Assessment of safety and efficacy of 6-phytase produced by *Komagataella phaffii* DSM 23036 (OptiPhos®) as a feed additive for breeding hens, turkeys for breeding and fattening, ornamental birds and other avian species, sows, pigs and minor pig species for fattening and piglets.

Reference number RP185

Regulated Products Risk Assessment Unit
Science, Evidence and Research Division, FSA

Risk Assessment Team
Science Division, FSS

Regulated Product Dossier Assessment

Safety Assessment finalised: 18/08/2023

Summary

An application was submitted to the Food Standards Agency in February 2021 from Huvepharma EOOD ("the applicant") for the authorisation of an additive (OptiPhos®), as a renewal and new authorisation under the category of 'zootechnical' additives, functional group 'digestibility enhancers'.

The additive is produced in three formulations: OptiPhos® 4000 G, solid, 22.79% phytase content with an activity of 20,000 Optiphos activity (OTU) /g; OptiPhos® 4000 CT, solid, 30% phytase content, activity of 20,000 OTU/g; OptiPhos® 8000 L, liquid, 20% phytase content, activity of 40,000 OTU/g. It is proposed to be used in concentrations between 125-250 OTU/kg of complete feed.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The AFFAJEG evaluated the literature review presented in the application and the previous EFSA opinion and concluded that the additive can be considered safe for the target species, the consumer and the environment. Further information was requested on skin sensitisation testing and, after re-evaluating the information provided, the AFFAJEG concluded that the additive should be considered to be a respiratory sensitiser and to not be a skin sensitiser or eye irritant.

No demonstration of efficacy was required for the renewal of authorisation. For the extension of use, the AFFAJEG concluded that previous data demonstrating efficacy could be extrapolated to breeding hens, ornamental birds, minor pig species for fattening/breeding and suckling piglets, at the doses proposed by the applicant.

The views of AFFAJEG and ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

1. Introduction

The FSA and FSS have undertaken a risk assessment for a feed additive (OptiPhos ®, Huvepharma NV., Uitbreidingstraat 80, 2600 Antwerp, Belgium) containing 6-phytase as an active substance, under Regulation (EC) No 1831/2003[1] under the category of 'zootechnical' additives, functional group 'digestibility enhancers' for the following conditions:

- A renewal of authorisation for use in chickens for fattening, chickens reared for laying, laying hens, turkeys for fattening, turkeys for breeding, other avian species, sows, pigs for fattening and piglets (weaned)
- A new authorisation for extension of use in breeding hens, ornamental birds, minor pig species for fattening/breeding and suckling piglets.

To support the safety assessment by FSA and FSS, the AFFAJEG and the ACAF provided advice to the FSA and FSS outlined in this document.

The dossier was evaluated on behalf of the FSA and FSS by the AFFAJEG. In line with Article 8 of 1831/2003, the assessment has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

With thanks to the members of the AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Dr. Katrina Campbell, Susan MacDonald, Professor Matthew Fisher, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Helen Warren and Dr. Nick Wheelhouse.

The dossier was evaluated by the AFFAJEG at their July 2021 and December 2021 meetings. Further information was provided by the applicant in September 2021, responding to queries by the FSA. The conclusions by the AFFAJEG were reviewed and approved by the ACAF at their October 2022 meeting.

This document outlines the discussion and conclusions of the AFFAJEG's assessment on the safety and efficacy of 6-phytase as a feed additive.

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

The additive's active substance is 6-phytase, seeking authorisation in three formulations: OptiPhos® 4000 G, solid, 22.79% phytase content with an activity of 20,000 OTU/g; OptiPhos® 4000 CT, solid, 30% phytase content, activity of 20,000 OTU/g; OptiPhos® 8000 L, liquid, 20% phytase content, activity of 40,000 OTU/g. The applicant provided data from several batches supporting the values outlined in Tables 1-3.

Table 1: Identity table OptiPhos® 4000 G

Composition	
6-phytase (20,000 OTU/g)	22.79%
Pregelatinised starch	0.90%
Wheat meal	Up to 100%
Appearance	
Beige granules	
Chemical-physical specifications	
Loss on drying	<10%
Phytase content	Not less than 4000
Dusting potential Particle size distribution	0.04 g/m3 <100 µm (0.66%); 100 – 1000 µm (99.34%)
Microbiological profile (CFU/g)	
Salmonella	Abs/25g
Heavy metals	
Arsenic	<4 mg/kg
Lead	<10 mg/kg
Cadmium	<0.5 mg/kg
Mercury	<0.2 mg/kg

Table 2: Identity table OptiPhos® 4000 CT

Composition	
6-phytase (20,000 OTU/g)	30%
Pregelatinised starch	5%
Wheat meal	38%
Distilled monoglyceride	13.5%
Palm oil	13.5%
Corn cobs	0-20%
Appearance	
Beige granules	
Chemical-physical specifications	
Loss on drying	<11%
Phytase content	Not less than 4000
Dusting potential Particle size distribution	0.11 g/m3 <630 µm (0%); 630 µm - 1400 µm (100%)
Microbiological profile (CFU/g)	
Salmonella	Abs/25g
Heavy metals	
Arsenic	<4 mg/kg
Lead	<10 mg/kg
Cadmium	<0.5 mg/kg
Mercury	<0.2 mg/kg

Table 3: Identity table OptiPhos® 8000 L

Composition	
6-phytase (40,000 OTU/g)	20%
Sucrose	30%
Sodium benzoate	0.5%
Water, purified	Up to 100%
Appearance	
Opalescent, greenish liquid	
Chemical-physical specifications	
Relative density	1.10-1.30
Phytase content	Not less than 8000
Microbiological profile (CFU/g)	
Salmonella	Abs/25g
Heavy metals	
Arsenic	<4 mg/kg
Lead	<10 mg/kg
Cadmium	<0.5 mg/kg
Mercury	<0.2 mg/kg

The enzyme is obtained through fermentation of a genetically modified organism (GMO) strain of *Komagataella phaffii*, which is considered to be a QPS (qualified presumption of safety) organism for the production of enzymes[2]. The application presented a whole genome sequence (WGS) analysis and evidence of absence of the microorganism or its DNA in the final product, in line with guidance recommendations. The AFFAJEG evaluated the data and did not raise any concerns over the identification of the microorganism. On request from FSA, the applicant confirmed that the composition, production process and impurities have not changed since the previous European authorisation. The additive is proposed to be used as shown in Table 4:

Table 4: Proposed mode of use of OptiPhos® as described in the application

Proposed mode of use in animal nutrition			
Additive		6-phytase	
CAS-number		9001-89-2	
Category(-ies) of additive		Zootechnical feed additive	
Functional group(s) of additive		Digestibility enhancer	
Description			
Conditions of use			
Species or category of animal	**Max. Age**	**Min. content**	**Other provisions**
All poultry species other than turkey species Ornamental birds Pigs for fattening Sows Minor pig species for fattening or breeding	--	125 OTU/kg	1. In the directions for use of the additive and premixture, indicate the storage temperature, storage life, and stability to pelleting. 2. Recommended maximum dose for all authorised species is 500 OTU/kg of complete feedingstuffs. 3. For use in feed rich in phytin-bound phosphorus. 4. For safety: breathing protection, safety goggles, protective gloves shall be used during handling
Turkeys for fattening Turkeys reared for breeding Weaned piglets Suckling piglets	--	250 OTU/kg	

2.1.1. Conclusions on Section II

The AFFAJEG concluded that the additive did not demonstrate stability when pelleted at 85°C, as no retention time was provided, but acknowledged that this was expected for the nature and formulation of the product.

No further concerns were raised for Section II of the application dossier.

2.2. Section III: Safety

The applicant conducted a literature review to support the evaluation of the safety of the additive and evidence from a previous EFSA opinion[3]. Members evaluated the literature review and concluded that no significant causes for concern were identified.

2.2.1. Safety for the target species

The applicant presented evidence of safety for the target species through their literature review and previous conclusions from EFSA, which stated that the additive is safe for the target species at the maximum dose of 500 OTU/kg, and up to 50,000 OTU/kg[3]. The AFFAJEG concluded that based on the information presented, the additive remains safe

for the target species proposed as a renewal of authorisation. The Group extended these conclusions to the proposed new uses of the additive.

2.2.2. Safety for the consumer

The applicant presented evidence of safety for the consumer through their literature review and previous conclusions from EFSA, which stated that the additive is safe for the consumer[3]. The AFFAJEG evaluated previous conclusions on a bacterial reverse mutation assay, an *in vitro* mammalian chromosomal aberration test, a mammalian erythrocyte micronucleus test and a subchronic oral toxicity study, none of which reported treatment-related adverse effects[3]. Based on these data and the absence of reports on adverse effects from the literature review, the AFFAJEG concluded that the additive remains safe for consumers.

2.2.3. Safety for the user

The applicant presented evidence of safety for the user through their literature review and previous conclusions from EFSA, which stated that the additive is not a skin/eye irritant or skin sensitiser but has the potential to be a respiratory sensitiser[3]. The AFFAJEG evaluated the data presented and concluded that the additive should be considered a potential respiratory sensitiser, due to its proteinaceous nature, and to be non-irritant to the eye. However, the Group discussed that the data presented were insufficient to conclude on the skin irritant and sensitiser potential of the additive and that further information would have to be provided by the applicant. Following this request by FSA, a GLP-compliant study report of an existing skin sensitising assay was provided and evaluated by the AFFAJEG at their December 2021 meeting. It was concluded that, based on the negative result of the Buhler test conducted, the additive could be considered to not be a skin sensitiser. Summarising the conclusions:

- The additive is a respiratory sensitiser
- Workers are not likely to be exposed to respirable dust
- The additive is not a skin sensitiser or a skin or eye irritant

2.2.4. Safety for the environment

The proteinaceous nature of the additive means it will largely be degraded in the gastro-intestinal tract, therefore, accumulation in the environment was not considered to be a risk by AFFAJEG.

2.2.5. Conclusions on safety

The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment. The additive should be considered to be a respiratory sensitiser and to not be a skin sensitiser or skin or eye irritant.

2.3. Section IV: Efficacy

For the renewal of authorisation, no demonstration of efficacy was required. The AFFAJEG evaluated the extrapolation of previous efficacy conclusions by EFSA to the newly proposed species for the extension of use. The Group concluded that the additive could be considered efficacious in breeding hens, ornamental birds, minor pig species for fattening/breeding and suckling piglets, at the doses proposed by the applicant (125 and 250 OTU/kg).

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Optiphos® 10^4:

For the quantification of phytase activity in feed additive, premixtures and feedingstuffs the applicant submitted the same single-laboratory validated and further verified colorimetric method evaluated by the EURL in the frame of the dossier FAD-2010-0008, based on the quantification of the inorganic phosphate released by the enzyme from the sodium phytate. Supplementary experimental results were provided for feedingstuffs for rainbow trout.

Based on the satisfactory performance characteristics available the EURL recommends for official control the colorimetric method mentioned above for the quantification of phytase activity in the feed additive, premixtures and feedingstuffs.

Further testing or validation of the methods is not considered necessary.

FSA/FSS accept the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The data presented in the application identified and characterised the additive adequately. AFFAJEG also concluded that the additive did not demonstrate stability when pelleted at 85°C, as no retention time was provided.

Data from the literature review provided, together with evidence from a previous EFSA opinion, are sufficient evidence to demonstrate the safety of the additive for the target species, consumers and the environment. The additive should be considered to be a respiratory sensitiser and to not be a skin sensitiser, skin irritant or eye irritant.

No demonstration of efficacy was required for the renewal of authorisation. For the extension of use, AFFAJEG concluded that previous data demonstrating efficacy could be extrapolated to breeding hens, ornamental birds, minor pig species for fattening/breeding and suckling piglets, at the doses proposed by the applicant (125 and 250 OTU/kg).

FSA/FSS accept the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

5. References

1. EC (European Commission), 2003. Regulation No 1831/2993 of the European Parliament and of the Council on additives for use in animal nutrition. Available at https://www.legislation.gov.uk/eur/2003/1831/contents

2. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2018. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until September 2017. EFSA Journal 2018;16(1):5131, 43 pp. https://doi.org/10.2903/j.efsa.2018.5131

3. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2011. Scientific Opinion on the safety and efficacy of Optiphos® (6-phytase) as a feed additive for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, laying hens, other birds for fattening and laying, weaned piglets, pigs for fattening and sows. EFSA Journal;9(11): 29. DOI: 10.2903/j.efsa.2011.2414

4. EURL-FA (European Reference Laboratory for Feed Additives), 2020. Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. 6-phytase. Available at: https://joint-research-centre.ec.europa.eu/publications/fad-2016-0019_en

6. Abbreviations

ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
CFU	Colony forming units
EC	European Commission
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
OTU	OptiPhos technical units (phytase activity)
GMO	Genetically modified organism
QPS	Qualified presumption of safety
WGS	Whole genome sequence

For enquiries about this publication, contact the Food Standards Agency.

Follow us on Twitter:
@foodgov

Find us on Facebook:
facebook.com/FoodStandardsAgency