SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 September 2022

doi: 10.2903/j.efsa.2022.7610

Safety and efficacy of a feed additive consisting of 6-phytase produced by *Trichoderma reesei* CBS 146250 (Axtra® PHY GOLD 30L, Axtra® PHY GOLD 30T, Axtra® PHY GOLD 65G) for all poultry species and all pigs (Danisco (UK) ltd)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Boet Glandorf, Giovanna Martelli, Luca Tosti, Montserrat Anguita, Rosella Brozzi, Jaume Galobart, Elisa Pettenati, Joana Revez, Jordi Tarrés-Call and Jordi Ortuño

Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of 6-phytase produced by the genetically modified strain *Trichoderma reesei* CBS 146250 (Axtra® PHY GOLD 30L, Axtra® PHY GOLD 30 T and Axtra® PHY GOLD 65G) as a zootechnical feed additive for all poultry species and all pigs. The FEEDAP Panel concluded that the genetic modification of the production strain does not give rise to safety concerns. Based on the no observed adverse effect level identified in a subchronic oral toxicity study in rats, the additive was considered safe for all poultry species and all pigs at the proposed conditions of use. The Panel also concluded that the use of the product as a feed additive does not give rise to concerns for consumers and the environment. Owing to the lack of data obtained with the final formulations, the Panel cannot conclude on the potential of the additive to be irritant to eyes or skin. Due to the proteinaceous nature of the active substance, it is considered a respiratory sensitiser. The panel concludes that the additive is efficacious in increasing the phosphorus utilisation when supplemented at 500 FTU/kg for all growing poultry species and all pigs, and at 300 FTU/kg in laying hens and other laying birds.

Keywords: zootechnical additives, digestibility enhancers, 6-phytase, Axtra PHY GOLD®, safety, efficacy, poultry and pigs

Requestor: European Commission

Question number: EFSA-Q-2020-00767

Correspondence: feedap@efsa.europa.eu

Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): FEEDAP Working Groups of Toxicology, Microbiology and Animal Nutrition, Matteo Innocenti, Yolanda García-Cazorla and Alberto Navarro.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Glandorf B, Martelli G, Tosti L, Anguita M, Brozzi R, Galobart J, Pettenati E, Revez J, Tarrés-Call J and Ortuño J, 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of 6-phytase produced by *Trichoderma reesei* CBS 146250 (Axtra® PHY GOLD 30L, Axtra® PHY GOLD 30T, Axtra® PHY GOLD 65G) for all poultry species and all pigs (Danisco (UK) ltd). EFSA Journal 2022;20(11):7610, 20 pp. https://doi.org/10.2903/j.efsa.2022.7610

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Danisco (UK) Ltd, represented in the European Union (EU) by Genencor International B.V.,[2] for the authorisation of the additive consisting of 6-phytase produced by *Trichoderma reesei* CBS 146250 (Axtra® PHY GOLD 30 L, Axtra® PHY GOLD 30 T, Axtra® PHY GOLD 65G), when used as a feed additive for all pigs and all poultry species (category: zootechnical 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 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 9 March 2021.

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 6-phytase produced by *Trichoderma reesei* CBS 146250 (Axtra® PHY GOLD 30 L, Axtra® PHY GOLD 30 T, Axtra® PHY GOLD 65G), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The product containing 6-phytase produced by the genetically modified microorganism *Trichoderma reesei* CBS 146250 is not authorised as a feed additive in the EU.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of 6-phytase produced by *Trichoderma reesei* CBS 146250 (Axtra® PHY GOLD 30L, Axtra® PHY GOLD 30T, Axtra® PHY GOLD 65G) as a feed additive.

The FEEDAP panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the 6-phytase in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of 6-phytase produced by *Trichoderma reesei* CBS 146250 (Axtra® PHY GOLD 30L, Axtra® PHY GOLD 30T, Axtra® PHY GOLD 65G) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed

[1] Regulation (EC) N° 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, pp. 29.
[2] Willem Einthovenstraat 4, 2,342 BH, Oegstgeest, The Netherlands.
[3] FEED dossier reference: FAD-2020-0083.
[4] The full report is available on the EU Science Hub website: https://joint-research-centre.ec.europa.eu/publications/fad-2020-0083_en
[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, pp. 1.

additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product containing 6-phytase produced by a genetically modified strain of *Trichoderma reesei* (CBS 146250) is intended for use as a zootechnical feed additive (functional group: digestibility enhancers) in feed for all poultry species (chickens/hens, turkeys and minor growing and reproductive) and all pigs (piglets, pigs for fattening, sows and minor growing and reproductive porcine species). The product will be hereafter referred to with its trade name Axtra® PHY GOLD.

3.1. Characterisation

3.1.1. Characterisation of the production strain

The production strain is a genetically modified *Trichoderma reesei* deposited at the Westerdijk Fungal Biodiversity Institute with deposit number CBS 146250.[6]

The taxonomic identification of the recipient strain [redacted] as *Trichoderma reesei* was confirmed by DNA sequencing analysis [redacted] and by phylogenomic analysis comparing its genome against available related genomes.[7] However, the analysis was not conducted on the production strain. The panel notes that the whole genome sequence (WGS) data of the production strain were available and should have been used for identification purposes.

[redacted][8]

3.1.2. Manufacturing process

[redacted]

[6] Technical dossier/Section II/Annex II_11.
[7] Technical dossier/SIn_200122/Annexes S1 – S4.
[8] Technical dossier/Section II/Annex II_12 and SIn_200122/Annex S1.

[redacted]

3.1.3. Characterisation of the additive

The active substance of Axtra® PHY GOLD is 6-phytase (myo-inositol-hexakisphosphate 4-phosphohydrolase; Enzyme Commission number 3.1.3.26). The three different commercial formulations differ in the enzyme activity and/or physical properties:

- Axtra® PHY GOLD 30L: brown liquid preparation (average viscosity = 9.5 cP at 25°C; average specific gravity = 1,220 kg/m^3) with a minimum enzyme activity of 30,000 FTU[9]/g.
- Axtra® PHY GOLD 30T: off-white to light tan granular preparation (average bulk density = 1,200 kg/m^3) with a minimum enzyme activity of 30,000 FTU/g.
- Axtra® PHY GOLD 65G: off-white to light tan granular preparation (average bulk density = 1,050 kg/m^3) with a minimum enzyme activity of 65,000 FTU/g.

The enzyme activity of five batches of Axtra® PHY GOLD 30 L (range: 41,884–47,648 FTU/g; average: 44,865 FTU/g); 30T (range: 42,858–47,161 FTU/g; average: 44,432 FTU/g); and 65G (range: 90,268–115,668 FTU/g; average: 104,344 FTU/g) showed compliance with the minimum specifications set by the applicant.[10] The range of total organic solids (TOS) coming from fermentation is estimated to be of [redacted] for the Axtra® PHY GOLD 30 L, 30 T and 65 G formulations, respectively.

Three batches from each formulation of the additive were analysed for chemical and microbiological impurities.[11] Values for mercury, cadmium and all mycotoxins analysed fell below the respective limit of quantification (LOQ)[12] in all formulations and batches analysed. Axtra® PHY GOLD 30L showed an average value of 0.15 mg/kg of lead and below LOQ for arsenic; 30T showed average values of 0.11 and 0.02 mg/kg for arsenic and lead, respectively; and the 65 G showed values below LOQ for lead and arsenic, except for 0.04 mg/kg of arsenic in one of the batches analysed. [redacted]

The same batches were analysed for microbiological impurities. *Escherichia coli* or *Salmonella* spp. were not detected in 25 g, and Enterobacteriaceae, total coliforms, and yeasts and filamentous fungi were < 10 CFU/g additive.

The detected amounts of the above-described analysis do not raise safety concerns.

The applicant submitted three sets of data to exclude the ability of the production strain to produce antimicrobials. The antimicrobial production was tested against reference strains[13] in the supernatant of the production strain,[14] in the enzyme fermentation product[15] and in each formulation of the additive[16] with disc-diffusion agar method. No antimicrobial activity was detected.

Some *Trichoderma* species are known to be capable of producing a variety of mycotoxins and antifungal metabolites. *T. reesei* seems to be unable to produce mycotoxins (EFSA, 2007; Frisvad et al., 2018; EFSA BIOHAZ Panel, 2020) but it is known to produce peptaibols, such as paracelsin A, C and D (Frisvad et al., 2018), and its genome has been shown to harbour genes for two peptaibol synthases (Kubicek et al., 2007). Those peptaibols are peptides with antimicrobial activity and are

[9] One FTU is the amount of enzyme that releases 1 μmol of inorganic orthophosphate from a sodium phytate substrate per minute at pH 5.5 and 37°C.
[10] Technical dossier/Section II/Annexes II_1 and 2.
[11] Technical dossier/Section II/Annex II_1.
[12] LOQs. Arsenic: 0.01–0.1; Lead: 0.01–0.05 mg/kg; Mercury: 0.005–0.01 mg/kg; Cadmium: 0.001–0.01 mg/kg; Aflatoxins (B1 + B2 + G1 + G2): 4–5 μg/kg; Ochratoxin A: 2 μg/kg; Zearalenone: 10–25 μg/kg; Fumonisins (B1 + B2): 40–100 μg/kg; Deoxynivalenol: 20–50 μg/kg.
[13] *Staphylococcus aureus* ATCC 6538, *Streptococcus pyogenes* ATCC 12344, *Bacillus cereus* ATCC 2, *Bacillus circulans* ATCC 4516, *Escherichia coli* ATCC 11229 and *Serratia marcescens* ATCC 14041.
[14] Technical dossier/SIn_180722/Annex S4_2.
[15] Technical dossier/SIn_180722/Annex S3_2.
[16] Technical dossier/Section II/Annex II_4.

mostly produced under stress conditions (Frisvad et al., 2018). The production predominantly occurs in solid fermentation and correlates with conidiation (Kubicek et al., 2007; Tisch and Schmoll, 2010). The lack of antimicrobial activity in the additive under assessment (described above) would indicate that if peptaibols are produced under the fermentation conditions, their concentration would be of no concern. This was confirmed in the recipient (*T. reesei* [redacted]) and an intermediate strain (*T. reesei* [redacted]) when tested for their ability to produce trichothecenes (trichodermin, trichodermol and harzianum A) and gliotoxin.[17]

The applicant did not provide information on the potential of the production strain to produce secondary metabolites except from data on the mycotoxin levels in the final forms of the additive (data shown above; all values below LOQ). However, safety concerns derived from the presence of other secondary metabolites potentially produced by *T. reesei* CBS 146250 are addressed by the toxicological studies conducted with the liquid enzyme concentrate (see Section 3.2.2).

The presence of viable cells of the production strain was investigated in triplicate in three batches of the liquid enzyme concentrate.[18] [redacted] The results obtained in the intermediate liquid enzyme concentrate allow conclusions on the final product.

The presence of recombinant DNA from the production strain *Trichoderma reesei* CBS 146250 was also analysed in triplicate in three batches of the liquid enzyme concentrate.[19] [redacted] No DNA of the production strain was detected.

The dusting potential of three batches of the solid forms of the additive was determined using the Stauber-Heubach method[20] and showed average values of 6.67 mg/m^3 (range 0–20 mg/m^3) and of 13.3 mg/m^3 (range 10–15 mg/m^3) for Axtra® PHY GOLD 30 T and 65 G, respectively. The particle size distribution was analysed by laser-diffraction method on the same three batches of the solid forms[21]; the results showed that 100% (v/v) of the particles have a diameter > 200 μm both for Axtra® PHY GOLD 30T and 65G.

3.1.4. Stability and homogeneity

Shelf-life

The shelf-life of the additive was studied in three batches of each formulation (initial activity of 52,938; 53,969 and 117,826 FTU/g for the Axtra® PHY GOLD 30L, 30T and 65G, respectively) when stored in a commercial packaging for up to 24 months at 25°C and 60% relative humidity (RH). The enzyme activity at the end of the storage period ranged 85.5–90.7% for Axtra® PHY GOLD 30L, 98.4–105.2% for Axtra® PHY GOLD 30T and 95.1–107.9% for Axtra® PHY GOLD 65G compared to the starting one.[22]

Stability

The stability of the solid forms of the additive (three batches for each form) in a commercial vitamin/mineral premixture for poultry was studied when supplemented at a minimum target dose of 0.667% for Axtra® PHY GOLD 30T and 0.307% for Axtra® PHY GOLD 65G (approximated minimum activity of 199,382 FTU/kg premix). The supplemented premix was stored in air-tight plastic containers at 25°C for up to 6 months. The enzyme activity at the end of the storage period ranged 90.3–91.8% for Axtra® PHY GOLD 30 T and 82.8–108.7% for 65 G compared to the starting one.[23]

The stability of the solid forms of the additive (one batch for each form) was measured in mash feeds when supplemented at a minimum phytase activity of 500 FTU/kg complete feed for piglets, sows and chickens for fattening or at 300 FTU/kg complete feed for laying hens. Samples were stored in small paper bags placed in larger paper bags lined with plastic at 25°C and 60% RH for up to

[17] Technical dossier/SIn_180722/Annex S1_2.
[18] Technical dossier/SIn_200122/Annex S5.
[19] Technical dossier/SIn_200122/Annex S6.
[20] Technical dossier/Section II/Annex II_10
[21] Technical dossier/Section II/Annex II_9
[22] Technical dossier/SIn_200121/Annexes II_24R, 25R and 26R
[23] Technical dossier/Section II/Annexes II_27 and 28

3 months. The enzyme activity at the end of the storage period ranged 83.4–94.5% for Axtra® PHY GOLD 30T and 82.3–100.1% for 65G compared to the starting one.[24]

The stability of all forms of the additive (one batch for each form) during pelleting was studied after mixing into mash feed for piglets, sows, laying hens and chickens for fattening to achieve a total phytase activity between 400 and 820 FTU/kg feed for Axtra® PHY GOLD 30L and 30T and between 500 and 1,000 FTU/kg feed for Axtra® PHY GOLD 65G. Mash feed samples containing Axtra® PHY GOLD 30L and 65G were then pelleted for 30 s at 95°C or at 75°C those containing Axtra® PHY GOLD 30T. The enzyme activity after the pelleting process ranged 80.1–91.7% for Axtra® PHY GOLD 30L, 80.7–110.7% for 30T and 81.9–103.9% for 65 G compared to the starting one.[25]

The storage stability after pelleting of the solid forms of the additive included in the mash feeds for piglets, sows, laying hens and chickens for fattening was studied. In contrast, Axtra® PHY GOLD 30L was sprayed onto pellets to obtain a minimum phytase activity of 500 FTU/kg complete feed for piglets, sows and chickens for fattening and of 300 FTU/kg complete feed for laying hens. The pelleted feeds were stored in small paper bags (closed with paper clips) placed in larger paper bags lined with plastic at 25°C and 60% RH for up to 3 months. The enzyme activity at the end of the storage period ranged 82.3–95.7% for Axtra® PHY GOLD 30L, 90.4–105.8% for 30T and 76.9–99.1% for 65G compared to the starting one.[26]

Homogeneity

The capacity for homogeneous distribution of the solid forms of the additive included in mash feeds for piglets, sows, laying hens and chickens for fattening was studied in 10 subsamples. The coefficient of variation ranged 5.8–10.2% and 7.9–10.0% in mash feed samples supplemented with Axtra® PHY GOLD 30 T and 65 G, respectively.[27] The capacity for homogeneous distribution of the 30L formulation of the additive sprayed onto pelleted feeds for piglets, sows, laying hens and chickens for fattening was studied in 10 subsamples.[28] The coefficient of variation ranged from 1.4 to 1.8%.

3.1.5. Conditions of use

The additive is intended for use in feed for all growing poultry species and all pigs (including minor growing and reproductive porcine species) at a proposed minimum level of 500 FTU/kg complete feed and for laying hens and other laying birds at a proposed minimum level of 300 FTU/kg complete feed. The applicant states that the liquid formulation is to be applied post-pelleting when the pelleting process exceeds 75°C.

3.2. Safety

3.2.1. Safety aspects of the genetic modification

In the view of the FEEDAP Panel, the identity of the production strain has been unambiguously established as *Trichoderma reesei*. [redacted] The introduced sequences raise no safety concerns. The presence of viable cells from the production strain and their recombinant DNA were not detected in the liquid enzyme concentrate used to formulate the final forms of the additive. The product Axtra® PHY GOLD manufactured with the production strain *T. reesei* CBS 146250 does not raise safety concerns with regard to the genetic modification of the production strain.

3.2.2. Toxicological studies

All the toxicological studies were performed with the liquid enzyme concentrate form of 6-phytase [redacted] from which the three final Axtra® PHY GOLD formulations are obtained. The test item is considered representative for the three final formulations of the additive.

[24] Technical dossier/Section II/Annexes II_29, 30, 31 and 32
[25] Technical dossier/Section II/Annexes II_39, 40 and 41
[26] Technical dossier/Section II/Annexes II_33, 34, 35, 36, 37 and 38
[27] Technical dossier/Section II/Annexes II_29, 30, 31 and 32
[28] Technical dossier/SIn_200121/Annex_S9

3.2.2.1. Genotoxicity studies

Bacterial reverse mutation assay (Ames test)

The test item was assessed for the induction of reverse mutations [redacted].[29] The experimental protocol was in line with the Organisation for Economic Co-operation and Development (OECD) guideline 471 following good laboratory practices (GLP). [redacted] No statistically significant increase in the number of revertant colonies was observed at any tested condition in any tester strain. Therefore, the test item did not induce gene mutations in bacteria under the experimental conditions applied in the study.

In vitro *mammalian chromosome aberration test*

The potential capacity of the test item to induce structural chromosome aberrations was evaluated *in vitro* in human peripheral blood lymphocytes in compliance with OECD TG 473 and following GLP.[30] [redacted] Comparable frequencies of chromosomal aberrations were detected in treated and control cultures. Thus, the test item did not induce structural chromosome aberrations in cultured human peripheral blood lymphocytes under the experimental conditions employed in the study.

In vitro *micronucleus test*

To evaluate the potential of the test item to induce chromosome damage, an *in vitro* micronucleus test was carried out in human whole blood according to OECD TG 487 and following GLP.[31] [redacted] No increase of the frequency of micronuclei was induced by treatment with the test item in any experimental condition. The test item did not induce structural and numerical chromosome aberrations in human lymphocytes under the experimental conditions employed in this study.

3.2.2.2. Subchronic oral toxicity study

[redacted] 0 (control), 250, 500 or 1,000 mg TOS per kg bw and day [redacted], corresponding to 0, 112,500, 225,000 and 450,000 FTU/kg bw and day.[32] The study was conducted in compliance with OECD guideline 408 and following GLP. [redacted][33]

[29] Technical dossier/Section III/Annex III_1
[30] Technical dossier/Section III/Annex III_2
[31] Technical dossier/SIn_200122/Annex S10
[32] Technical dossier/Section III/Annex III_3
[33] Haematocrit, haemoglobin, erythrocyte count, reticulocyte count, total leucocyte count (including differential), platelet count, mean corpuscular haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin concentration, prothrombin and activated partial thromboplastin time

[redacted][34][redacted][35][redacted]

No treatment-related clinical signs were observed. One male animal died in the 250 mg TOS/kg bw per day group, but the death was unrelated to treatment. No effects were observed on survival, behaviour, body weight, feed intake, haematology, clinical parameters, urine analysis, gross pathology and histological examination. From this study, it was derived an NOAEL of 1,000 mg TOS/kg bw per day, the highest dose tested, corresponding to 450,000 FTU/kg bw per day.

3.2.2.3. Conclusions on the toxicological studies

The FEEDAP Panel concludes that the intermediate product used for the formulation of the additive showed no genotoxicity potential in tests addressing gene mutations, numerical and structural chromosome aberrations. Moreover, the results obtained in a subchronic oral toxicity study raised no concerns regarding the product and allowed to derive an NOAEL of 450,000 FTU/kg bw and day, the highest dose tested.

3.2.3. Safety for the target species

No tolerance studies in relevant target species were submitted. To support the safety of the additive for the target species, the applicant referred to the 90-day toxicity study described above (see Section 3.2.2.2). The NOAEL identified (450,000 FTU/kg bw and day) was used to calculate the maximum safe level in chickens and turkeys for fattening, laying hens, piglets, pigs for fattening and lactating sows in accordance with the procedure described in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b), and the results are shown in Table 1. The values obtained are higher than the recommended use level of 500 FTU/kg complete feed for chickens and turkeys for fattening, piglets, pigs for fattening and lactating sows, and of 300 FTU/kg for laying hens. Therefore, the panel concludes that the additive is safe at 500 FTU/kg for all growing poultry species and all pigs, and at 300 FTU/kg in laying hens and other laying birds.

Table 1: Maximum safe concentration of Axtra® PHY GOLD in the feed of the major target species

Animal category	Default values for daily feed intake (g DM per kg bw)	Maximum safe level in feed (FTU/kg complete feed)
Chickens for fattening	79	50,127
Turkeys for fattening	59	67,500
Laying hens	53	74,717
Piglets	44	90,000
Pigs for fattening	37	108,000
Lactating sows	30	131,250

3.2.4. Safety for the consumer

The results obtained with the liquid enzyme concentrate, considered representative of the final formulations of the additive, in the genotoxicity studies and the subchronic oral toxicity study, do not indicate any reason for concern for consumer safety arising from the use of the product as a feed additive.

3.3. Safety for the user

Effect on the respiratory system

The applicant provided no specific studies regarding the effects of the additive on the respiratory system. The proteinaceous nature of the active substance implies that the additive is considered a respiratory sensitiser.

[34] Sodium, potassium, glucose, cholesterol, urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, calcium, phosphorus, triglycerides, albumin/globulin ratio, chloride, globulin, total cholesterol, high/low density lipoprotein, creatine phosphokinase T3, T4 and TSH

[35] Appearance, volume, specific gravity, pH, protein, glucose, ketone, sediments, urobilinogen, nitrite and blood cells.

Effect on eyes and skin

The skin[36] and eye[37] irritation potential of the liquid enzyme concentrate was tested in valid studies performed according to OECD guidelines 439 and 492 following GLP, respectively, showing that the test item is not a skin or an eye irritant.

The results of a valid local lymph-node assay (LLNA) performed with the liquid enzyme concentrate following OECD guidelines 429 and GLP showed that the test item is not a skin sensitiser.[38]

3.3.1. Conclusions on safety for the user

The panel notes that the final formulations were not tested. Based on the studies provided, the liquid enzyme concentrate used as test item is not a skin or eye irritant nor a skin sensitiser. In the absence of data obtained with the final formulations, the panel cannot conclude on the potential of the additive to be an irritant to the skin and eyes or a skin sensitiser. Due to the proteinaceous nature of the active substance (6-phytase), the additive is considered a respiratory sensitiser.

3.3.2. Safety for the environment

The production strain and its DNA were not detected in the liquid enzyme concentrate used to prepare the final forms of the additive. The additive does not raise safety concerns for the environment regarding the genetic modification of the production strain. The active substance is a protein; thus, it will be degraded/inactivated during the passage through the digestive tract of animals or in the environment. Therefore, no risks to the environment are expected when the additive is used on poultry and pigs.

3.4. Efficacy

The test article used in the studies to demonstrate the efficacy of Axtra® PHY Gold was the liquid enzyme concentrate 6-phytase sprayed onto a wheat carrier.

3.4.1. Efficacy for poultry

3.4.1.1. Chickens for fattening

The applicant provided three short-term balance trials sharing a similar design. All trials were designed to study the effect of the 6-phytase contained in the additive on the retention of dietary phosphorus and included bone mineralisation parameters. The main results of the trials are presented in Table 2.

[redacted][39][redacted][40][redacted][41][redacted]

[36] Technical dossier/Section III/Annex III_4
[37] Technical dossier/Section III/Annex III_5
[38] Technical dossier/Section III/Annex III_6
[39] Technical dossier/Section IV/Annex IV_1
[40] Technical dossier/Section IV/Annex IV_2
[41] Technical dossier/Section IV/Annex IV_3

[redacted]

The birds receiving the 6-phytase at 500 FTU/kg feed showed significantly higher P retention compared to the control diet in the three trials, and also in comparison with the positive control diet in trials 1 and 3. The improvement of the P utilisation at the minimum use level was also reflected in higher ash bone content in the three trials, and on P bone content in trial 1.

Table 2: Effect of Axtra® PHY GOLD on the phosphorus retention and bone mineralisation of the chickens for fattening in the short-term trials

Trial	Diets			P retention (%)	Bone content	
	Phytase (FTU/kg feed)	P/Ca (%)	Form		Ash (% of DM)	P (% of ash)
1	0	[redacted]	Mash	44.3^{c}	35.8^{d}	16.3^{c}
	500	[redacted]	Mash	71.2^{a}	44.0^{b}	17.0^{b}
	PC	[redacted]	Mash	50.7^{b}	46.8^{a}	17.0^{b}
	0	[redacted]	Pellet	48.0^{bc}	41.6^{c}	16.6^{c}
	500	[redacted]	Pellet	66.3^{a}	48.6^{a}	17.4^{a}
	PC	[redacted]	Pellet	46.4^{bc}	47.7^{a}	17.1^{ab}
2	0	[redacted]	Mash	43.9^{b}	30.3^{b}	–
	500	[redacted]	Mash	62.6^{a}	36.5^{a}	–
	1,000	[redacted]	Mash	67.6^{a}	38.4^{a}	–
	PC	[redacted]	Mash	57.7^{a}	37.7^{a}	–
3	0	[redacted]	Pellet	55.7^{b}	40.7^{b}	–
	500	[redacted]	Pellet	66.4^{a}	43.7^{a}	–
	PC	[redacted]	Pellet	54.4^{b}	44.5^{a}	–

^{a,b,c}: Values within the same trial and column with different superscript are significantly different ($P < 0.05$).

3.4.1.2. Laying hens

The applicant provided two short-term (balance) trials and one long-term efficacy trial including a balance study in laying hens. The main results of the phosphorus utilisation in the three trials are presented in Table 3.

The two short-term trials followed a similar design. [redacted][42] [redacted][43] [redacted]

[42] Technical dossier/Section IV/Annex IV_4
[43] Technical dossier/Section IV/Annex IV_5

None of the hens died during any of the studies. The birds receiving the phytase at 300 FTU/kg feed showed significantly higher P utilisation in comparison with the control diet in both trials. There was no effect of the additive on the egg phosphorus content.

In trial 3,[44]

The panel notes that the husbandry conditions applied in trial 3 did not reflect those in which the birds are raised in the EU and were not in line with Directive 1999/74/EC[45] (regarding the lighting program and the available surface/usable area per hen). Therefore, the results of the performance parameters recorded were disregarded. However, the panel considers that the data on the phosphorus utilisation could be retained considering that the husbandry conditions would have a lower impact on this endpoint.

Regarding the results of the balance trial, the birds receiving the phytase at 300 FTU/kg feed showed significantly higher phosphorus utilisation in comparison with the control diet. There was no effect of the additive on the tibia ash content. None of the hens died during the study.

Table 3: Effect of Axtra® PHY GOLD on the P and Ca utilisation and internal egg P content (trials 1 and 2) and tibia ash content (trial 3) in the short-term trials in laying hens

Trial	Phytase (FTU/kg feed)	P/Ca (%)	P utilisation(1) (%)	Internal Egg P (%)	Tibia ash content (%)
1	0		27.3^{d}	0.19	–
	300		35.6^{b}	0.20	–
	600		38.1^{a}	0.20	–
	900		38.8^{a}	0.20	–
	PC		31.7^{c}	0.20	–
2	0		25.5^{b}	0.17	–
	300		36.1^{a}	0.18	–
	PC		25.1^{b}	0.17	–
3	0		22.9^{b}/19.2^{b}	–	42.4
	300		33.1^{a}/29.9^{a}	–	43.6

^{a,b,c}: Values within the same trial and column with different superscript are significantly different.
(1): In trial 3, X/Y values in P utilisation refer to results obtained by different methods ( ).

[44] Technical dossier/Section IV/Annex IV_6
[45] Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens, OJ L 203 03.08.1999, pp. 53

3.4.2. Efficacy for pigs

3.4.2.1. Weaned piglets

Four short-term trials were provided in weaned piglets. The main results of the trials are presented in Table 4.

Trials 1[46] and 2[47] shared a similar design. [redacted] The animals receiving the 6-phytase additive from 500 FTU/kg feed showed significantly higher P retention in comparison with the control diet in both trials. No effect on the metacarpi ash content was found in trial 1.

In trial 3,[48] [redacted]

The supplementation of the diet with the 6-phytase from 500 FTU/kg feed showed higher P retention than the control. However, the results provided for the P retention calculated by the different methods ([redacted]) showed substantial discrepancies. Therefore, the results of this study were considered unreliable as evidence of efficacy.

In trial 4,[49] [redacted]

[46] Technical dossier/Section IV/Annex IV_10
[47] Technical dossier/Section IV/Annex IV_11
[48] Technical dossier/Section IV/Annex IV_12, SIn_200122 and SIn_180722
[49] Technical dossier/SIn_180722/Annex_S5

[redacted] Regardless of the dietary P content, the supplementation with the additive from 500 FTU/kg feed showed higher ATTD P and P bone content than the control diet.

Table 4: Effect of Axtra® PHY GOLD on the apparent total tract digestibility (ATTD) of P, P retention and bone mineral content in weaned piglets

Trial	Diets		Retention/ATTD P(1) (%)	Bone mineral content(2) (%)
	Phytase (FTU/kg feed)	Total P–Ca (%)		
1	0	[redacted]	43.2^{c}	43.3
	500	[redacted]	55.3^{b}	43.1
	1,000	[redacted]	67.1^{a}	45.3
2	0	[redacted]	41.4^{b}	–
	500	[redacted]	66.1^{a}	–
4(3)	0	[redacted]	21.1^{c}/23.4^{d}/24.4^{c}	7.83^{b}/7.41^{b}/7.33^{b}
	500	[redacted]	56.4^{b}/51.2^{c}/48.6^{b}	9.22^{a}/8.91^{a}/9.43^{a}
	1,000	[redacted]	67.9^{a}/59.6^{b}/60.4^{a}	9.30^{a}/9.43^{a}/9.57^{a}
	2,000	[redacted]	68.9^{a}/72.2^{a}/66.9^{a}	9.53^{a}/9.66^{a}/9.73^{a}
	4,000	[redacted]	74.3^{a}/74.4^{a}/67.0^{a}	9.19^{a}/9.75^{a}/9.58^{a}

^{a,b,c}: Values within the same trial, column and phosphorus level (trial 4) with different superscript are significantly different ($P < 0.05$).
(1): P retention in trials 1 and 2/ATTD in trial 4.
(2): Ash metacarpi content in trial 1/P femur content in trial 4.
(3): In trial 4, X/Y/Z data in each column represent values for the different levels of phytate-bound phosphorus tested ([redacted]).

3.4.2.2. Sows

Three short-term trials were provided, one carried out in the lactation and two during the gestations phase. The results of the three trials are presented in Table 5.

In the trial performed in lactating sows,[50] [redacted]

[50] Technical dossier/Section IV/Annex IV_7

[redacted] The sows receiving the 6-phytase contained in the additive at 500 FTU/kg feed showed higher ATTD of P in comparison with the control and PC diets.

The two trials in gestating sows shared a similar design. [redacted][51][52] In both trials, the sows receiving the 6-phytase contained in the additive at 500 FTU/kg feed showed improved ATTD of P in comparison with the control group.

Table 5: Effect of Axtra® PHY GOLD on the apparent total tract digestibility (ATTD) of P in sows

Trial	Diets		ATTD P (%)
	Phytase (FTU/kg feed)	Total P/Ca (%)	
1	0	[redacted]	27.9[c]
	500	[redacted]	44.3[a]
	PC	[redacted]	32.4[b]
2	0	[redacted]	19.7[c]
	500	[redacted]	34.2[a]
	PC	[redacted]	28.8[b]
3	0	[redacted]	27.8[b]
	500	[redacted]	35.4[a]
	PC	[redacted]	34.3[a]

[a,b,c] Values within the same trial and column with different superscript are significantly different ($P < 0.05$).

3.4.3. Conclusion on efficacy

The panel concludes that the additive is efficacious in increasing the phosphorus utilisation when supplemented at 500 FTU/kg in the diets of chickens for fattening, weaned piglets and sows and at 300 FTU/kg in laying hens. The conclusions reached in chickens for fattening, weaned piglets and sows allow concluding on all pigs and all growing poultry species at 500 FTU/kg, and the conclusions reached in laying hens on other poultry species for laying at 300 FTU/kg feed.

[51] Technical dossier/Section IV/Annex IV_8
[52] Technical dossier/Section IV/Annex IV_9

3.5. 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 Regulation[53] and good manufacturing practice.

4. Conclusions

The additive does not give rise to safety concerns with regard to the genetic modification of the production strain. The production strain and recombinant DNA were not detected in the fermentation product used to formulate the additive.

Axtra® PHY GOLD is safe for all growing poultry species and all pigs at the minimum recommended level of 500 FTU/kg complete feed and for laying hens and other poultry species for laying at 300 FTU/kg complete feed.

The use of Axtra® PHY GOLD in animal nutrition is of no concern for consumer safety.

The use of the additive as a feed additive in poultry and pigs is considered safe for the environment.

The panel cannot conclude on the potential of the additive to be an irritant to eyes or skin or a skin sensitiser. Due to the proteinaceous nature of the active substance (6-phytase), the additive is considered a respiratory sensitiser.

The panel concludes that the additive is efficacious in increasing the phosphorus utilisation when supplemented at 500 FTU/kg for all growing poultry species and all pigs, and at 300 FTU/kg in laying hens and other laying birds.

5. Documentation provided to EFSA/chronology

Date	Event
23/10/2020	Dossier received by EFSA. Axtra® PHY GOLD 30 L, Axtra® PHY GOLD 30 T, Axtra® PHY GOLD 65 G (6- phytase) for all pigs, all poultry species. Submitted by Danisco UK Ltd. represented by Genencor International B.V.
13/11/2020	Reception mandate from the European Commission
09/03/2021	Application validated by EFSA – Start of the scientific assessment
28/05/2021	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 of the additive/consumer safety/user safety/efficacy*
09/06/2021	Comments received from Member States
09/09/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
20/01/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
18/02/2022	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 of the additive*
24/02/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
15/03/2022	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 of the additive*
21/03/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
17/05/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: purity / efficacy*
18/07/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
27/09/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[53] 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, pp. 1.

References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. https://doi.org/10.2903/j.efsa.2007.587.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020. Scientific Opinion on the update of the list of QPS recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017a. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017b. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017c. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018a. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Karenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018b. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, Lopez-Alonso M, Lopez Puente S, Marcon F, Mayo B, Pechova A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

Frisvad JC, Moller LLH, Larsen TO, Kumar R and Arnau J, 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus Niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. Applied Microbiology and Biotechnology, 102, 9481–9515.

Kubicek CP, Komoń-Zelazowska M, Sándor E and Druzhinina IS, 2007. Facts and challenges in the understanding of the biosynthesis of peptaibols by *Trichoderma*. Chemistry & Biodiversity, 4, 1068–1082.

Tisch D and Schmoll M, 2010. Light regulation of metabolic pathways in fungi. Applied Microbiology and Biotechnology, 85, 1259–1277.

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ADI	average daily intake
BW	body weight
CAS	Chemical Abstracts Service

CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
EC	European Commission
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FCR	feed conversion ratio
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FGE	food group evaluation
GC–MS	gas chromatography–mass spectrometry
HACCP	hazard analysis and critical control points
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MRL	maximum residue limit
MW	molecular weight
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity
SCAN	Scientific Committee on Animal Nutrition
UF	uncertainty factor

Appendix A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for a preparation of 6-phytase (EC 3.1.3.26)

In the current application, authorisation of a *preparation of 6-phytase (EC 3.1.3.26)* is sought under Article 4 for all poultry and all pig species under the category/functional group 4(a) 'zootechnical additives'/'digestibility enhancers' according to Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, the active agent of the product is *6-phytase*, produced by fermentation of the genetically modified strain *Trichoderma reesei* CBS 146250. Other *preparation of 6-phytase* from a different *Trichoderma reesei* strain is currently authorised as *feed additive*.

The activity of *6-phytase* is expressed in phytase units (FTU) where 'one FTU is the amount of enzyme which releases one micromole of inorganic orthophosphate from a sodium phytate substrate per minute at pH 5.5 and 37 °C'. This definition is in agreement with the phytase activity unit as described in EN ISO 30024.

The product is intended to be marketed in three different formulations namely *Axtra® PHY GOLD 30L* (liquid), *Axtra® PHY GOLD 30T* (granular and thermostable) and *Axtra® PHY GOLD 65G* (granular) with a guaranteed minimum *6-phytase* activity of 30,000 FTU/g for the *Axtra® PHY GOLD 30L* and the *Axtra® PHY GOLD 30T* formulations and 65,000 FTU/g for the *Axtra® PHY GOLD 65G* formulation. The product is intended to be included through *premixtures* or directly in *feedingstuffs* to obtain a minimum recommended activity of 300 FTU/kg *feedingstuffs* for laying hens and other birds for laying and 500 FTU/kg *feedingstuffs* for the other target species.

The Applicant submitted single-laboratory validated and further verified methods for the quantification of the *phytase* activity in the *product (Axtra® PHY GOLD), premixtures* and *feedingstuffs*. The submitted methods are very similar to the ring-trial validated EN ISO 30024 method.

Upon request of the EURL, the Applicant compared both protocols confirming that equivalent results are obtained when applying the slightly different methods to *feedingstuffs* containing the product *(Axtra® PHY GOLD)*.

Additionally, the EURL is aware of other ring-trial validated VDLUFA methods specifically describing the preparation of *premixtures* (VDLUFA 27.1.3) and *feed additives* (VDLUFA 27.1.4) for the quantification of their *phytase* activity according to EN ISO 30024.

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated EN ISO and VDLUFA colorimetric methods mentioned above for the quantification of the phytase activity in the *product*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.