Assessment of the application for renewal of authorisation of PHYZYME® XP 5000 G/L (6-phytase) for chickens for fattening, laying hens, turkeys for fattening, ducks for fattening, weaned piglets, pigs for fattening and sows for reproduction

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Abstract

PHYZYME® XP 5000 G/L is a feed additive that contains 6-phytase produced by a genetically modified strain of Schizosaccharomyces pombe. The applicant requested for the renewal of the authorisation for PHYZYME® XP 5000 G and L to be used as a feed additive in chickens for fattening, laying hens, turkeys for fattening, ducks for fattening, piglets (weaned), pigs for fattening and sows. This scientific opinion concerns the renewal of the authorisation of the additive for those species. To support the request, the applicant provided evidence that the additive in the market complies with the conditions of the authorisation. According to the information provided by the applicant, no new evidence has been identified that would make the FEEDAP Panel reconsider the previous conclusions regarding the safety for the target species, consumer, user and environment. The application for renewal of the authorisation did not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there was no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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Keywords: zootechnical additives, digestibility enhancers, phytase, safety, efficacy, poultry, pigs

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from Danisco Animal Nutrition for renewal of the authorisation of the product PHYZYME® XP 5000 G/L (6-phytase), when used as a feed additive for chickens, turkeys and ducks for fattening, laying hens, weaned piglets, pigs for fattening and sows (category: zootchnical additives; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 14(1) (renewal of the authorisation). The particulars and documents in support of the application were considered valid by EFSA as of 24 October 2016.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product PHYZYME® XP 5000 G/L (6-phytase), when used under the proposed conditions of use (see Section 3.2.1).

1.2. Additional information

The additive PHYZYME® XP 5000 G/L is a preparation of 6-phytase produced by a genetically modified strain of Schizosaccharomyces pombe (ATCC 5233). EFSA issued two opinions which considered the safety and efficacy of the enzyme preparation when used in chickens for fattening, laying hens, turkeys for fattening, ducks for fattening, piglets (weaned), pigs for fattening and sows, which included the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification (EFSA, 2006a,b). A third opinion was adopted regarding the use of a more concentrated form, PHYZYME® XP 10000 G and L (EFSA, 2008), and a fourth opinion on the extension of use of the additive for minor poultry species was adopted in 2012 (EFSA FEEDAP Panel, 2012a).

PHYZYME® XP 5000 XP G/L is authorised for use in chickens for fattening, laying hens, turkeys for fattening, ducks for fattening, piglets (weaned), pigs for fattening and sows3,4 and all avian species5. The applicant requested the renewal of the authorisation for PHYZYME® XP 5000 G and L to be used as a feed additive in chickens for fattening, laying hens, turkeys for fattening, ducks for fattening, piglets (weaned), pigs for fattening and sows.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier6 in support of the authorisation request for the use of PHYZYME® XP 5000 G/L as a feed additive.

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1 Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
2 Danisco (UK) Ltd., trading as Danisco Animal Nutrition, PO Box 777, SN8 1AA Marlborough, UK.
3 Commission Regulation (EC) No 785/2007 of 4 July 2007 concerning the authorisation of 6-phytase EC 3.1.3.26 (Phyzyme XP 5000G Phyzyme XP 5000L) as a feed additive. OJ L 175, 25.7.2007, p. 29.
4 Commission Regulation (EC) No 379/2009 of 8 May 2009 concerning the authorisation of a new use of 6-phytase EC 3.1.3.26 as a feed additive for chickens for fattening, turkeys for fattening, laying hens, ducks for fattening, piglets (weaned), pigs for fattening and sows (holder of the authorisation Danisco Animal Nutrition, Legal Entity Danisco (UK) Limited. OJ L 116, 9.5.2009, p. 6. Holder of the Authorisation modified to 'Danisco (UK) Ltd, trading as Danisco Animal Nutrition and represented by Genencor International B.V.' by Commission Implementing Regulation (EU) 2019/221 of 6 February 2019.
5 Commission Implementing Regulation (EU) No 840/2012 of 18 September 2012 concerning the authorisation of 6-phytase (EC 3.1.3.26) produced by Schizosaccharomyces pombe (ATCC 5233) as a feed additive for all avian species for fattening other than chickens for fattening, turkeys for fattening and ducks for fattening and all avian species for laying other than laying hens (holder of authorisation Danisco Animal Nutrition). OJ L 252, 19.9.2012, p. 14. Holder of the Authorisation modified to 'Danisco (UK) Ltd, trading as Danisco Animal Nutrition and represented by Genencor International B.V.' by Commission Implementing Regulation (EU) 2019/221 of 6 February 2019.
6 FEED dossier reference: FAD-2016-0045.
The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance are valid and applicable for the current application.\(^7\)

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of PHYZYME® XP 5000 G/L is in line with the principles laid down in Regulation (EC) No 429/2008\(^8\) and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013), Guidance on zootchnical additives (EFSA FEEDAP Panel, 2012a,b) and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

### 3. Assessment

The additive PHYZYME® XP 5000 G/L is a feed additive that contains 6-phytase produced by a genetically modified strain of *S. pombe* (ATCC 5233). This additive is authorised for use in all avian species for fattening and laying, weaned piglets, pigs for fattening and sows. This opinion deals with the renewal of the authorisation of PHYZYME® XP 5000 G/L as a zootchnical additive (functional group of digestibility enhancers) for chickens for fattening, laying hens, turkeys for fattening, ducks for fattening, piglets (weaned), pigs for fattening and sows.

#### 3.1. Characterisation of the additive

The Panel considers that these modifications do not have an impact on the final product and data regarding the characterisation supports this conclusion. The applicant declared that no antibiotics are used during the manufacturing process.\(^10\)

PHYZYME® XP 5000 G contains the phytase (solids 1.4–2.1%), calcium propionate (0.1–0.3%), citric acid (0.2–0.8%) and wheat flour (up to 100%). The composition of this formulation is basically the same as the one previously related with minor modifications on the amounts used (e.g. citric acid from 0.4–1.0% to 0.2–0.8%). PHYZYME® XP 5000 L contains the phytase (solids 1.5–2.3% in the final additive), sorbitol (2–8%), sodium chloride (11–17%), sodium citrate (0.5–1.0%), sodium benzoate (0.1–0.2%), potassium sorbate (0.07–0.11%) and water.

The two formulations ensure a minimum guaranteed enzyme activity of 5,000 FTU\(^11\)/g. The batch-to-batch variation was studied in three recent batches of each formulation. The mean enzyme activity in the PHYZYME® XP 5000 G was 6,198 FTU/g (range 5,969–6,637 FTU/g) and in PHYZYME® XP 5000 L was 6,343 FTU/g (range 5,890–6,902).\(^12\)

Purity specifications for the two formulations include chemical compounds lead (\(\leq 5\) mg/kg), cadmium (\(\leq 0.5\) mg/kg), mercury (\(\leq 0.1\) mg/kg) and arsenic (\(\leq 2\) mg/kg) as well as absence of coliform (per gram of product), and absence of *Escherichia coli* and *Salmonella* spp. (negative in 25 mL). Compliance with these specifications was demonstrated in at least three recent batches of each formulation.\(^13\)

Purity data submitted included also information on mycotoxins; aflatoxin B1...
(< 0.1 µg/kg for solid, < 1 µg/kg for the liquid), ochratoxin A (< 0.2 µg/kg for the solid and < 2 µg/kg for liquid), zearalenone (< 25 µg/kg), T2-toxin (< 10 µg/kg only in the solid), fumonisins (< 600 µg/kg only in the liquid) and deoxynivalenol (< 330 µg/kg). Microbiological purity also included total viable cells (< 1.7 × 10⁵ colony forming units (CFU)/g solid formulation, < 7 CFU/g for liquid formulation). No antimicrobial activity was found in three batches of either formulation.¹⁴

The 6-phytase present in the additive is obtained by fermentation with a genetically modified strain of S. pombe which is deposited at the American Type Culture Collection under deposition number SD-5233.¹⁵ The taxonomic classification of the strain as S. pombe was confirmed. The assessment of the genetic modification was performed in a previous opinion (EFSA, 2006a) and the Panel concluded that the genetic modification does not raise any safety concern. The production strain has not been subject to any further genetic modification.

The presence of viable cells of the production strain was investigated in three batches of the liquid formulation and three of the solid formulation (analysis of three samples for each batch in duplicate).¹⁷ The samples of the liquid formulation were plated (1 mL) in a proprietary in-house medium appropriate for S. pombe and incubated at 30°C for 72 h. The samples of the solid formulation were first dissolved (approximately 1:10) and then 1 mL was plated in yeast extract glucose agar at 25°C for 120 h. No cells were detected. A positive control was included in the analysis.

The presence of recombinant DNA was analysed in triplicate in three batches of the liquid formulation.¹⁸ The primers targeted the recombinant phytase gene, with an amplicon size of 1,182 bp and the methodology included a lysis step. The analysis showed no amplification in the samples (while positive polymerase chain reaction (PCR) control gave amplification). The limit of detection in samples spiked with DNA was < 1 ng/mL. The results in the liquid formulation apply also to the solid formulation.

### 3.1.1. Conditions of use

The additive is authorised at a minimum recommended level of 150 FTU/kg feed for laying hens, 250 FTU/kg feed for chickens, turkeys and ducks for fattening, weaned piglets and pigs for fattening, and at 500 FTU/kg feed for sows. The applicant does not ask for a modification of these conditions of use.

Under the other provisions, it is stated that ‘in the directions for use of the additive and premixture, indicate the storage temperature, storage life, and stability to pelleting. For use in feed containing more than 0.23% phytin bound phosphorus. For piglets (weaned) up to 35 kg of body weight.’

### 3.2. Safety

#### 3.2.1. Previous assessments

EFSA issued two opinions (EFSA, 2006a,b) which considered the safety and efficacy of the enzyme preparation when used in chickens for fattening, laying hens, turkeys for fattening, ducks for fattening, piglets (weaned), pigs for fattening and sows, which included the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification. The Panel concluded that the additive is safe for the target species at the recommended conditions of use, that the genetic modification of the production strain is of no concern and the use of the product as a feed additive would be of no concern for the consumers of products derived from animals fed with the additive, or for the environment. Concerns for the user were limited to its potential as a respiratory sensitiser.

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¹⁴ Technical dossier/Supplementary information June 2018/Annex II.6.
¹⁷ Technical dossier/Supplementary information August 2018/Annex 4.
¹⁸ Technical dossier/Supplementary information August 2018/Annex 5.
3.2.2. Further evidence

In 2007, EFSA established the Qualified Presumption of Safety (QPS) approach to safety assessment for microorganisms (EFSA, 2007). The production strain of the additive belongs to a species, *S. pombe*, that is considered to qualify for the QPS approach to safety assessment (EFSA, 2007 and EFSA BIOHAZ Panel, 2017). The taxonomic classification of the strain has been unambiguously established and the genetic modification raised no concerns. Therefore, the fermentation product obtained from the production strain does not raise safety concerns for the consumer and the environment.

In line with the requirements established in the EFSA guidance on the renewal (EFSA FEEDAP Panel, 2013), the applicant performed a literature search in order to provide evidence that in the light of the current knowledge the additive remains safe under the approved conditions for target species, consumers, users and the environment. The literature search was conducted in July 2016 and covered the period from 2005 to 2016.19 Search terms used, and the strategy followed were reported. The search terms included several key words including those for the production strain (*Schizosaccharomyces pombe* and deposit number), words related to safety (e.g. safe* and toxic*, poison*) as well as the target species. The search was conducted using three databases, CAB Abstracts, Veterinary Science Database and Medline. The literature search retrieved 38 publications but in none of these publications adverse events or safety issues concerning the additive were reported (See Appendix A).

The applicant claims that no adverse effects have been reported in the framework of its global monitoring plan.20

3.2.3. Conclusions on the safety

Based on the above and the fact that the additive and the conditions of use for the species/categories for which the additive is authorised have not been modified, the Panel considers that there is no evidence to reconsider the conclusions reached in previous assessments. Therefore, the additive is safe for chickens, turkeys and ducks for fattening, laying hens, weaned piglets, pigs for fattening and sows, consumers of products from animals fed the additive and the environment but it is considered a potential respiratory sensitiser.

3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation21 and Good Manufacturing Practice.

4. Conclusions

The additive currently in the market complies with the existing conditions of authorisation.

The FEEDAP Panel concludes that PHYZYME® 5000 G/L remains safe for chickens, turkeys and ducks for fattening, laying hens, weaned piglets, pigs for fattening and sows, consumers of products from animals fed the additive and the environment under the approved conditions of authorisation. The additive is a potential respiratory sensitiser.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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19 Technical dossier/Section III/References.
20 Technical dossier/Section III/Annex III.1.
21 Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.
Documentation provided to EFSA/Chronology

| Date         | Event                                                                                     |
|--------------|-------------------------------------------------------------------------------------------|
| 01/08/2016   | Dossier received by EFSA. PHYZYME® XP 5000 G/L Renewal. Submitted by Danisco Ltd.          |
| 12/09/2016   | Reception mandate from the European Commission                                             |
| 24/10/2016   | Application validated by EFSA – Start of the scientific assessment                         |
| 07/04/2017   | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation |
| 24/01/2017   | Comments received from Member States                                                       |
| 31/05/2018   | Clarification teleconference during risk assessment with the applicant according to the “EFSA’s Catalogue of support initiatives during the life-cycle of applications for regulated products” |
| 15/06/2018   | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 29/06/2018   | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended Issues: Characterisation |
| 27/08/2018   | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 04/04/2019   | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment                      |

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Abbreviations

CFU: colony forming unit
EURL: European Union Reference Laboratory
FEEDAP: EFSA Panel on Additives and Products or Substances used in Animal Feed
PCR: polymerase chain reaction
QPS: Qualified Presumption of Safety
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