Safety Assessment of LumiVida® (Hen Egg White Lysozyme Hydrolysate)

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Novel Food Classification: Class 1.2(e)

Introduction

An application for the authorisation of hen egg white lysozyme hydrolysate (lumiVida®) was submitted to the Food Safety Authority of Ireland (FSAI) by DSM Nutritional Products Ltd. of the United Kingdom in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on August 31st, 2016.

Hen egg white lysozyme is a non-glycosylated protein comprised of 129 amino acids and has a molecular weight of approximately 14.4 kD. It was the first protein to be sequenced and to have its three-dimensional structure completely analysed. Lysozymes from different sources are very similar, displaying only minor variations in amino acid sequence and three-dimensional structures. Lysozyme is authorised as a food additive (E1105) for the EU market with preservative anti-microbial effects, for example in the cheese and wine manufacturing industries. The novel ingredient comprises a distinct set of peptides of varying amino acid length (primarily di- and tri-peptides) and composition, many of which are rich in tryptophan. It is produced by the hydrolysis of commercially available hen egg white lysozyme in a manufacturing process that incorporates a food grade protease (subtilisin). The applicant proposes to market the novel ingredient in food supplements at doses up to 1,000mg/day and as a food fortification ingredient, particularly in non-alcoholic beverages.

Lysozyme that occurs naturally in chicken eggs has a significant history of consumption within the EU and around the world. However, the hydrolysate of hen egg white lysozyme is considered to be a novel food ingredient in the EU, and classified by the applicant in accordance with *Article 1.2(e)* of the novel food Regulation (EC) No 258/97: *"foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use*". In order to assess wholesomeness, the

application dossier was prepared pursuant to Class 2 of Commission Recommendation 97/618/EC; *"Complex NF from non-GM source",* and subclass (1); *"the source of the NF has a history of food use in the Community".*

I. Specification of the novel food

The novel ingredient is a white to light yellow powder, predominantly comprised of protein (≥80%) along with relatively minor levels of ash (<10%), sodium (<6%) and moisture (<5%). The source lysozyme is hydrolysed (99.2%) to various peptides, at least ten of which contain the amino acid tryptophan (≥5.5%) and a smaller number of long chain neutral amino acids (LNAA).

Physical and Chemical Tests		
	Specification	**Test Method**
Appearance	Powder	visual
Color	White to light yellow	visual
Protein (TN*x5.30)	≥ 80%	ISO 8968 -1
Tryptophan (Trp)	≥ 5.5%	C2529[$]
Ratio Trp/LNAA	≥ 0.18	C2425[$]
Moisture	< 5%	methode 3B Landbouwkwaliteitsregeling beschikking poedervormige melkproducten[&]
Ash	< 10%	AOAC Official Mehods nr. 930.30
Sodium	< 6%	ICP-AES[#]

*TN: total nitrogen; [$]: validated manufacturer method: [&]: Method ISO-certified Qlip, Zutphen, The Netherlands
[#]: Method ISO-certified NIZO, Ede, The Netherlands

The novel ingredient is stable for up to 36 months when stored in a closed container at ambient temperature (below 25°C). Different storage temperatures were found to have no impact on the physical and chemical properties of the novel ingredient, though differences in the sensory profile were observed when the product was stored at 40°C.

II. Effect of the production process applied to the novel food

The novel ingredient is produced by a standard production processes using commercially available hen egg white lysozyme as the starting material. Solubilised protein is enzymatically hydrolysed by subtilisin, a well characterised protease derived from *Bacillus licheniformis* and commonly used in the food industry. Crude hydrolysate is subjected to

filtration, centrifugation, ultrafiltration, chromatography, pasteurisation and spray drying before packaging. The applicant has demonstrated that the production process reproduces a consistent peptide profile with no intact lysozyme remaining (99.2% conversion).

III. History of the organism used for the novel food

Chicken eggs, and therefore lysozyme, have a long history of safe food use worldwide. In addition, lysozyme is an EU-authorised food additive (E1190) with a preservative role in some foods.

IX. Anticipated intake/extent of use of the novel food

The novel ingredient is to be added to food supplements and other food products as outlined in Table 1, with the aim of raising plasma levels of a particular amino acid (tryptophan). The consumption of foods high in tryptophan is associated with certain health benefits, but this is not addressed in this assessment. The applicant has clarified that the foods to which the novel ingredient will be added will be low protein foods in order to maintain a favourable tryptophan/LNAA ratio which is required to achieve the purported health benefits. Adults and adolescents are the primary target population groups, though consumption by other age groups cannot be discounted. Food supplements containing up to 500mg/serving of the novel ingredient will be in the form of tablets, capsules, soft gels, gel caps, liquids or powders with a recommended daily intake up to 1,000 mg/day. Non-alcoholic beverages to which the novel ingredient is to be added include near water beverages, energy drinks and shots, juices and instant drink powders.

The applicant anticipates that the consumption of foods containing the recommended dose of the novel ingredient (500 mg) will be self-limiting due to the costs of the ingredient. The amount of lumiVida® to be consumed through food supplements will also be managed by means of the mandatory instructions of use (0.5 – 1.0 g/day) as set out in *Article 6.3*(b) and (c) of the food supplements Directive 2002/46/EC.

The applicant derives intake estimates of lumiVida® using 4 data sources,

i) The UK National Diet and Nutrition Survey (NDNS; 2008-09)

ii) The Dutch Nutrition and Food Consumption Survey (DNFCS; 2007-2010)

iii) EFSA FAIM assessments

iv) Probabilistic modelling of intakes using DNFCS

Table 1: Proposed dosage of Hen Egg White Lysozyme Hydrolysate (lumiVida®)

Food category	Quantity per serving (mg)
Supplements	500*
Non-alcoholic beverages	500
Chewing gum	500
Sweets	500
Chocolates	500
Low-protein sorbets	500
Other applications	500

*Dose to be recommended to supplement producers: 500mg
Maximum anticipated supplemental dose: 1000mg

The proposed NOAEL for lumiVida® is 5,790 mg/kg bw/day based on rat toxicity studies, which means a maximum safe dose of 57.9mg/kg bw/day or 4g/day for a 70Kg person considering a margin of safety of 100. This equates approximately to 240 mg/day L-tryptophan based on the calculations provided by the applicant (500g lumiVida ~ 30mg L-tryptophan).

The intake of lumiVida® from supplements is estimated to be approximately 1 g/day (2 x 500g supplemental doses), which for a 70Kg person would mean 14.3 mg/kg bw/day (maximum safe dose 57.9 mg/kg bw/day).

Worst case scenario estimates were calculated by the addition of 1g/day (or 14.3 mg/kg bw/day for a 70 kg adult) of the novel ingredient to the highest mean and high level use intakes as estimated by the FAIM model for both adolescents and adults. The combined high use levels were estimated as 85.2 mg/kg bw/day for adolescents and 77.7 mg/kg bw/day for adults, both of which exceed the maximum safe dose of 57.9 mg/kg bw/day. However, such a combination of supplement usage and P95 intakes from non-alcoholic beverages is not considered very likely, and so not a particular concern.

The applicant also carried out probabilistic modelling using Dutch national food consumption data to assess the normal intake level of lumiVida® and to characterise the proportion of the population likely to exceed the maximum safe dose. Normal intakes were calculated including the relative impact of coffees, teas and infusions which had not been factored hitherto. Using Monte-Carlo simulations, predicted daily intake of the novel ingredient

through consumption of non-alcoholic beverages at 0.5g/300ml serving size did not exceed 1.8g/day, with >99% of the population estimated to have a normal intake of <1g/day.

Intake estimates for the novel ingredient are conservative with respect to non-alcoholic beverages and supplements and do not suggest a risk in the two populations studied. While, the relative contributions from other proposed food groups to total intake and by age group are unclear, it could be argued that they are factored in by the conservative nature of the overall intake assessments for supplements and non-alcoholic beverages.

X. Information from previous human exposure to the novel food or its source

As a constituent of hen egg white, lysozyme has a long history of consumption worldwide. About 60% of the weight of an egg is made up of egg white, of which lysozyme is approximately 0.3%. Lysozyme is an authorised food additive in the EU with preservative antimicrobial effects used by the cheese and wine industries. Lysozyme has GRAS ('generally regarded as safe') status in the USA where hen egg lysozyme hydrolysate is registered with the FDA as a new dietary ingredient for supplements.

XI. Nutritional information on the novel food

The novel ingredient is comprised of ≥80% protein, with the remainder comprised of ash (<10%), sodium (<6%) and moisture (<5%). The amino acid profile of hen egg white lysozyme hydrolysate (lumiVida®) is essentially the same as that for hen egg white lysozyme. However, due to some amino acid loss during hydrolysis, the novel ingredient contains up to 20% less protein/gram compared to intact hen egg white lysozyme. The caloric value of the novel ingredient is approximately 16kcal/day at the maximal safe dose of 4g/day (assuming 4kcal/g of protein). The applicant estimates that the proposed dietary intake of tryptophan from the novel ingredient would have a relatively minor impact on the total daily intake of that amino acid.

The applicant estimates that in general, regular egg consumers would have an intake of 126mg/day of intact lysozyme, while intake of lysozyme as a food additive from lysozyme-treated cheese would range from 2.5- 28 mg/day in adults. Therefore, the proposed intakes of lysozyme as a hydrolysate (0.5-1 g/day) may be higher than the amounts of intact lysozyme typically consumed through eggs. However, in terms of overall dietary protein intake, the proposed intake of protein from lumiVida® (at around 2% of an average adult daily intake of protein) is unlikely to have any significant impact on overall protein intake.

XII. Microbiological information on the novel food

Batch analysis demonstrates the microbiological purity of the novel ingredient. Product specifications are set out in relation to total combined yeasts/moulds count, *Enterobacteriaceae, Salmonella Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* The applicant states that routine microbial analysis will be performed to ensure compliance with product specifications.

XIII. Toxicological information on the novel food

Metabolic fate

The applicant makes the reasonable argument that specific ADME studies (Absorption, Distribution, Metabolism and Excretion) are not necessary for the novel ingredient as it is a hydrolysed form of a normal dietary protein. An overview of available information on protein and peptone ADME was carried out by the applicant to support this position. Hen egg white lysozyme is rich in the essential amino acid tryptophan. The literature indicates that the majority of dietary tryptophan is used for protein and kynurenine synthesis in the body, while approximately 3% is used as a precursor for serotonin synthesis. Tryptophan crosses the blood-brain barrier where it is metabolised to serotonin, a neurotransmitter involved in a number of neurological processes. The hydrolysed form of lysozyme (di- and tri-peptides) present in lumiVida® provides a more bioavailable form of tryptophan facilitating quicker metabolism. Large neutral amino acids (LNAAs) such as valine, isoleucine, leucine, methionine, tyrosine and phenylalanine are closely related to tryptophan and cross the blood-brain-barrier through the same transporter system. Therefore, because LNAAs compete with tryptophan for uptake into the brain, it is important that they are proportionate in the blood to enhance tryptophan absorption into the CNS.

Toxicological Studies

Because the source of the novel ingredient is a routinely consumed naturally occurring protein (hen egg white lysozyme), no specific studies were conducted in relation to acute toxicity, carcinogenicity or reproductive toxicity.

Repeat-dose toxicity

A dose range-finding oral toxicity study in rats carried out in accordance with OECD guidelines 407 and 408 did not reveal any treatment-related or toxicologically significant observations, with the highest dose of 5,790 mg/kg bw being considered the 'no-observed-adverse-effect-level' (NOAEL). A sub-chronic repeat dose oral toxicity study was performed

at three dose groups of ten male and ten female Wistar rats received the lumiVida® preparation once daily for 90 consecutive days, by oral gavage at dosages of 580, 1,740, and 5,790 mg/kg bw/day. Administration of the test substance by gavage did not lead to any adverse findings on general health, growth, food consumption, neurological findings, hematological and biochemical parameters and histopathology. In mid and high dose female groups, a treatment-related increase in weekly body weights and net weight gain was observed. Statistically significant higher terminal fasting body weights were observed in mid and high dose females (dose-correlated). These changes were considered treatment related, but not toxicologically relevant, and therefore the highest dose of 5,790 mg/kg bw was considered to be the 'no-observed-adverse-effect-level' (NOAEL).

Genotoxicity

An *in vitro* bacterial reverse mutation test with tryptophan rich protein hydrolysate was carried out under GLP conditions in accordance with OECD guideline 471. The applicant concluded that the lysozyme hydrolysate was not mutagenic under the conditions employed in the study in any test strains assessed either in the presence or absence of S9 fractions (+/- metabolic activation). Following an *in vitro* mammalian chromosome aberration test (OECD guideline no 473), the applicant concluded that lysozyme hydrolysate was cytotoxic but not clastogenic to cultured human lymphocytes both in the presence and absence of S9 fractions (+/- metabolic activation).

Skin irritation

An *in vitro* skin irritation test using a human skin model (OECD guideline 439) was conducted under GLP conditions. The applicant concluded that the test was valid, and that the novel ingredient was non-irritant under the experimental conditions described.

Human Safety Data

The literature reports three human studies applying a bolus dose of 12g of lumiVida® with no adverse effects associated with the intervention.

The applicant provided information on the history of use of L-tryptophan as a food supplement where adverse effects (eosinophilia-myalgia syndrome) were observed following consumption of synthetic tryptophan in the late 1980s in the USA and in Europe. However, a direct cause and effect between the adverse effects observed and L-tryptophan consumption was never established. In addition, a number of subsequent studies along with other confounding factors have diminished the possible link between eosinophilia-myalgia

syndrome and tryptophan consumption, which has ultimately led to a relaxation of the marketing restrictions initially applied to tryptophan-containing supplements in the USA.

Allergenicity

The results of allergenicity studies on the novel ingredient indicate that while the hydrolysis of hen egg lysozyme may affect the number of potentially allergenic epitopes, the egg allergy threat is not removed by hydrolysis. In any case, all lumiVida®-containing products must declare the presence of egg as an allergen in line with the requirements of *Article 9.1(c)* of Regulation (EU) No 1169/2011.

Conclusions

Hen egg white lysozyme has a long history of safe consumption in the EU and around the world as a constituent in eggs, as well as an EU-authorised additive with preservative effects. The hysdolysate of hen egg white lysozyme is almost identical to the lysozyme itself in terms of composition, though some amino acid loss may occur due to the hydrolysis process. The obvious difference is that lysozyme is a 129 amino acid protein molecule while the hydrolysate is composed of a mix or primarily di-and tri-peptides. While the metabolism of peptides generally follows the same pathway as their larger protein counterparts, the desired feature of this novel ingredient is the greater bioavailability afforded by the smaller size of peptides compared to larger protein molecules. This size feature facilitates greater transfer of tryptophan across the blood-brain barrier for one of its function as a serotonin precursor. Intake estimates for the novel ingredient are conservative with respect to non-alcoholic beverages and supplements and do not suggest a risk in the two populations studied. While, the relative contributions from other proposed food groups to total intake and by age group are not as well defined in this application, it could be argued that they are factored in by the conservative nature of the overall intake assessments for supplements and non-alcoholic beverages.

Recommendation

The Food Safety Authority of Ireland has not identified any safety concerns associated with the consumption of hen egg white lysozyme hydrolysate (lumiVida®) at the proposed use levels in foods, food supplements or non-alcoholic beverages containing the novel ingredient. Therefore the FSAI is of the opinion that the novel ingredient meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.