
[21] FAO (2013) Dietary protein quality evaluation in human nutrition. *Available at*: 35978-02317b979a686a57aa4593304ffc17f06.pdf.

iii. OECD (2018), Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

iv. OECD (2020), Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

We trust that the feedback above is responsive to your questions. We welcome any other questions you may have.

Sincerely,



Natalie E. Rainer

4866-7128-8067, v. 6

Arla Foods Ingredients
Discovering the wonders of whey

# Lacprodan® DI-BLG 100 Neutral and Lacprodan® DI-BLG 100 Acidic amino acids

Supplier: Arla Foods Ingredients Group P/S
Plant: Danmark Protein
Product: Lacprodan® DI-BLG 100 Neutral and Lacprodan® DI-BLG 100 Acidic
Date of issue: 23 November 2021

The amino acid profiles are given as g amino acid(AA) per 100 g protein. In the tables below are given data from five batch of Beta-lactoglubulin (BLG), brand name Lacprodan® DI-BLG 100 Neutral and Lacprodan® DI-BLG 100 Acidic.

| **Amino acids g AA/ 100g protein** | **Lacprodan® DI-BLG 100 Neutral Batch no.** | | | | |
|---|---|---|---|---|---|
| | J26017 | J30020 | J35024 | J42027 | J47031 |
| Alanine | 7,13 | 6,97 | 7,43 | 7,05 | 7,11 |
| Arginine | 2,79 | 2,73 | 2,89 | 2,76 | 2,77 |
| Aspartic acid | 11,65 | 11,53 | 12,14 | 11,60 | 11,83 |
| Glutamic acid | 20,02 | 19,86 | 20,99 | 20,06 | 20,36 |
| Glycine | 1,41 | 1,33 | 1,49 | 1,37 | 1,36 |
| Histidine | 1,64 | 1,61 | 1,74 | 1,67 | 1,67 |
| Isoleucine | 6,25 | 6,21 | 6,53 | 6,31 | 6,37 |
| Leucine | 16,00 | 15,91 | 16,83 | 16,15 | 16,20 |
| Lysine | 12,29 | 12,17 | 12,89 | 12,25 | 12,47 |
| Phenylalanine | 3,59 | 3,53 | 3,79 | 3,59 | 3,60 |
| Proline | 5,31 | 5,27 | 5,55 | 5,46 | 5,47 |
| Serine | 3,82 | 3,74 | 4,06 | 3,79 | 3,86 |
| Threonine | 5,30 | 5,17 | 5,44 | 5,16 | 5,29 |
| Tyrosine | 3,76 | 3,70 | 3,93 | 3,76 | 3,83 |
| Valin | 6,03 | 5,86 | 6,29 | 6,02 | 6,01 |
| Cysteine + Cystine | 2,72 | 2,49 | 2,72 | 2,86 | 2,76 |
| Methionine | 3,17 | 2,83 | 3,07 | 3,20 | 3,21 |
| Tryptophan | 2,27 | 2,28 | 2,23 | 2,25 | 2,39 |

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| Amino acids g AA/ 100g protein | Lacprodan® DI-BLG 100 Acidic Batch no. | | | | |
|---|---|---|---|---|---|
| | J36071 | J41042 | J47013 | J50114 | J54095 |
| Alanine | 6,99 | 6,93 | 6,87 | 6,49 | 6,76 |
| Arginine | 2,80 | 2,83 | 2,73 | 2,56 | 2,58 |
| Aspartic acid | 11,61 | 11,52 | 11,16 | 11,02 | 11,19 |
| Glutamic acid | 20,51 | 20,41 | 19,83 | 18,98 | 20,01 |
| Glycine | 1,38 | 1,38 | 1,38 | 1,28 | 1,34 |
| Histidine | 1,61 | 1,58 | 1,58 | 1,54 | 1,59 |
| Isoleucine | 6,32 | 6,24 | 6,15 | 5,80 | 6,04 |
| Leucine | 15,74 | 15,58 | 15,28 | 14,62 | 15,28 |
| Lysine | 12,27 | 12,29 | 12,13 | 11,56 | 11,94 |
| Phenylalanine | 3,48 | 3,48 | 3,51 | 3,23 | 3,34 |
| Proline | 5,52 | 5,44 | 5,35 | 5,19 | 5,22 |
| Serine | 3,87 | 3,70 | 3,65 | 3,56 | 3,73 |
| Threonine | 5,34 | 5,29 | 5,23 | 5,00 | 5,12 |
| Tyrosine | 3,67 | 3,65 | 3,68 | 3,43 | 3,53 |
| Valin | 6,01 | 5,95 | 5,92 | 5,52 | 5,85 |
| Cysteine + Cystine | 2,89 | 2,89 | 2,88 | 2,43 | 2,84 |
| Methionine | 3,01 | 3,08 | 3,03 | 2,93 | 2,99 |
| Tryptophan | 2,30 | 2,22 | 2,21 | 2,12 | 2,15 |

Best regards,
Arla Foods Ingredients Group P/S

Marie-Louise R. W. Hansen
Global QEHS Officer

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Arla Foods Ingredients
Discovering the wonders of whey

## Amino acid composition of commercially available WPI and WPC

Supplier: Arla Foods Ingredients Group P/S
Plant: Danmark Protein
Date of issue: 7 December 2021

Parameters below are monitored once per year by Arla Foods Ingredients Group P/S.
The amino acid profiles are given as g amino acid (AA) per 100 g protein. In the tables below are given data from the last three years for whey protein isolate (WPI) and for whey protein concentrate (WPC).

| | | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| Alanine | 6,1 | 6,4 | 5,9 |
| Arginine | 2,2 | 2,5 | 2,2 |
| Aspartic acid | 11,6 | 13,0 | 11,2 |
| Glutamic acid | 20,0 | 21,5 | 19,3 |
| Glycine | 1,6 | 1,6 | 1,6 |
| Histidine | 1,6 | 1,8 | 1,6 |
| Isoleucine | 7,0 | 7,4 | 6,8 |
| Leucine | 12,0 | 12,5 | 11,6 |
| Lysine | 10,5 | 11,1 | 10,3 |
| Phenylalanine | 3,1 | 3,3 | 3,0 |
| Proline | 6,8 | 6,8 | 6,8 |
| Serine | 5,2 | 5,5 | 5,0 |
| Threonine | 7,8 | 8,4 | 7,6 |
| Tyrosine | 2,9 | 3,1 | 2,9 |
| Valin | 6,5 | 6,8 | 6,3 |
| Cysteine + Cystine | 2,5 | 1,9 | 2,2 |
| Methionine | 2,6 | 2,5 | 2,4 |
| Tryptophan | 2,1 | 1,9 | 1,8 |

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| Amino acids g AA/ 100g protein | WPC Batch no. | | |
|---|---|---|---|
| | A | B | C |
| Alanine | 5,5 | 5,4 | 5,5 |
| Arginine | 2,6 | 2,6 | 2,7 |
| Aspartic acid | 11,7 | 11,9 | 12,0 |
| Glutamic acid | 18,7 | 19,0 | 19,0 |
| Glycine | 1,9 | 1,9 | 1,9 |
| Histidine | 1,9 | 1,9 | 1,9 |
| Isoleucine | 6,9 | 6,7 | 6,8 |
| Leucine | 11,3 | 11,1 | 11,5 |
| Lysine | 10,0 | 10,3 | 10,1 |
| Phenylalanine | 3,4 | 3,3 | 3,4 |
| Proline | 6,4 | 6,4 | 6,6 |
| Serine | 5,6 | 5,6 | 5,7 |
| Threonine | 7,8 | 7,7 | 7,8 |
| Tyrosine | 3,1 | 2,9 | 2,9 |
| Valin | 6,2 | 6,1 | 6,3 |
| Cysteine + Cystine | 2,3 | 2,0 | 1,9 |
| Methionine | 2,1 | 2,4 | 2,1 |
| Tryptophan | 2,1 | 1,9 | 2,0 |

Best regards,
Arla Foods Ingredients Group P/S

Marie-Louise R. W. Hansen
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