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Safety of Beta-lactoglobulin as a Novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on beta-lactoglobulin (BLG) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF (≥ 90% w/w dry matter protein) consists of BLG as primary component (≥ 90% of total protein), which is equivalent to BLG present in bovine milk and whey protein isolate (WPI). The NF is produced from bovine whey by crystallisation under acidic or neutral conditions. The NF is proposed to be used as a food ingredient in isotonic and sport drinks, whey powder and milk-based drinks and similar products, and in food for special medical purposes as defined in Regulation (EU) No 609/2013. The target population is the general population. The highest daily intake of the NF was estimated for children of 3 to < 10 years of age as 667 mg/kg body weight (bw) per day. The NF presents proximate composition and content of essential amino acids similar to those in WPI. The Panel notes that the highest mean and highest 95th percentile daily protein intakes from the NF are below the protein population reference intakes for all population groups. Although a tolerable upper intake level has not been derived for protein, the protein intake from the NF may nevertheless further contribute to an already high dietary protein intake in Europe. The exposure to the reported minerals does not raise concerns. The Panel considers that the consumption of the NF is not nutritionally disadvantageous. No genotoxic concerns were identified from the standard *in vitro* test battery. No adverse effects were observed in the subchronic toxicity study, up to the highest dose tested, i.e. 1,000 mg NF/kg bw per day. The Panel concludes that the NF is safe under the proposed conditions of use.

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Keywords: beta-lactoglobulin, novel food, whey protein isolate

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 21 July 2020, the company Arla Foods Ingredients Group P/S submitted a request to the Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place on the EU market beta-lactoglobulin (BLG).

BLG is intended to be used in a number of foods and in food for special medical purposes (FSMP) as defined by Regulation (EU) No 609/2013[2].

The applicant has requested data protection according to the provisions of Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on BLG.

2. Data and methodologies

2.1. Data

The safety assessment of this novel food (NF) is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information.

During the assessment, the Panel identified additional data which were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[3].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (both in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise: toxicological information (*in vitro* genotoxicity studies, 14-day and 90-day repeated dose oral toxicity studies; Table 9); certificates of analysis of chloride and potassium levels in 23 batches of the NF (Section 3.4) and 20 batches of commercially available whey protein isolate (WPI); and certificates of analysis of total plate count levels in 7 batches of the NF (Table 5).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469. The legal provisions for the assessment of FSMP are laid down in Regulation (EU) No 609/2013 and in Commission Delegated Regulation (EU) 2016/128[4].

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit. Furthermore, this assessment is not an assessment on whether the NF is suitable as stipulated by Regulation (EU) No 609/2013.

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

[2] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

[3] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, p. 64–71.

[4] Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes. OJ L 25, 2.2.2016, p. 30–43.

3. Assessment

3.1. Introduction

The NF contains ≥ 90% w/w dry matter (DM) protein, which is primarily BLG, the dominant non-casein protein in bovine milk. BLG accounts for about 10% of the total protein in bovine milk and 50–58% w/w of the total bovine whey protein (Chatterton et al., 2006; Jovanovic et al., 2007; Madureira et al., 2007). The NF is produced from bovine whey by crystallisation under acidic or neutral conditions. The NF is intended to be used as an ingredient in isotonic and sport drinks, whey powder and milk-based drinks and similar products, and in FSMP as defined in Regulation (EU) No 609/2013. The target population is the general population.

According to Article 3(2)(a) of Regulation (EU) 2015/2283, the NF falls under the following category: 'food consisting of, isolated from or produced from animals or their parts, except for animals obtained by traditional breeding practices which have been used for food production within the Union before 15 May 1997 and the food from those animals has a history of safe food use within the Union'.

3.2. Identity of the NF

The NF (≥ 90% w/w DM protein) is a white-to-cream powder mainly composed of BLG (≥ 90% of total protein) and produced from bovine whey by crystallisation under acidic (pH 3.5–4.0) or neutral[5] (pH 6.0–8.0) conditions. BLG is a globular compact protein and the most abundant bovine milk whey protein (Table 1). It naturally occurs in milk from many mammalian species, but not in human milk. The protein is present in milk as a 36.7-kDa dimer with 162 amino acid residues per polypeptide subunit (McKenzie et al., 1972). BLG is thermolabile and relatively resistant to acid and enzymatic hydrolysis (EFSA, 2004; EFSA NDA Panel, 2014). BLG belongs to the lipocalin protein family and is considered as a retinol-binding protein (Papiz et al., 1986; Brownlow et al., 1997). Crystallographic studies have revealed a very similar folding or β-barrel structure for lipocalin proteins, with the same arrangements of 8 (or 10) antiparallel β-sheets (EFSA, 2004; EFSA NDA Panel, 2014). Overall, the structure of BLG consists of 45% β-sheets, 8% α-helices and 47% random coils (Sawyer, 2013).

Although BLG is found in 13 genetic variants, 2 main isoforms are present in bovine milk, the variants A and B, which differ in two-point mutations at amino acids 64 and 118 (Restani et al., 2006; Gai et al., 2021), and contain two disulfide bonds at Cys66–Cys160 and Cys106–Cys119 and one sulfhydryl group on Cys121 within the protein structure (McKenzie et al., 1972; Papiz et al., 1986; Ng-Kwai-Hang and Grosclaude, 2003). Surrounding pH, temperature, ionic strength, crystal packing and protein concentration have been reported to affect the structure of BLG (Brownlow et al., 1997; Adams et al., 2006). Under physiological conditions, bovine BLG exists as a dimer, which has been demonstrated to dissociate into monomers at a pH below 3.5 and above 7.5, and at a low ionic strength (Uhrínová et al., 1998; Mercadante et al., 2012). For instance, in solution, when the pH is reduced from 6.2 to 2.6, under a low salt concentration, BLG undergoes a transition from a dimer to a monomer, which has a secondary structure very similar to the dimeric one, as shown by Nuclear Magnetic Resonance (NMR) spectroscopy (Fugate and Song, 1980; Molinari et al., 1996; Uhrínová et al., 2000).

Table 1: Chemical identity of BLG

Chemical substance	
Chemical (IUPAC) name	N/A
Common name	Beta-lactoglobulin
Abbreviations	BLG, β-Lg, b-Lg, B-LG
Alternative chemical names	N/A
Synonyms, trade names	β-Lactoglobulin, Lacprodan® BLG
CAS Number	9045-23-2
Molecular formula	N/A
Molecular weight	36.7 kDa (dimer); 18.4 kDa (monomer)

[5] The pH range corresponds to 'neutral to slightly basic' conditions, but it will be henceforth named as 'neutral' upon request of the applicant.

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The identity of BLG produced under acidic and neutral conditions has been confirmed by reverse phase high-performance liquid chromatography with UV detection (RP-HPLC/UV), by comparison with a commercial standard of BLG. In the RP-HPLC/UV chromatograms, the elution of the BLG protein as two separate peaks has been attributed to the genetic variants A and B, with retention times of 6.8 and 6.9 min, respectively. A minor peak at 5.8 min corresponds to alpha-lactalbumin (ALA). RP-HPLC/UV chromatograms also showed similar retention times and peak shapes for the BLG contained in the NF, produced under acidic and neutral conditions, and the BLG present in a commercial WPI.

Upon EFSA's request, the applicant provided the results of size exclusion chromatography (SEC) analysis under denaturing and reducing conditions and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The SEC chromatograms for the NF produced under acidic and neutral conditions and commercial WPI showed a single monodisperse peak for BLG, eluting after 34.5 min, immediately followed by a minor peak for ALA. In addition, SDS-PAGE analysis revealed similar migration distance when comparing the BLG obtained under acidic and neutral conditions at different storage periods and the BLG present in (i) a commercial standard, (ii) bovine milk (Costa et al., 2014), (iii) the mother liquor after crystallisation at different pH values and (iv) whey protein concentrate used as raw material in the production of the NF.

Considering the data submitted by the applicant and the literature data reported above, the Panel concludes that BLG in the NF produced under both acidic and neutral conditions is chemically and structurally equivalent to the BLG present in bovine milk and WPI.

3.3. Production process

According to the information provided, the NF is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The starting material for the manufacturing process of the NF is whey obtained from bovine milk. After filtration and concentration steps, the concentrated whey protein solution is subjected to crystallisation by adjusting the pH to 5.0–6.0, while ensuring low conductivity. BLG is precipitated as crystals and separated from the remaining whey protein solution. The BLG crystals are redissolved and the pH adjusted to either 3.5–4.0 or 6.0–8.0. The resulting solutions are then concentrated prior to spray-drying. The emulsifier soy lecithin may be added during spray-drying for improved solubility of powders sold for e.g. shakes. In this case, the final product consists of approximately 1.15% soy lecithin.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.4. Compositional data

The NF (≥ 90% w/w DM protein) contains BLG as primary constituent (≥ 90% of total protein) and small amounts of lactose (≤ 0.2% or ≤ 1.0% for the NF produced under acidic or neutral conditions, respectively), fat (≤ 0.5% or ≤ 1.0%, for the NF produced under acidic or neutral conditions, respectively) and moisture (≤ 5.5%), with less than 1% of ALA.

All analyses were conducted following internationally recognised standard methods (e.g. ISO) or otherwise, validated internal methods (e.g. RP-HPLC/UV for BLG content). Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with the required characteristics, the applicant provided analytical information for 10 batches of the NF, 5 produced under acidic conditions (Table 2) and 5 produced under neutral conditions (Table 3).

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Table 2: Batch-to-batch analysis of the NF produced under acidic conditions

Parameter	Batch number					Method of analysis
	#1-A	#2-A	#3-A	#4-A	#5-A	
Physico-chemical parameters						
Insolubility index (mL)	0.09	0.09	0.09	0.09	0.09	ISO 8156:2005/IDF 129:2005
pH (10% solution)	3.7	3.8	3.7	3.8	3.8	ISO 5546:2010/IDF 115:2010
Composition						
Protein 'as is' (N × 6.38) (%)	92.13	91.14	92.30	91.67	92.94	Kjeldahl; ISO 8968-3:2004/IDF 20-3:2004
Protein in DM (N × 6.38) (%)	95.43	94.63	95.85	95.21	96.23	Calculation[(1)]
BLG[(2)] (% of protein)	100.97	103.00	104.69	106.29	102.65	RP-HPLC/UV (validated internal method)
Lactose (%)	0.09	0.09	0.09	0.09	0.09	Enzymatic; ISO 5765-2/IDF 79-2:2002
Fat (%)	0.06	0.06	0.11	0.22	0.09	Gravimetry; ISO 1736/IDF 9:2008
Ash (%)	0.14	0.09	0.09	0.09	0.09	Gravimetry; NMKL 173:2005
Moisture (%)	3.46	3.69	3.70	3.72	3.42	Gravimetry; ISO 6731:2010/IDF 21:2010
Minerals						
Aluminium (mg/kg)	< 0.5	0.6	< 0.5	< 0.5	< 0.5	EN ISO 17294-2-E29 (DE Food)/ ICP-MS
Chromium (mg/kg)	–	–	0.32	< 0.20	< 0.20	DS/EN 13805m:2014, DS/EN ISO 17294m:2016/ICP-MS
Chloride (%)	1.53	1.45	1.61	1.52	1.52	Potentiometric titration; ISO 5943:2006/IDF 88:2006
Calcium (%)	0.025	0.025	0.025	0.025	0.025	ICP (internal method)
Magnesium (%)	0.003	0.003	0.003	0.003	0.003	ICP (internal method)
Phosphorus (%)	0.025	0.025	0.025	0.025	0.025	ICP (internal method)
Potassium (%)	0.025	0.025	0.025	0.025	0.025	ICP (internal method)
Sodium (%)	0.025	0.025	0.025	0.025	0.025	ICP (internal method)
Contaminants						
Arsenic (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	DS/EN 13805m:2014, DS/EN ISO 17294m:2016/ICP-MS
Cadmium (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	ICP-MS ISO 17294-2:2016
Lead (mg/kg)	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	ICP-MS ISO 17294-2:2016
Mercury (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ICP-MS ISO 17294-2:2016
Aflatoxin M1 (μg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	HPLC-FLD (internal method)
Microbial parameters						
Total plate count (CFU/g)	< 10	< 10	110	20	< 10	ISO 4833-1:2013
Yeast and mould (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 6611:2004/IDF 94:2004
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21528-2:2017
Salmonella (in 25 g)	ND	ND	ND	ND	ND	ISO 6579
Bacillus cereus (CFU/g)	140	80	10	< 10	50	ISO 7932:2004
Listeria monocytogenes (in 25 g)	ND	ND	ND	ND	ND	ISO 11290
Staphylococcus aureus (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 6888-1:1999/Amd.1:2003
Sulfite-reducing clostridia (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 15213:2003

'–': Not reported; 'A': NF produced under acidic conditions; ADPI: American Dairy Products Institute; Amd.: Amendment; BLG: Beta-lactoglobulin; CFU: Colony forming unit; DE Food: German Food and Feed Code; DM: Dry matter; DS: Danish standard; EN: European norm; ICP: Inductively coupled plasma; IDF: International Dairy Federation; ISO: International Organisation for

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Standardisation; HPLC/FLD: High-performance liquid chromatography with fluorescence detection; MS: Mass spectrometry; ND: Not detected; NMKL: Nordic Committee on Food Analysis; RP-HPLC/UV: Reversed phase-high performance liquid chromatography with UV detection.
(1): Protein in DM = (Protein 'as is'/DM) × 100%.
(2): The levels of BLG, expressed as % of the total protein, exceed 100% in some batches, which can be attributed to the uncertainty of the method (RP-HPLC/UV) used for the quantification of BLG using a commercial standard with 90% purity.

Table 3: Batch-to-batch analysis of the NF produced under neutral conditions

Parameter	Batch number					Method of analysis
	#1-N	#2-N	#3-N	#4-N	#5-N	
Physico-chemical parameters						
Insolubility index (mL)	0.09	0.09	0.09	0.09	0.09	ISO 8156:2005/IDF 129:2005
pH (10% solution)	7.1	7.1	7.1	7.1	7.1	ISO 5546:2010/IDF 115:2010
Composition						
Protein 'as is' (N × 6.38) (%)	94.39	93.67	93.87	92.24	93.83	Kjeldahl; ISO 8968-3:2004/IDF 20-3:2004
Protein in DM (N × 6.38) (%)	98.30	97.73	97.54	95.90	97.41	Calculation(1)
BLG(2) (% of protein)	97.33	98.50	100.04	102.48	97.58	RP-HPLC/UV (validated internal method)
Lactose (%)	0.09	0.09	0.09	0.09	0.09	Enzymatic; ISO 5765-2/IDF 79-2:2002
Fat (%)	0.09	0.09	0.04	0.04	0.04	Gravimetry; ISO 1736/IDF 9:2008
Ash (%)	1.86	1.87	1.80	1.76	1.82	Gravimetry; NMKL 173:2005
Moisture (%)	3.98	4.16	3.76	3.82	3.68	Gravimetry; ISO 6731:2010/IDF 21:2010
Minerals						
Aluminium (mg/kg)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	EN ISO 17294-2-E29 (DE Food)/ ICP-MS
Chromium (mg/kg)	0.38	0.32	0.34	0.33	0.36	DS/EN 13805m:2014, DS/EN ISO 17294m:2016/ICP-MS
Chloride (%)	0.04	0.04	0.04	0.04	0.04	Potentiometric titration; ISO 5943:2006/IDF 88:2006
Calcium (%)	0.01	0.01	0.01	0.01	0.01	ICP (internal method)
Magnesium (%)	0.003	0.003	0.003	0.003	0.003	ICP (internal method)
Phosphorus (%)	0.03	0.03	0.03	0.03	0.03	ICP (internal method)
Potassium (%)	0.67	0.68	0.68	0.66	0.68	ICP (internal method)
Sodium (%)	0.27	0.28	0.28	0.27	0.28	ICP (internal method)
Contaminants						
Arsenic (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	DS/EN 13805m:2014, DS/EN ISO 17294m:2016/ICP-MS
Cadmium (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	ICP-MS ISO 17294-2:2016
Lead (mg/kg)	0.004	0.008	0.004	< 0.003	< 0.003	ICP-MS ISO 17294-2:2016
Mercury (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	ICP-MS ISO 17294-2:2016
Aflatoxin M1 (μg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	HPLC-FLD (internal method)
Microbial parameters						
Total plate count (CFU/g)	< 10	70	< 10	< 10	< 10	ISO 4833-1:2013
Yeast and mould (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 6611:2004/IDF 94:2004
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21528-2:2017
Salmonella (in 25 g)	ND	ND	ND	ND	ND	ISO 6579
B. cereus (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 7932:2004
L. monocytogenes (in 25 g)	ND	ND	ND	ND	ND	ISO 11290
S. aureus (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 6888-1:1999/Amd.1:2003

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Parameter	Batch number					Method of analysis
	#1-N	#2-N	#3-N	#4-N	#5-N	
Sulfite-reducing clostridia (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 15213:2003

ADPI: American Dairy Products Institute; Amd.: Amendment; CFU: Colony forming unit; DE Food: German Food and Feed Code; DM: Dry matter; DS: Danish standard; EN: European norm; ICP: Inductively coupled plasma; IDF: International Dairy Federation; ISO: International Organisation for Standardisation; HPLC/FLD: High-performance liquid chromatography with fluorescence detection; MS: Mass spectrometry; ND: Not detected; 'N': NF produced under neutral conditions; NMKL: Nordic Committee on Food Analysis; RP-HPLC/UV: Reversed phase high-performance liquid chromatography with UV detection.
(1): Protein in DM = (Protein 'as is'/DM) × 100%.
(2): The levels of BLG, expressed as % of the total protein, exceed 100% in some batches, which can be attributed to the uncertainty of the method (RP-HPLC/UV) used for the quantification of BLG using a commercial standard with 90% purity.

Following a request from EFSA, the applicant reported the concentration of aflatoxin M1 in one batch of bovine milk (< 0.01 μg/kg).

Due to the variability of *B. cereus* load in the NF produced under acidic conditions, which in one batch exceeded the proposed specification (< 100 CFU/g), the applicant was requested to provide additional data. The reported *B. cereus* load was below the proposed specification for this parameter in five additional batches of the NF produced under acidic conditions (Table 4).

Table 4: *B. cereus* load in five additional batches of the NF produced under acidic conditions

Parameter	Batch number					Method of analysis
	#6-A	#7-A	#8-A	#9-A	#10-A	
B. cereus (CFU/g)	< 10	< 10	20	< 10	< 10	ISO 7932:2004

'A': NF produced under acidic conditions; CFU: Colony forming unit; ISO: International Organisation for Standardisation.

In response to a request from EFSA to lower the initially proposed specifications (≤ 10,000 CFU/g) for total plate count levels in the NF, the applicant provided additional data for seven batches of the NF (claimed as proprietary by the applicant), three produced under acidic conditions, two under neutral conditions and two for which the pH has not been reported (Table 5). The Panel noted the high variability in total plate count between the batches, with two exceeding the initially proposed specifications. This parameter is considered a process hygiene indicator and could affect the safety of the NF (see Section 3.5).

Table 5: Total plate count levels in seven additional batches of the NF

Parameter	Batch number							Method of analysis
	#11-A	#12-A	#13-A	#6-N	#7-N	#1-U	#2-U	
Total plate count (CFU/g)	440	530	88,000	17,000	420	6,000	< 1,000	ISO 4833-1:2013

'A': NF produced under acidic conditions; CFU: Colony forming unit; ISO: International Organisation for Standardisation; 'N': NF produced under neutral conditions; 'U': pH of the NF unknown.

The differences in the mineral content were attributed to the different pH regulators used in the manufacture of the NF under acidic or neutral conditions and to the overall protein charge. Considering the isoelectric (5.13) and isoionic points (5.35 and 5.41, respectively) of the BLG variants A and B (Farrell et al., 2004), the NF produced under acidic conditions will be positively charged, with a higher concentration of counterions such as chloride. In contrast, the charge of the NF will be slightly negative when produced under neutral conditions, resulting in higher concentrations of counterions such as sodium and potassium. Upon EFSA's request to lower the initially proposed specifications for chloride (≤ 1.9%) and potassium (≤ 1.8%) levels in the NF produced under acidic and neutral conditions, respectively, the applicant provided additional data for 23 batches of the NF (claimed as proprietary by the applicant), 18 produced under acidic conditions and 5 under neutral conditions. Chloride levels ranged between 1.45% and 1.85% in the 18 batches of the NF produced under acidic conditions, and potassium levels up to 0.83% were reported in the 5 batches of the NF produced under neutral conditions (see Section 3.5).

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Particle size distribution was determined by laser diffraction in one batch of the NF produced under acidic conditions. Overall, 99% of all the particles were smaller than 60.34 ± 2.40 μm.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

3.4.1. Stability

Stability of the NF

The applicant performed stability tests with five batches of the NF produced under acidic conditions. The tests were carried out for 18 months at normal storage conditions (21 ± 2°C and 45 ± 5% relative humidity (RH), in polyethylene bags) in a dark cabinet. The batches were analysed for total protein, BLG, moisture and microbial parameters (total plate count, *Enterobacteriaceae*, yeast and mould). Following a request from EFSA, the applicant provided stability data up to 12 months for five batches of the NF produced under neutral conditions.

After 18 months of storage, a total loss of BLG of 7.9% (on average) and increase in moisture content (from 3.6 to 6.6%, on average) were observed in the NF produced under acidic conditions, while no appreciable changes in microbiological indicators were noticed. With regard to the NF produced under neutral conditions, a similar trend for BLG (6.4% total loss, on average) and moisture content (increase from 3.9% to 6.2%, on average) was observed after 12-month storage, with no change over time in microbial parameters. The applicant proposes an 18-month shelf-life under ambient conditions for the NF produced under acidic and neutral conditions.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for 18 months.

Stability of the NF under the intended conditions of use

The applicant performed stability tests with five batches of the NF produced under acidic conditions in a clear acidic beverage. The NF (4.4% w/w) was mixed with water, sugar and food additives and the pH adjusted to 3.7 with food-grade phosphoric acid. The beverage was pasteurised at 120°C for 15 s and stored in cans for 6 months at 21 ± 2°C and 45 ± 5% RH in a dark cabinet. The batches were analysed for total protein, BLG, dry matter and pH after 3- and 6-month storage. The initial level of BLG in the acidic beverage was on average 88.0% of the total protein, which indicates that about 10% of BLG was denatured during the heat treatment. After 6 months, no significant changes in the total protein content were observed, although the concentration of intact BLG decreased by 11.6% on average. The applicant claims this reduction in the BLG content is reasonably comparable to that observed in the NF produced under acidic conditions (7.9% on average) by the end of the proposed shelf-life (18 months), considering the accelerated degradation of BLG in acidic aqueous solution. The applicant concludes that the clear acidic beverage can be considered stable for a 6-month period.

The Panel considers that the provided information is sufficient with respect to the stability of the NF in relevant food matrices.

3.5. Specifications

The specifications of the NF are indicated in Table 6.

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Table 6: Specifications of the NF

Description: Beta-lactoglobulin is a white to cream powder produced from bovine whey by crystallisation under acidic or neutral conditions.

Source: Bovine whey

Parameter	Specification		
	Acidic conditions	**Neutral conditions**	**Combined**
Physico-chemical parameters			
Insolubility index (mL)	≤ 0.3	≤ 0.3	≤ 0.3
pH (10% solution)	3.5–4.0	6.0–8.0	3.5–8.0
Composition			
Protein 'as is' (N × 6.38) (%)	≥ 86.0	≥ 86.0	≥ 86.0
Protein in DM(1) (N × 6.38) (%)	≥ 90.0	≥ 90.0	≥ 90.0
BLG (% of protein)	≥ 90.0	≥ 90.0	≥ 90.0
Lactose (%)	≤ 0.2	≤ 1.0	≤ 1.0
Fat (%)	≤ 0.5	≤ 1.0	≤ 1.0
Ash (%)	≤ 4.5	≤ 5.0	≤ 5.0
Moisture (%)	≤ 5.5	≤ 5.5	≤ 5.5
Minerals			
Calcium (%)	≤ 0.05	≤ 0.15	≤ 0.15
Chloride (%)	≤ 1.9	≤ 1.0	≤ 1.9
Magnesium (%)	≤ 0.1	≤ 0.1	≤ 0.1
Phosphorus (%)	≤ 0.03	≤ 0.03	≤ 0.03
Potassium (%)	≤ 0.1	≤ 1.2	≤ 1.2
Sodium (%)	≤ 0.1	≤ 0.5	≤ 0.5
Contaminants			
Cadmium (mg/kg)	< 0.2	< 0.2	< 0.2
Lead (mg/kg)	< 0.1	< 0.1	< 0.1
Mercury (mg/kg)	< 0.01	< 0.01	< 0.01
Aflatoxin M1 (μg/kg)	< 0.01	< 0.01	< 0.01
Microbial parameters			
Total plate count (CFU/g)	≤ 5,000	≤ 5,000	≤ 5,000
Yeast and mould (CFU/g)	< 10	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10
Salmonella (in 25 g)	ND	ND	ND
B. cereus (CFU/g)	< 100	< 100	< 100
L. monocytogenes (in 25 g)	ND	ND	ND
S. aureus (CFU/g)	< 10	< 10	< 10
Sulfite-reducing clostridia (CFU/g)	< 10	< 10	< 10

BLG: Beta-lactoglobulin; CFU: Colony forming unit; DM: Dry matter; ND: Not detected.
(1): Protein in DM = (Protein 'as is'/DM) × 100%.

The applicant proposed a specification for total plate count as ≤ 10,000 CFU/g. The Panel notes that total plate count is considered a process hygiene indicator and could affect the safety of the NF. Therefore, a lower specification should be met and the criterion proposed by the Panel is ≤ 5,000 CFU/g.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

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3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

The NF (≥ 90% w/w DM protein) contains BLG as primary constituent (≥ 90% of total protein), which represents about 10% of the total protein in bovine milk and 50–58% w/w of the total bovine whey protein (Chatterton et al., 2006; Jovanovic et al., 2007; Madureira et al., 2007). Bovine whey, which is a by-product from cheese manufacturing, is the raw material used in the production of the NF.

Bovine whey, whey protein concentrates, isolates and hydrolysates and demineralised whey powder are currently consumed in the EU, as well as whey protein fractions, e.g. different mixtures of isolates of BLG, ALA, bovine serum albumin, lactoferrin and/or immunoglobulins. The production and use of whey products in the EU have been reported as 55.5 million tonnes in 2020 (Eurostat, 2021). EFSA has published a few scientific opinions on the safety of bovine milk proteins as NFs: bovine whey basic protein isolate, with bovine lactoferrin and lactoperoxidase as main constituents (EFSA NDA Panel, 2018, 2019), and bovine lactoferrin (EFSA NDA Panel, 2012a,b), both included in the Union list of NFs (Commission Implementing Regulation (EU) 2017/2470[6]).

Whey protein concentrates, whey basic protein isolates, milk protein concentrates and milk protein isolates have also been granted GRAS (Generally Recognised As Safe) status in the United States (US) (GRAS notices GRN 37, 504, 633 and 809) (US FDA, 2000, 2014, 2016, 2019). The per-capita consumption of whey and whey protein concentrate in the US has been estimated as 2.0 pounds (DM) per person in 2020 (USDA-ERS, 2021).

3.6.2. History of use of the NF

There is no history of use of the NF, i.e. BLG produced from bovine whey by crystallisation under acidic or neutral conditions. A related product, BLG produced by submerged fermentation with the filamentous fungus *Trichoderma reesei*, was granted GRAS status (GRN 863) in US in 2020, as a substitute for dairy and plant-derived proteins in foods (US FDA, 2020).

In relation to non-food uses, BLG is available in the market as a chemical reagent and certified reference material. The potential use of BLG as a carrier for hydrophobic and acid labile drugs for oral administration has been demonstrated (Barbiroli et al., 2010; Izadi et al., 2016; Bijari et al., 2019).

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population.

3.7.2. Proposed uses and use levels

The NF is intended to be used as an ingredient in isotonic and sport drinks, whey powder and milk-based drinks and similar products. These food products defined using the FoodEx2[7] hierarchy and the maximum use levels are reported in Table 7.

In addition, the NF is intended to be used in FSMP as defined in Regulation (EU) No 609/2013, for children above 3 years of age (excluding pregnant and lactating women). The use of the NF in FSMP will be determined by health professionals in accordance with the particular nutritional requirements of the persons for whom the products are intended, according to Regulation (EU) No 609/2013.

[6] Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, p. 72–201.

[7] FoodEx2 is an EFSA standardised food classification and description system https://www.efsa.europa.eu/en/data/data-standardisation

Table 7: Food categories according to FoodEx2 hierarchy and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)
A03GB	L4	Isotonic and sport drinks	25
A02PN	L4	Whey powder	8
A02NR	L4	Probiotic milk-like drinks	12

3.7.3. Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 7), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intake of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 8.

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the Excel file annexed to this scientific opinion (under supporting information).

Table 8: Intake estimate for the NF resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	1.3	40.5	0.0	159
Young children[8]	1 to < 3	1.5	65.3	0.0	500
Other children	3 to < 10	2.3	122	0.0	667
Adolescents	10 to < 18	1.8	76.1	0.0	463
Adults[(c)]	≥ 18	9.2	43.0	0.0	431

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 8 February 2022. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 8 February 2022. The lowest and the highest P95th observed among all EU surveys are reported in these columns.
(c): Includes elderly, very elderly, pregnant and lactating women.

3.8. Absorption, distribution, metabolism and excretion (ADME)

No ADME data have been provided for the NF. However, the applicant provided references to ADME studies on BLG or bovine whey protein.

BLG is relatively pepsin-resistant throughout the gastric phase and has a fast-gastric emptying, followed by rapid intestinal hydrolysis (*in vitro* and *in vivo* data) leading to absorption of amino acids in the proximal intestine (Mahé et al., 1995, 1996; Rieu et al., 2007; Farnfield et al., 2009; Sanchón et al., 2018; Mackie et al., 2019). Systemic absorption of undigested BLG may occur in infants, owing to gut transient permeability to BLG when cow's milk-based formula is started (Kuitunen et al., 1994).

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF.

The NF (≥ 90% w/w DM protein) is produced from bovine whey and is mainly composed of BLG (≥ 90% of total protein). Proximate composition and lactose content in the NF are similar to those in WPI (see Appendix A).

The applicant provided data on the amino acid profile of the NF produced under acidic and neutral conditions, which was compared to that of WPI, egg white and milk whey (see Appendix B). Results show that the NF and commercial WPI have a similar content of essential (58.2 vs. 52.6 g/100 g protein) and branched-chain (27.2 vs. 23.9 g/100 g protein) amino acids. In comparison with egg white and milk whey, the histidine content is lower and represents the first limiting amino acid for both

[8] Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

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the NF and WPI, compared to the indispensable amino acid reference pattern recommended by FAO (2013). Assuming a similar digestibility as reported in the literature for WPI (Mathai et al., 2017), calculation of Digestible Indispensable Amino Acid Scores (DIAAS) would yield a value of about 72 for children (6 months to 3 years) and 90, for older children, adolescents and adults.

Considering the highest protein level (94.39% w/w 'as is') reported in the batch-to-batch analysis of the NF, the highest mean and highest 95th percentile daily protein intake from the NF have been estimated, respectively, as 0.04 and 0.15 g/kg bw per day in infants (0 to < 1 year), 0.06 and 0.47 g/kg bw per day in young children (1 to < 3 years), 0.12 and 0.63 g/kg bw per day in children of 3 to < 10 years, 0.07 and 0.44 g/kg bw per day in adolescents (10 to < 18 years), and 0.04 and 0.41 g/kg bw per day in adults (including adult, elderly and very elderly subjects, and pregnant and lactating women). The Panel notes that the protein intake from the NF remains below the population reference intakes (PRIs) for protein for all age groups. Although a tolerable upper intake level (UL) has not been derived for protein (EFSA NDA Panel, 2012c), the protein intake from the NF may nevertheless further contribute to an already high dietary protein intake in Europe (EFSA NDA Panel, 2012c).

According to the composition and specifications of the NF (Tables 2, 3 and 6), the analysed mineral content includes aluminium (up to 0.6 mg/kg), calcium (≤ 0.15%), chloride (≤ 1.9%), chromium (up to 0.38 mg/kg), magnesium (≤ 0.1%), phosphorus (≤ 0.03%), potassium (≤ 1.2%) and sodium (≤ 0.5%). Considering the highest mean and highest 95th percentile daily intake estimates of the NF, the intake from the NF of those elements does not exceed the respective dietary reference values (DRVs).

The Panel considers that, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided three toxicological studies on the NF produced under acidic conditions (Batch No. J44DIMNI005), which were conducted in compliance with Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998) and in accordance with the test guidelines (TG) No 471 (OECD, 1997), 487 (OECD, 2016) and 408 (OECD, 2018). An additional preliminary *in vivo* repeated dose study was also carried out. These studies, which were claimed proprietary by the applicant, are listed in Table 9.

Table 9: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Study No. 190013 (Unpublished, 2019a)	Bacterial reverse mutation assay (GLP, OECD TG 471)	*Salmonella* Typhimurium TA98, TA100, TA1535 and TA1537. *Escherichia coli* WP2 uvrA	• 31.6–5,000 μg/plate • Plate incorporation and pre-incubation tests with/without metabolic activation via S9 mix
Study No. 190014 (Unpublished, 2019b)	*In vitro* mammalian micronucleus test in human lymphocytes (GLP, OECD TG 487)	Human peripheral blood lymphocytes	• 4 h: With (500–2,000 μg/mL) and without (1000–2,000 μg/mL) metabolic activation (S9 mix) • 44 h: Without metabolic activation (1,000–2,000 μg/mL)
Study No. 190015 (Unpublished, 2019c)	14-day repeated dose oral range-finding toxicity study	Healthy Wistar rats, Crl:WI (Han) (Full Barrier)	100, 300, 1,000 mg/kg bw per day
Study No. 190016 (Unpublished, 2020)	90-day repeated dose oral toxicity study (GLP, OECD TG 408 – 2018)	Healthy Wistar rats, Crl:WI (Han) (Full Barrier)	100, 300, 1,000 mg/kg bw per day

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3.10.1. Genotoxicity

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test and an *in vitro* mammalian cell micronucleus test (Unpublished study report, 2019a,b). These studies were conducted in compliance with OECD principles of GLP (OECD, 1998) and in accordance with the OECD TG No 471 and 487 of 1997 and 2016, respectively.

The assessment of the mutagenic potential of the NF (Unpublished study report, 2019a) was performed according to the plate incorporation and pre-incubation tests, using *S*. Typhimurium (TA98, TA100, TA1535 and TA1537) and *E*. *coli* WP2 uvrA strains, which were exposed to the NF at concentrations up to 5,000 μg/plate, either in the presence or absence of liver microsomal fractions (S9). No reproducible or dose-related increases in revertant colony numbers over control counts were observed for any of the strains, following exposure to the NF at any concentration (irrespective of the presence or absence of S9), for both the plate incorporation and the pre-incubation methods. No evidence of precipitation or toxic effects of the test item towards the bacterial test strains was obtained following exposure to the NF. Therefore, under the experimental conditions applied, the NF was considered to be non-mutagenic at concentrations up to 5,000 μg/plate, in the absence or presence of metabolic activation.

In the *in vitro* mammalian cell micronucleus test (Unpublished study report, 2019b), NF concentrations up to 2,000 μg/mL were tested in cultured human peripheral blood lymphocytes following 4-h exposure in the presence or absence of metabolic activation (S9 fraction), or 44-h exposure in the absence of metabolic activation. No cytotoxicity and no solid precipitates were observed. Neither a statistically significant nor a biologically relevant increase in the micronucleus frequency were observed following the short- or long-term exposure to the NF (irrespective of the presence or absence of S9). A statistically significant increase was recorded in the mean micronucleus frequency only at the mid concentration (1,500 μg/mL) for the 4-h treatment without S9 fraction, which was attributed to the high micronucleus count in one of the two evaluated cultures, but was not considered as biologically relevant. Any result with the test item was within the range of negative historical controls. Under the experimental conditions applied, the NF did not show any evidence of clastogenicity or aneugenicity in the absence or presence of metabolic activation.

Taking into account the test results provided and considering the nature, source and production process of the NF, the Panel considers that the available evidence does not raise concern regarding genotoxicity.

3.10.2. Subacute toxicity

The applicant submitted a 14-day toxicity study (Unpublished study report, 2019c), which was designed as a dose range finding study for the 90-day repeated oral dose toxicity study. Healthy Wistar rats were divided into four groups (six rats per group, three male and three female) and were orally administered 0, 100, 300 and 1,000 mg/kg bw of the NF per day. No mortality or clinical signs were observed in this study. There were no test item-related effects on body weight and food consumption. White blood cell (WBC) count was statistically significantly and dose-dependently increased in mid- and high-dose male rats at day 15. No such effect on WBC count was seen in females. There were no test item-related effects observed in the remaining haematology parameters, i.e. red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT) and platelet (PLT) levels. The study considered the elevated WBC count in male rats to be possibly related to a mild immunological reaction to the test item. However, the Panel notes that such an effect on the WBC count was not observed in the rat 90-day study (see Section 3.10.3). Mean creatinine and mean urea levels were statistically significantly decreased in male rats of the high-dose group only. However, these observations were not reproduced in the 90-day study, and the values were considered to be within historical controls (see Section 3.10.3). In male rats, 2/3 of the low-dose group and 2/3 of the high-dose group had pale kidneys (both sides) vs. 0/3 in the mid-dose group and 0/3 in the control group. 1/3 of the low-dose males had several red foci in the thymus vs. none in any other group. The authors considered these findings to be incidental and not related to treatment with the test item. In females, there were no macroscopic findings in any of the assessed organs and tissues.

3.10.3. Subchronic toxicity

A 90-day repeated oral dose toxicity study (Unpublished study report, 2020) was conducted following OECD TG No 408 (OECD, 2018) and in compliance with GLP standards. Healthy Wistar rats

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were divided into four groups (10 animals per sex per group) and were orally administered 0, 100, 300 and 1,000 mg/kg bw of the NF per day. There were no mortality and no statistically significant changes in body weight, food consumption, functional observation battery, ophthalmoscopic examination, urinalysis and fertility parameters (i.e. sex hormones, mean sperm parameters, mean testis weight, oestrus cycle, macroscopic and microscopic evaluation of reproductive organs and tissues).

The following effects were statistically significantly different from control in a dose-related manner or at the highest dose: (i) Moving of the bedding was seen in all male and female high-dose group animals on 3 treatment days, which was considered to be a sign of local reaction after test item application and not an adverse systemic effect of the test item. In addition, males and females of the mid- and high-dose groups showed statistically significantly less sleep and increased moving in the cage at some weeks; (ii) Total bilirubin levels were statistically significantly increased in mid- and high-dose females at study end, but not in males. Thyroid-stimulating hormone (TSH) levels were statistically significantly lower in high-dose male rats compared to the control group. There were no histopathological findings in any organs (assessed in control and high-dose groups); (iii) In female rats of the high-dose group, there was a statistically significant increase in mean RBC, mean HGB and mean HCT at study end. In male rats, there was only a statistically significant increase in mean HGB at the mid dose. As these findings were within the normal range of variation, they were not considered of toxicological relevance. In contrast to the 14-day dose range finding study, there were no statistically significant changes in WBC count from control in males and female rats; (iv) In female rats only, there was a statistically significant increase in absolute kidney weight at the highest dose, and in relative kidney/brain weight at the mid and highest doses. There were no differences in relative kidney/body weight. Statistically significant organ weight changes were noted for a decrease of heart weight in males. However, absolute weights of heart (males) and kidneys (females) lie within the historical control range; and (v) Isolated macroscopic findings in single animals of all groups did not correlate with adverse histopathological findings and no test item-related effects were considered.

The Panel considers that the highest dose tested (i.e. 1,000 mg/kg bw per day) is the no observed adverse effect level (NOAEL) of this study.

3.10.4. Human data

There are no human studies conducted with the NF. However, the applicant provided references to human studies on BLG or bovine whey protein (Axelsson et al., 1986; Mahé et al., 1996; Farnfield et al., 2009; Weinheimer et al., 2012; Areta et al., 2013; Poppitt et al., 2013; Chungchunlam et al., 2017).

Only three of the studies provided by the applicant included BLG treatment, given in single dosages of 54 g (Chungchunlam et al., 2017), 25 g (Poppit et al., 2013) and 13 and 69 g (Mahé et al., 1996). All of them were on small numbers of subjects and did not include safety-related parameters, apart from nausea (as secondary outcome) considered in the studies by Chungchunlam et al. (2017) and Poppitt et al. (2013). In the study by Chungchunlam et al. (2017), there were no differences in nausea visual analogue scale-rated feelings following a meal providing either BLG isolate, WPI or ALA. In the study by Poppitt et al. (2013), there were no significant reports of nausea following consumption of BLG. The other studies were on whey protein/WPI.

The available human data do not indicate safety concerns. However, due to the study designs (small number of subjects, single doses, other substance than the NF tested), the Panel notes that their contribution to the safety assessment of the NF is limited/of little relevance.

3.11. Allergenicity

The source of the NF is bovine milk, which is considered a common allergenic food by European Regulation[9]. The EFSA NDA Panel has previously summarised the allergenic properties of BLG (Bos d 5) and considered it as a major allergen in milk (EFSA NDA Panel, 2014). BLG belongs to the lipocalin protein family. Lipocalins have a high allergenic potential, and several allergens of animal origin belong to this family. They share a well-conserved sequence homology in their N-terminus moiety and the

[9] Regulation (EU) No 1169/2011 of the European parliament and of the council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304, 22.11.2011, p. 18–63.

invariable presence of tryptophan at position 19 (Papiz et al., 1986; Brownlow et al., 1997). Several reports have described that immunoglobulin E (IgE) epitopes are located on the surface of the protein (Chatchatee et al., 2001; Geiselhart et al., 2021; Jaiswal and Worku, 2021).

The applicant did not carry out any specific study to determine the potential allergenicity of this NF, but declares that because BLG is a well-known allergen, products containing the NF will follow the requirements defined in Regulation (EU) No 1169/2011 regarding the labelling of certain substances or products causing allergies. Furthermore, it is noted that soy lecithin may be used as an emulsifier during spray-drying and in those cases, the requirements defined in Regulation (EU) No 1169/2011 should also apply.

The Panel concludes that the intake of the NF can cause allergic reactions similar to those arising from consuming milk and dairy products.

4. Discussion

The NF (≥ 90% w/w DM protein) contains BLG as primary constituent (≥ 90% of total protein). BLG in the NF produced under both acidic and neutral conditions has been demonstrated to be chemically and structurally equivalent to the BLG present in bovine milk and WPI.

The applicant intends to market the NF as an ingredient in isotonic and sport drinks, whey powder and milk-based drinks and similar products, and in FSMP as defined in Regulation (EU) No 609/2013. The target population proposed by the applicant is the general population.

Intake estimates for the NF consumed via foods in which it would be added as an ingredient were performed for the general population, based on the EFSA Comprehensive European Food Consumption Database. The highest intake among population groups on a bw basis was calculated for children of 3 to < 10 years of age as 667 mg NF/kg bw per day at the 95th percentile.

The NF presents proximate composition, lactose levels and content of essential amino acids similar to those in WPI, with histidine being the first limiting amino acid in both, the NF and WPI. The Panel notes that the highest mean and highest 95th percentile daily protein intakes from the NF are both below the protein population reference intakes (PRIs) for all the population groups. Although a tolerable upper intake level (UL) has not been derived for protein (EFSA NDA Panel, 2012c), the protein intake from the NF may nevertheless further contribute to an already high dietary protein intake in Europe (EFSA NDA Panel, 2012c). The exposure to the analysed minerals does not raise concerns.

The submitted toxicity studies did not raise safety concerns. No adverse effects were observed in the subchronic rat study, up to the highest dose tested, i.e. 1,000 mg NF/kg bw per day.

Taking into account the similarities to WPI, the source, the production process, the nature of the NF, the history of safe use as a major component of bovine milk and the lack of toxicity in the experimental studies, the Panel considers that the consumption of the NF does not raise safety concerns.

5. Conclusions

The Panel concludes that the NF, beta-lactoglobulin, is safe under the proposed conditions of use.

5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant: toxicological information (*in vitro* genotoxicity studies, 14-day and 90-day repeated dose oral toxicity studies; Table 9); certificates of analysis of chloride and potassium levels in 23 additional batches of the NF (Section 3.4) and 20 batches of commercial WPI; and certificates of analysis of total plate count levels in 7 additional batches of the NF (Table 5).

6. Steps taken by EFSA

1) On 5 November 2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of beta-lactoglobulin as a NF pursuant to Regulation (EU) 2015/2283. Ref. Ares(2020)6383251 – 5 November 2020.
2) On 5 November 2020, a valid application on beta-lactoglobulin, which was submitted by Arla Foods Ingredients Group P/S, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1707) and the scientific evaluation procedure was initiated.

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3) On 16 March 2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 30 August 2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 18 November 2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 23 February 2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) During its meeting on 28 February 2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of beta-lactoglobulin as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
ADPI	American Dairy Products Institute
ALA	Alpha-lactalbumin

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Amd.	Amendment
BLG	Beta-lactoglobulin
bw	Body weight
CAS	Chemical Abstracts Service
CFU	Colony forming unit
Crl:WI(Han) rats	Charles River laboratories: Han Wistar rats
DE Food	German Food and Feed Code
DIAAS	Digestible Indispensable Amino Acid Scores
DM	Dry matter
DRV	Dietary reference value
DS	Danish standard
EN	European norm
Eurostat	European Statistical Office
FAO	Food and Agriculture Organisation of the United Nations
FoodEx2	EFSA standardised food classification and description system
FSMP	Food for special medical purposes
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRAS	Generally Recognised As Safe
GRN	GRAS Notice
HACCP	Hazard Analysis Critical Control Points
HCT	Haematocrit
HGB	Haemoglobin
HPLC/FLD	High performance liquid chromatography with fluorescence detection
ICP	Inductively coupled plasma
IC-UV	Ion chromatography with UV detection
IDF	International Dairy Federation
IgE	Immunoglobulin E
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
MS	Mass spectrometry
ND	Not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	Novel food
NMKL	Nordic Committee on Food Analysis
NMR	Nuclear magnetic resonance spectroscopy
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PLT	Platelets
PRI	Population reference intake
RBC	Red blood cell
RH	Relative humidity
RP-HPLC/UV	Reverse phase-high performance liquid chromatography with UV detection
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
TG	Test guidelines
TSH	Thyroid-stimulating hormone
UL	Tolerable upper intake level
US	United States
US FDA	US Food and Drug Administration
USDA-ERS	US Department of Agriculture-Economic Research Service
WBC	White blood cell
WPI	Whey protein isolate
w/w	weight per weight

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Appendix A – Proximate composition and mineral content of the NF and commercial WPI

Parameter	NF(1) (acidic)	NF(1) (neutral)	Commercial WPI(2)	Method of analysis
Proximate composition				
Protein 'as is' (N × 6.38) (%)	92.04 ± 0.68	93.60 ± 0.81	89.20 ± 0.27	Kjeldahl; ISO 8968-3:2004/IDF 20-3:2004
Protein in DM (N × 6.38) (%)	95.47 ± 0.61	97.38 ± 0.89	93.81 ± 0.39	Calculation(3)
BLG(4) (% of protein)	103.52 ± 2.04	99.19 ± 2.13	49.06(5)	RP-HPLC/UV (validated internal method)
Lactose (%)	0.09 ± 0.00	0.09 ± 0.00	0.10 ± 0.00	Enzymatic; ISO 5765-2/IDF 79-2:2002
Fat (%)	0.11 ± 0.07	0.06 ± 0.03	0.12 ± 0.04	Gravimetry; ISO 1736/IDF 9:2008
Ash (%)	0.10 ± 0.02	1.82 ± 0.04	3.78 ± 0.04	Gravimetry; NMKL 173:2005
Moisture (%)	3.60 ± 0.15	3.88 ± 0.19	4.92 ± 0.16	Gravimetry; ISO 6731:2010/IDF 21:2010
Mineral content				
Chloride (%)	1.53 ± 0.06	0.04 ± 0.00	0.07 ± 0.02	Potentiometric titration; ISO 5943: 2006/IDF 88:2006
Calcium (%)	0.025 ± 0.000	0.01 ± 0.00	0.06 ± 0.01	ICP (internal method)
Magnesium (%)	0.003 ± 0.000	0.003 ± 0.000	0.01 ± 0.00	ICP (internal method)
Phosphorus (%)	0.025 ± 0.000	0.03 ± 0.00	0.20 ± 0.00	ICP (internal method)
Potassium (%)	0.025 ± 0.000	0.67 ± 0.01	1.17 ± 0.05	ICP (internal method)
Sodium (%)	0.025 ± 0.000	0.28 ± 0.01	0.49 ± 0.01	ICP (internal method)

DM: Dry matter; ICP: Inductively coupled plasma; IDF: International Dairy Federation; ISO: International Organisation for Standardisation; NF: Novel food; NMKL: Nordic Committee on Food Analysis; RP-HPLC/UV: Reversed phase-high performance liquid chromatography with UV detection; WPI: Whey protein isolate.

(1): Average of five batches of the NF (Tables 2 and 3).

(2): Average of five (fully characterised) batches of commercial WPI.

(3): Protein in DM = (Protein 'as is'/DM) × 100%.

(4): The levels of BLG, expressed as % of the total protein, exceed 100% in some batches, which can be attributed to the uncertainty of the method (RP-HPLC/UV) used for the quantification of BLG using a commercial standard with 90% purity.

(5): Calculated as 55% of total protein.

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Appendix B – Amino acid profile of the NF, egg white, milk whey and commercial WPI

Parameter	NF[(1),(4)] (acidic)	NF[(1),(4)] (neutral)	Egg white[(2)]	Milk whey[(2)]	Commercial WPI[(3),(4)]
Amino acids (g/100 g protein)					
Alanine	6.3 ± 0.2	6.7 ± 0.2	6.3	5.4	5.5 ± 0.2
Arginine	2.5 ± 0.1	2.6 ± 0.1	6.1	3.2	2.1 ± 0.1
Aspartic acid	10.4 ± 0.2	11.0 ± 0.3	11.0	12.4	10.8 ± 0.8
Cystine	2.6 ± 0.2	2.5 ± 0.1	3.0	3.0	1.7 ± 0.2
Glutamine	18.4 ± 0.6	19.0 ± 0.5	13.9	18.1	18.3 ± 1.0
Glycine	1.2 ± 0.0	1.3 ± 0.1	3.8	2.2	1.5 ± 0.1
Histidine	1.5 ± 0.0	1.6 ± 0.0	2.4	2.5	1.5 ± 0.1
Isoleucine	5.6 ± 0.2	5.9 ± 0.1	5.4	6.0	6.5 ± 0.3
Leucine	14.1 ± 0.4	15.2 ± 0.4	8.9	13.5	10.8 ± 0.4
Lysine	11.1 ± 0.3	11.6 ± 0.3	7.3	11.0	9.7 ± 0.4
Methionine	2.8 ± 0.0	2.9 ± 0.1	4.1	2.3	2.1 ± 0.1
Phenylalanine	3.1 ± 0.1	3.4 ± 0.1	6.2	4.0	2.9 ± 0.2
Proline	4.9 ± 0.1	5.1 ± 0.1	4.0	5.3	6.2 ± 0.2
Serine	3.4 ± 0.1	3.6 ± 0.1	7.1	5.1	4.7 ± 0.2
Threonine	4.8 ± 0.1	4.9 ± 0.1	4.8	5.7	7.2 ± 0.4
Tryptophan	2.0 ± 0.1	2.1 ± 0.1	1.5	2.4	1.7 ± 0.0
Tyrosine	3.3 ± 0.1	3.6 ± 0.1	4.3	4.0	2.7 ± 0.1
Valine	5.4 ± 0.2	5.7 ± 0.2	7.2	5.8	5.9 ± 0.2

HPLC-FLD: High-performance liquid chromatography with fluorescence detection; IC-UV: Ion chromatography with UV detection; ISO: International Organisation for Standardisation; NF: Novel food; WPI: Whey protein isolate.

(1): Average of five batches of the NF.

(2): Calculated from Matsuoka et al. (2019).

(3): Average of three (fully characterised) batches of commercial WPI.

(4): The method used for the analytical determination corresponds to ISO 13903:2005/IC-UV, except for tryptophan where EU No 152/2009/HPLC-FLD was applied.

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Annex A – Dietary exposure estimates to the Novel Food for each population group from each EU dietary survey

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.7204#support-information-section).

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