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Safety and efficacy of a feed additive consisting of 6-phytase produced by *Komagataella phaffii* CGMCC 7.370 (VTR-phytase powder/liquid) for all pigs and all avian species (Victory Enzymes GmbH)

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, Gabriele Aquilina, Paul Brantom, Noël Dierick, Giovanna Martelli, Kettil Svensson, Montserrat Anguita, Rosella Brozzi, Jaume Galobart, Jordi Ortuño Casanova, Elisa Pettenati and Jordi Tarrés-Call

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 (VTR-phytase) as zootechnical feed additive for all pigs and all avian species. The additive VTR-phytase consists of 6-phytase and it is available in solid and liquid forms. VTR phytase (liquid/solid) was produced by a genetically modified strain of *Komagataella phaffii* (CGMCC 7.370). The genetic modification of the production strain does not give rise to safety concerns. Viable cells of the production strain and its DNA were not detected in the final products. The additive does not pose any safety concern regarding the production strain. VTR phytase (liquid/solid) produced by *Komagataella phaffii* CGMCC 7.370 is safe for all Suidae and all avian species at the proposed conditions of use. The use of both forms of the additive under assessment in animal nutrition under the proposed conditions of use raises no safety concerns for consumers or for the environment. The liquid VTR phytase and powder VTR phytase are non-irritant to skin or eyes but should be considered skin and respiratory sensitisers. The additive has the potential to be efficacious in laying hens at 1,000 U phytase/kg complete feed. The conclusion can be extrapolated to other birds for egg production or breeding. The FEEDAP Panel cannot conclude on the efficacy of all pigs or growing poultry species.

Keywords: zootechnical additives, digestibility enhancers, 6-phytase, *Komagataella phaffii* CGMCC 7.370, safety, efficacy

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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

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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 two requests from Victory Enzymes GmbH[2] for the authorisation of the additive consisting of 6-phytase produced by *Komagataella phaffii* CGMCC 7.370 (VTR-phytase liquid/powder), when used as a feed additive: one request was for all pigs, and the other for all avian species (category: zootechnical additive; 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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 16 July 2021 for all avian species and as of 4 August 2021 for all pigs.

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 feed additive consisting of 6-phytase produced by *K. phaffii* CGMCC 7.370 (VTR-phytase liquid/powder), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The subject of the assessment is the feed additive consisting of 6-phytase produced by *K. phaffii* CGMCC 7.370 (VTR-phytase liquid/powder), intended for use as a zootechnical additive (functional group: digestibility enhancers) for all pigs and all avian species. The product is not authorised as a feed additive in the European Union.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of two technical dossiers[3] in support of the authorisation request for the use of 6-phytase produced by *K. phaffii* CGMCC 7.370 (VTR-phytase liquid/powder) as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance 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 *K. phaffii* CGMCC 7.370 (VTR-phytase liquid/powder) 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 assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a); guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b); guidance on the assessment of the safety of feed additives for the target species (EFSA

[1] Regulation (EC) No 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, p. 29.
[2] Victory Enzymes GmbH (Fürschlag 3, D-91564 Neuendettelsau, Germany).
[3] FEED dossier reference: FAD-2021-0066 for all avian species and FAD-2021-0067 for al pigs.
[4] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2021-00660067_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, p. 1.

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); guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product containing 6-phytase (phytase; Enzyme Commission (EC) number 3.1.3.26) produced by *K. phaffii* CGMCC 7.370 (VTR-phytase liquid/powder) is intended to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for all pigs and all avian species. It will be hereafter referred to as VTR-phytase liquid and powder.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The 6-phytase is produced by fermentation with a genetically modified strain of *K. phaffii*, deposited in the China General Microbiological Culture Collection Center (CGMCC) with deposition number CGMCC 7.370.[6]

The taxonomical identification of the production strain CGMCC 7.370 as *K. phaffii* was confirmed [redacted][7].

3.1.1.1. Information related to the genetically modified microorganism

Characterisation of the parental or recipient microorganism

The recipient strain [redacted].[8]

Description of the genetic modification

[redacted].

The absence of plasmids in the production strain was confirmed [redacted].

[6] Technical dossier FAD-2021-0066/Section II/Annexes_Sect_II/Annex_II_2_1_2_4.
[7] Technical dossier FAD-2021-0066/Section II/Annexes_Sect_II/Annex_II_2_1_2_1, Annex_II_2_1_2_2 and Annex_II_2_1_2_3.
[8] Technical dossier FAD-2020-0066/Section II/Annexes_Sect_II/Annex_II_2_1_2_1.
[9] Technical dossier FAD-2020-0066/Section II/Annexes_Sect_II/Annex_II_2_1_2_1 and Supplementary information July 2022/Annex_1.

3.1.2. Manufacturing process

The enzyme is produced by fermentation with the production strain.[10] [redacted].[11]

The applicant declared that no antimicrobials had been used in the production process, and the final products do not contain antimicrobial substances.[12]

3.1.3. Characterisation of the additive

The additive VTR-phytase contains 6-phytase as active substance. It is intended to be marketed in two formulations: powder VTR-phytase containing ≥ 50,000 U[13]/g of additive; and liquid VTR-phytase containing ≥ 5,000 U/g of additive. The amount of carrier ([redacted]) in the powder VTR phytase is stated to range from [redacted].[14]

Analytical data to confirm the specifications were provided for five batches of each form of the additive. The liquid VTR phytase showed an average enzymatic activity of 5,696 U/g of additive (range 5,030–6,720 U/g). Total organic solids (TOS) analysed in three batches ranged from 0.22 to 0.23%. The powder VTR phytase showed an average enzymatic activity of 51,140 U/g of additive (range 39,800–59,000 U/g). It is noted that two out of the five batches analysed did not reach the minimum specified enzymatic activity. Moisture was on average 6.9% (range 6.1–7.4%).[15] TOS analysed in three batches ranged 2.5–2.8%.[16]

Three batches of each formulation of the additive were analysed for chemical and microbiological impurities. The liquid VTR phytase showed cadmium, lead, mercury and arsenic concentrations below the limit of detection (LOD) of the analytical methods.[17] In the three batches analysed of the powder VTR phytase, lead ranged from 0.035 to 0.039 mg/kg; cadmium ranged from 0.0011 to 0.0012 mg/kg; arsenic ranged from 0.011 to 0.013 mg/kg; mercury was found below the LOD in all three batches.[18] Methanol residual concentration was below limit of quantification (LOQ) in the liquid VTR phytase and ranged 22.7–24.8 mg/kg in the powder VTR phytase.[19] Dioxins, dioxin-like PCBs and non-dioxin-like PCBs were not analysed in the liquid form. In the powder VTR phytase, polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (dl-PCBs) were found below the corresponding limit of quantification (LOQ). The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.0855 pg WHO-PCDD/F-TEQ/kg and 0.0987 pg WHO-PCDD/F-PCB-TEQ/kg, respectively (in all three batches).[20] Non-dioxin-like PCBs were also analysed and found below the LOD. Regarding mycotoxins, both final forms of the additive showed values of ocratoxin A, zearalenone, fumonisins B1, B2, B3, HT2-toxin, T2-toxin, deoxynivalenol and aflatoxins (B1, B2, G1, G2) below the LOD/LOQ.[21] Microbiological contamination was analysed in three batches by determination of Enterobacteriaceae,

[10] Technical dossier FAD-2021-0066/Section II/Annex II_3_1.
[11] Technical dossier FAD-2021-0066/Supplementary information July 2022/Annex 2 manufacture CONF.
[12] Technical dossier FAD-2021-0066/Supplementary information July 2022/1 EFSA Sin 16NOV2021 reply/reply to question 2.
[13] The enzymatic activity is expressed in units (U) where one unit is defined as the amount of enzyme needed to release 1 μmol of inorganic phosphorus per minute from 5.0 mmol/L sodium phytate solution at 37°C and pH 5.50.
[14] Technical dossier FAD-2021-0066/Section II/Annex II.1.3.1.
[15] Technical dossier FAD-2021-0066/Section II/Annex II.1.4.1 and supplementary information July 2022/Annex 5.1. Phytase activity analysed by method DIN EN ISO 30024.
[16] Technical dossier FAD-2021-0066/Section II/Annex II.1.3.3.
[17] Technical dossier FAD-2021-0066/Section II/Annex II.1.4.1. LOD in mg/kg was 0.002 for arsenic, 0.0001 for cadmium, 0.0003 for lead and 0.0007 for mercury.
[18] Technical dossier FAD-2021-0066/Section II/Annex II.4.1.1. LOD in mg/kg was 0.0007 for mercury.
[19] Technical dossier FAD-2021-0066/Supplementary information July 2022/Annex 5.1. LOQ of methanol in mg/kg was 10.
[20] Technical dossier FAD-2021-0066/Section II/Annex II.4.1.1. LOQ in ng/kg were 0.02–0.1 for dioxins; and 0.05–0.3 for dioxin-like PCBs, depending on the parameter analysed. LOD in μg/kg were 0.01–0.05 for no dioxin-like PCBs, depending on the parameter analysed.
[21] Technical dossier FAD-2021-0066/Section II/Annex II.1.4.1 and supplementary information July 2022/Annex 5.1. LOD in μg/kg was 0.03 for ocratoxin A; 0.7 for zearalenone, HT2-toxin and T2-toxin; for fumonisin B1, B2 and B3; and 3 for deoxynivalenol. LOQ was 0.1 for aflatoxins (B1, B2, G1 and G2).

Salmonella spp., filamentous fungi and yeasts. *Salmonella* spp. was not detected in 25 g samples of any of the final forms of the additive. The liquid VTR phytase showed values of Enterobacteriaceae, filamentous fungi and yeasts below the LOD of the analytical methods.[22] In the powder VTR phytase, Enterobacteriaceae ranged from 60 to 90 colony forming units (CFU)/g, filamentous fungi ranged from < LOD to 2 × 10^2 CFU/g and yeasts ranged from 4 × 10^2 to 1.6 × 10^3 CFU/g.[23]

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

The presence of viable cells of the production strain in the final product was investigated in three batches of VTR-phytase liquid and three batches of VTR-phytase powder, [redacted][24] no viable cells of the production strain were found in VTR-phytase liquid nor in VTR-phytase powder.

The presence of recombinant DNA from the production strain in the final product was tested [redacted][25]. No recombinant DNA of the production strain was detected in VTR-xylanase powder nor in VTR-xylanase liquid.

3.1.3.1. Physical properties of the additive

The liquid VTR phytase appears as a yellowish to light brown liquid, its pH (five batches analysed) ranges from 5.3 to 5.6. Vapour pressure at 20°C was 2.22 kPa (average of six batches). Viscosity and specific weight (measured in five batches at 20°C) showed average values of 1.8 mPa·s and 1,100 kg/m^3, respectively.[26]

The powder VTR phytase appears as a greyish white powder. It is stated to be soluble in water.[27] Solid density and bulk density (measured in three batches) had average values of 1,476 kg/m^3 and 680 kg/m^3, respectively. The dusting potential of three batches was determined using the Stauber-Heubach method and showed values ranging from 5.0 to 7.9 g/m^3. The particle size distribution (laser diffraction method) showed that the percentage of particles with a diameter < 10, < 50 and < 100 μm of diameter ranged 8–9%, 48–50% and 56–58% (v/v), respectively.[28] No particles with a diameter < 1 μm were found in the batches analysed.

3.1.3.2. Stability and homogeneity

The shelf-life of the liquid VTR phytase (three batches) was studied when stored at (5°C) in brown plastic bottles for 3 months.[29] No losses of phytase activity were observed at the end of the storage period. The shelf-life of the powder VTR-phytase (three batches) was studied when stored at 25°C in non-transparent packs for 18 months.[30] Losses of phytase activity at the end of the storage period ranged from 12 to 15%.

The stability of the powder VTR-phytase (one batch) in a vitamin/mineral premixture for chickens for fattening (containing 600 mg choline chloride/kg) was studied when supplemented at 0.2% and stored at 25°C in plastic bags for 6 months. Losses of phytase activity at the end of the storage period were 20%.[31]

[22] Technical dossier FAD-2021-0066/Section II/Annex II.1.4.1. LOD in CFU/g was 10 for Enterobacteriaceae and 100 for yeast and filamentous fungi.
[23] Technical dossier FAD-2021-0066/Section II/Annex II.1.4.1. LOD in cfu/g was 100 Filamentous fungi.
[24] Technical dossier FAD-2020-0066/Section II/Annexes_Sect_II/Annex_ II_2_2_2_2_1 and Annex_II_2_2_2_2_2.
[25] Technical dossier FAD-2020-0066/Supplementary information July 2022/Annex_6_1 and Annex_6_2.
[26] Technical dossier FAD-2021-0066/Section II/Annex II.1.5.2.
[27] Technical dossier FAD-2021-0066/Section II/Annex II.5.2.1.
[28] Technical dossier FAD-2021-0066/Section II/Annex II.1.5.1.
[29] Technical dossier FAD-2021-0066/Section II/Annex II.4.1.2.
[30] Technical dossier FAD-2021-0066/Section II/Annex II.4.1.1.
[31] Technical dossier FAD-2021-0066/Section II/Annex II.4.1.3.

The stability of the powder VTR-phytase (one batch) in three complete feeds for chickens for fattening and one complete feed for weaned piglets (mash and pellet forms each) was studied when supplemented via the above-mentioned premixture at 1% to achieve a final concentration of 1,000 U/kg feed, stored at 25°C in plastic bags for 3 months. The pelleting process was performed at 60°C and represented losses ranging from 3 to 55% depending on the compound feed considered. Losses of phytase activity at the end of the storage period ranged from 5 to 42% in mash; and in pelleted feed, an additional loss of 6–73% was observed, depending on the feed considered.[31]

The VTR-phytase liquid (one batch) was sprayed on top of the four different pelleted feeds described above (that were not supplemented with enzymes) to achieve a phytase activity of 1,000 U/kg feed. Samples were stored at 25°C in plastic bags for 3 months. Losses of phytase activity at the end of the storage period ranged from 48 to 72%, depending on the batch considered.[31]

The capacity for homogeneous distribution of the powder VTR-phytase in mash and pelleted feed was studied in one of the complete feeds for chickens for fattening (described above). Ten subsamples of the meal and the pelleted forms were analysed for total phytase activity. The CV was 5% in mash and 6% in pelleted feed.[32] The capacity for homogeneous distribution of the liquid VTR-phytase in the pelleted complete feed for chickens for fattening described above was studied in 10 subsamples. Total phytase activity was analysed and the coefficient of variation (CV) was 4%.[33]

3.1.4. Conditions of use

The additive is intended for use in feed for all pigs and all avian species at a proposed minimum use level of 500 U/kg feed.

3.2. Safety

3.2.1. Safety of the production organism

The production organism belongs to *K. phaffii*, which is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment when used for enzyme production (EFSA BIOHAZ Panel, 2007; 2020). The production strain was identified as *K. phaffii* and differed from the parental strain in [redacted] No complete genes of concern were introduced by the genetic modification. No viable cells nor recombinant DNA of the production strain were detected in the final products.

Therefore, the final products of the additive under assessment do not pose any safety concern regarding the production strain.

3.2.2. Toxicological studies

The applicant submitted two genotoxicity tests and a 90-day oral repeated dose toxicity test performed with the intermediate liquid concentrate form of phytase ([redacted]) from which the two final VTR phytase formulations were obtained. This test item is considered representative of the two final forms of the additive.

3.2.2.1. Genotoxicity studies including mutagenicity

3.2.2.1.1. Bacterial reverse mutation test

The potential of VTR-phytase (liquid concentrate) to induce gene mutations was investigated in a bacterial reverse mutation test, performed according to the OECD Test Guideline (TG) 471 and in compliance with good laboratory practice (GLP) principles.[34]

[redacted]

[32] Technical dossier FAD-2021-0066/Section II/Annex II.4.2
[33] Technical dossier FAD-2021-0066/Section II/Annex II.4.2.
[34] Technical dossier FAD-2021-0066/Section III/Annex_III.2.2.2.1 CONF.

[redacted] The Panel concluded that the test item did not induce gene mutations in bacteria under the experimental conditions applied in this study.

3.2.2.1.2. In vitro mammalian cell micronucleus test

VTR-phytase (liquid concentrate) was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy, in compliance with the OECD TG 487 and GLP principles.[35]

[redacted], no evidence of chromosomal damage or aneuploidy was observed.

3.2.2.2. 90-day repeated dose toxicity study test

Sprague Dawley rats (10/sex/group) received VTR-phytase (liquid concentrate) at dose levels of [redacted] mg/kg body weight (bw) per day by oral gavage for 90 consecutive days. The study was conducted in compliance with OECD TG 408.[36]

[redacted]

From this study, an NOAEL of [redacted], was derived.

3.2.2.3. Conclusions on toxicology

The intermediate product used for the formulation of the additive showed no genotoxicity potential in tests addressing gene mutation, and numerical and structural chromosome aberrations. Moreover,

[35] Technical dossier FAD-2021-0066/Section III/Annex III.2.2.2.2 CONF.
[36] Technical dossier FAD-2021-0066/Section III/Annex III.2.2.3.CONF.

the results obtained in a 90-day repeated oral toxicity study raised no concerns regarding the product and allowed to derive an NOAEL of [redacted].

3.2.3. Safety for the target species

No tolerance studies in relevant target species were submitted. In order 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 ([redacted]) was used to calculate the maximum safe level in piglets, pigs for fattening, lactating sows, chickens and turkeys for fattening and laying hens in accordance with the procedure described in the guidance on the safety for the target species (EFSA FEEDAP Panel, 2017c), and the results are shown in Table 1. The maximum safe levels obtained are higher than the recommended minimum use level of 500 U/kg feed for all pigs and all avian species. Therefore, the Panel concludes that the additive is safe for all Suidae and for all avian species.

Table 1: Safe concentration in feed for the target species

Animal category	Default value for feed intake (g DM/kg bw)	Safe concentration in feed (Units/kg complete feed DM 88%)
Piglet	44	9,525
Pig for fattening	37	11,430
Sow lactating	30	13,891
Chicken for fattening	79	5,305
Laying hen	53	7,908
Turkey for fattening	59	7,144

3.2.3.1. Conclusions on safety for the target species

The 6-phytase produced by *Komagataella phaffii* CGMCC 7.370 (VTR phytase liquid/solid) is safe for all pigs and all avian species at the proposed conditions of use.

3.2.4. Safety for the consumer

The enzyme is produced by a genetically modified strain of *K. phaffii*. This species is considered to qualify for the QPS approach to safety assessment when used for enzyme production. The identity of the strain was established, and the genetic modification of the production strain raises no concerns. Therefore, the production strain is presumed safe for production purposes and no concerns would raise for the consumer from the fermentation product obtained from this strain. The results obtained in the genotoxicity studies and the 90-day oral toxicity test support this conclusion.

3.2.5. Safety for the user

3.2.5.1. Effects on the respiratory system

The dusting potential of the powder VTR phytase is up to 8 g/m^3, and therefore, exposure by inhalation is very likely. Owing to the proteinaceous nature of the active substance, the additive is considered as a respiratory sensitiser.

3.2.5.2. Effect on skin and eyes

The potential of the intermediate liquid concentrate used to formulate the final products to be irritant to skin was tested [redacted][37] [redacted] Based on the results obtained, the intermediate liquid concentrate is classified as non-irritant in accordance with the UN GHS 'No Category'.

The potential of the liquid VTR phytase and the powder VTR phytase to be irritant to eyes was tested *in vitro* using a reconstructed human cornea model.[38] The studies were conducted in compliance with GLP following the OECD TG 492. The results showed that, under the specified

[37] Technical dossier FAD-2021-0066/Section III/Annex III.3.1.2.Conf.
[38] Technical dossier FAD-2021-0066/Supplementary information July 2022/Annex 8.3a and Annex 8.3b.

experimental conditions, both additives should be classified in accordance with the UN GHS as 'No Category'.

The skin sensitisation potential of the liquid and powder forms of the additive was investigated *in vitro* using the keratinocyte-based ARE-Nrf2 luciferase reporter gene test method.[39] The studies were conducted in compliance with GLP, following the OECD TG 442D. The results showed that both forms of VTR phytase can induce ARE-dependent gene expression.

The skin sensitisation potential of the liquid and powder forms of the additive was further investigated *in vitro* using the human cell line activation test (h-CLAT) method.[40] The studies were conducted in compliance with GLP and following the OECD TG 442 E. The results showed that both formulations of the additive are predicted to be skin sensitisers.

3.2.5.3. Conclusions on safety for the user

On the basis of the results of the studies submitted, the FEEDAP Panel considered liquid VTR phytase and powder VTR phytase to be non-irritant to skin or eyes but should be considered skin and respiratory sensitisers.

3.2.6. Safety for the environment

Neither the production strain nor its recombinant DNA was detected in the final formulations of the additive. The final products do not pose any environmental safety concern associated with the genetic modification of the production strain. The active substance of the additive is a protein, and as such will probably be degraded/inactivated during passage through the digestive tract or in the environment. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

3.3.1. Efficacy in poultry

3.3.1.1. Efficacy in chickens for fattening

The applicant submitted three long-term trials in chickens for fattening aiming at assessing the effect of the additive on the zootechnical performance, also including a balance trial to evaluate the effect on the phosphorus retention. However, one of the trials[41] was not considered further due to the high mortality registered (overall mortality of 11% during the finisher phase, and up to 20% in the control group for the whole experimental period). The other two trials are described below.

In trial 1,[42] a total of 540 1-day-old male Cobb 500 chickens for fattening were distributed in 36 pens in groups of 15 birds per pen and allocated to four dietary treatments (nine replicates per treatment). Two basal diets (starter, from day 1 to 14; grower, from day 15 to 35) based on soya bean meal, maize, wheat and barley were either not supplemented (control) or supplemented with VTR-phytase powder to provide 500, 750 or 1,000 U/kg complete feed. The enzyme activity and the calcium/phosphorus content of the diets were confirmed analytically (see Table 2). The experimental diets were offered ad libitum in mash form for 35 days. The grower diet contained an external marker for the retention analysis. Mortality and health status were checked daily. Pen body weight and feed intake were registered weekly, and the average daily feed intake, average daily gain and feed to gain ratio were calculated. At day 29, 18 birds per treatment were selected (based on body weights closest to the average of their corresponding treatment group) and moved to metabolic cages in pairs (nine replicates per treatment). From day 32 to 35, excreta samples were collected by the total collection method and pooled per cage. Feed and excreta samples were analysed for dry matter, external marker and mineral content (ash, calcium, phosphorus), and the P retention calculated. At day 35, the same birds used for the balance study were killed and left tibia were collected and analysed for the mineral (ash, calcium, phosphorus) content. The experimental data were analysed with an ANOVA, including the treatment as fixed effect. Mean groups were compared with Tukey's test. Significance level was set at 0.05. Mortality was 5.2, 2.2, 1.5 and 1.5% for the control, 500, 750 and 1,000 U/kg feed,

[39] Technical dossier FAD-2021-0066/Supplementary information July 2022/Annex 8.1a and Annex 8.1b.
[40] Technical dossier FAD-2021-0066/Supplementary information July 2022/Annex 8.2a and Annex 8.2b.
[41] Technical dossier FAD-2021-0066/Section IV/Annex IV.3.2.
[42] Technical dossier FAD-2021-0066/Section IV/Annex IV.3.1 and supplementary information July 2022 /1 EFSA Sin 16NOV2021 reply/Reply to question 9.

respectively. The zootechnical performance of the animals was lower than the expected for the breed (69% of the expected), and therefore, the results on the zootechnical performance were not considered further in the assessment. The supplementation of the feed of chickens for fattening at the minimum recommended level showed higher P retention in comparison with the control diet (see Table 2).

In trial 2,[43] 208 1-day-old Ross 308 male chickens for fattening were distributed in pairs in 104 cages and randomly allocated into four treatment groups (26 replicates per treatment). At day 21, half of the animals were removed from the trial and the remaining birds were raised in the same cages individually until the end of the trial. Two basal diets (starter, from day 1 to 21; and grower, from day 22 to 35) based on maize, soya and wheat were either not supplemented (control) or supplemented with VTR-phytase powder to provide 500, 750 or 1,000 U/kg complete feed. The enzyme activity and Ca/P content in the diets were confirmed analytically (see Table 2). The experimental diets were offered ad libitum in pellets for 35 days. Mortality and health status were checked daily. Body weight and feed intake were registered weekly, and the average daily feed intake, average daily gain and feed to gain ratio were calculated. From day 28 to 32, excreta samples were collected from 10 birds per diet by the total collection method. Feed and excreta samples were analysed for dry matter, external marker and mineral content (ash, calcium, phosphorus), and the P retention calculated. The experimental data were analysed with an ANOVA, including the treatment as fixed effect. Mean groups were compared with Tukey's test. Significance level was set at 0.05. Mortality was 1.9, 1.9, 0 and 3.8% for the control, 500, 750 and 1,000 U/kg feed, respectively. The zootechnical performance of the animals was not considered in the assessment because the animals were in cages, which is not in line with Directive 2007/43/EC. The supplementation of the feed of chickens for fattening at the minimum recommended level showed no differences in the P retention in comparison with the control diet (see Table 2).

Table 2: Trial design, enzyme activity, Ca/P content in diets and effect of VTR-phytase on the phosphorus retention and bone mineralisation in chickens for fattening

Trial	Groups	Diets Starter/Grower		Retention	Bone mineralisation
		Enzyme activity (U/kg)	Ca-P(1) (%)	P (%)	P (g/kg DM)
1	0	102/< 100	0.78/0.66–0.30/0.38	48.4[b]	43.2
	500	721/590	0.76/0.65–0.31/0.38	64.1[a]	44.7
	750	1,170/989	0.80/0.66–0.32/0.38	63.8[a]	44.8
	1,000	1,430/1,380	0.82/0.65–0.33/0.38	63.9[a]	45.4
2	0	147/141	1.45/1.04–0.57/0.61	46.8	n/a
	500	612/529		47.1	
	750	821/811		51.6	
	1,000	1,200/1,130		51.6	

[a,b]: Mean values within a trial and within a column with a different superscript are significantly different $P < 0.05$.
n/a: not analysed.
(1): In trial 2, the analysis were performed in the starter/finisher basal diets.

3.3.1.2. Efficacy for laying hens

The applicant submitted three short-term balance trials to assess the effect of the additive on the phosphorus retention when included in the diet of laying hens. The experimental design of the different trials is shown in Table 3 and the main results of the phosphorus utilisation in Table 4.

In all trials, the hens were randomly allocated to four treatments: the basal diet either not supplemented (control) or supplemented with VTR-phytase powder to provide 500, 750 or 1,000 U/kg complete feed. The enzyme activity and the Ca/P content of the diets were analytically confirmed in the respective experimental feeds (see Table 3). In trials 1 and 2, the feeds included an external marker for the digestibility analysis. The general health status of the birds was monitored daily, and the mortality (including culls) was recorded as it occurred, including the most likely cause of death. In all trials, body weight, feed intake and the laying performance were monitored throughout the experiment.

[43] Technical dossier FAD-2021-0066/Annex IV.3.3. and Supplementary information July 2022/1 EFSA Sin 16NOV2021 reply/Reply to question 10.

Table 3: Trial design and analysed enzyme activity of the diets of the efficacy trials performed in laying hens

Trial	Total n° of animals (animals/replicate) Replicates/treatment	Breed (age) Duration	Composition feed (mash form)	Groups (U/kg feed)	
				Intended	Analysed
1[44]	200 (5) 10	Lohmann Brown (22 weeks) 54 days	Maize, soya bean meal, barley, wheat	0 500 750 1,000	502 1,000 1,370 1,690
2[45]	200 (5) 10	Lohmann Brown (26 weeks) 54 days	Maize, soya bean meal, barley, wheat	0 500 750 1,000	488 981 1,260 1,600
3[46]	96 (3) 8	Bábolna Tetra SL-LL (23 weeks) 28 days	Maize, soya bean meal, wheat	0 500 750 1,000	110 756 1,190 1,450

In trials 1 and 2, from day 60 to 63, excreta samples were collected from 10 hens per treatment (one per pen, selected based on the body weight closest to the average of the corresponding group). Feed and excreta samples were analysed for the content of dry matter, external marker and mineral (ash, Ca, P), and the P utilisation was calculated. At the end of the experiment (day 63), the hens used for the digestibility trial were killed, and the left tibia sampled and analysed for dry matter and the mineral (ash, Ca, P) content. In trial 3, from day 26 to 28, excreta samples were collected from all cages. Feed and excreta samples were analysed for the dry matter and mineral (ash, Ca, P) content, and the P utilisation was calculated. At the end of the experiment (day 28), one hen per cage was killed, and femur sampled and analysed for dry matter and mineral (ash, Ca, P) content. In comparison with the control group, the laying hens that received the additive showed higher P utilisation with phytase levels of 500 U/kg feed in trials 1 and 2, and of 1,000 U/kg in trial 3. Moreover, a higher bone P content was observed in trials 2 and 3 from 750 U/kg (see Table 4).

Table 4: Effect of VTR-phytase on the phosphorus utilisation and bone mineralisation of the laying hens

Trial	Groups	Diet	Utilisation		Bone mineralisation	
	(U/kg feed)	Ca/P %	% P	% Ca	P (g/kg DM)	Ca (g/kg DM)
1(1)	0	3.10/0.32	25.7^{a}	43.2^{c}	45.7	78.8
	500	3.11/0.33	29.9^{b}	49.4^{b}	46.5	82.1
	750	3.12/0.32	33.5^{bc}	54.1^{a}	46.1	83.2
	1,000	3.11/0.32	35.5^{c}	54.8^{a}	46.3	85.1
2(1)	0	3.10/0.32	26.6^{a}	41.8^{c}	52.6^{b}	78.4^{b}
	500	3.11/0.33	31.5^{b}	47.8^{b}	53.1^{b}	85.9^{a}
	750	3.12/0.32	33.9^{bc}	51.2^{ab}	58.7^{a}	89.1^{a}
	1,000	3.11/0.32	36.2^{c}	52.6^{a}	60.7^{a}	90.4^{a}
3	0	3.66/0.71	25.5^{bc}	56.2^{b}	73.1^{c}	164^{b}
	500	3.60/0.69	25.0^{c}	56.5^{ab}	74.4^{bc}	164^{b}
	750	3.56/0.71	30.4^{ab}	57.7^{ab}	76.6^{ab}	168^{ab}
	1,000	3.68/0.70	31.2^{a}	59.8^{a}	79.1^{a}	170^{a}

^{a,b,c}: Mean values within a trial and within a column with a different superscript are significantly different $P < 0.05$.

(1): The analytical data on bone mineralisation in studies 1 and 2 were about half the values obtained in study 3. Studies 1 and 2 were conducted in the same laboratory, and study 3 in a different one, and the methods used in the laboratories were different.

[44] Technical dossier FAD-2021-0066/Section IV/Annexes IV.2.1.
[45] Technical dossier FAD-2021-0066/Section IV/Annexes IV.2.2.
[46] Technical dossier FAD-2021-0066/Section IV/Annexes IV.2.3.

3.3.2. Efficacy in pigs

3.3.2.1. Efficacy in weaned piglets

The applicant submitted two long-term trials and one short-term trial to support the efficacy of the additive in weaned piglets on the zootechnical performance (trials 1 and 2) and the phosphorus retention (trials 2 and 3). However, the data reported in trial 3 suggested a poor adaptation of the piglets to the experimental conditions, reflected by the removal of a relevant number of animals from all groups from the statistical analysis due to health-related issues, a level-dependent worsening in daily weight gain and feed conversion rate and inconsistency in the P retention values at the different levels tested. Therefore, trial 3 was not considered further as supporting evidence of the efficacy.

The two long-term trials shared a similar design (see Table 5). In both trials, the piglets were distributed in four experimental groups, being the basal diet either not supplemented (control) or supplemented with VTR-phytase powder to provide 500, 750 or 1,000 U/kg feed. The experimental feeds were offered ad libitum, and the enzyme activity was confirmed analytically for phase I and II diets in each trial. The phase II feeds in trial 2 included an external marker for the digestibility analysis. The details of the experimental design of the trial are included in Table 5, and the results of the zootechnical performance are in Table 6.

Table 5: Trial design and analysed enzyme activity of the diets of the efficacy trials performed in weaned piglets

Trial	Total N (piglets/rep.) Rep/treat	Breed Sex (duration)	Duration (Phase I/Phase II)(1)	Composition feed (form)	Groups (units/kg feed)	
					Intended	Analysed Pre-starter/starter
1[47]	192 (4) 12	(DE × DL) × Pi 50:50 barrow: gilt	42 days (0–21/22–42)	Maize, soya bean meal (pelleted)	0 500 750 1,000	564/148 724/606 752/838 947/1210
2[48]	80 (2) 10	DanBred × Duroc 50:50 barrow: gilt	42 days (0–14/15–42)	Optigrain(2), soya bean meal (mash)	0 500 750 1,000	133/280 671/897 1,140/1,310 1,340/1,990

(1): Phase I = pre-starter (trial 1) or starter (trial 2) / Phase II = starter (trial 1) or grower (trial 2).
(2): 50% corn, 25% barley, 25% wheat, purified & heat treated (80°C).

In both trials, the general health status of the animals was monitored daily, and the mortality (including culls) was recorded as it occurred, including the most likely cause of death. The weight of the animals was recorded individually (trial 1) or on a pen basis (trial 2) at the start of the trial. Thereafter, body weight and feed intake were measured weekly. The average daily feed intake, average daily gain and feed to gain ratio were calculated and corrected for mortality for phases I and II, and the overall period. In trial 1, at the end of the experimental period, six pigs per treatment were killed and the right femur and tibia bones were sampled and analysed for the mineral content (ash, Ca and P). In trial 2, from day 39 to 42, faeces and urine were collected by the total collection method. The feed, faecal and urinary samples were analysed for the content of dry matter, the external marker and P, and the P retention calculated. The experimental data of both trials were statistically analysed with ANOVA considering the treatment as fixed effect. Mean comparison between treatments was evaluated with Tukey's test. The significance level applied was set at 0.05. In trial 1, three piglets from the 500 U/kg group were removed during the trial: one found death and two due to health reasons. In trial 2, no dead or culled animal was removed from the experiment. The supplementation of the diets with VTR-phytase showed an improved growing performance of the piglets in trial 1 from 750 U/kg feed (higher average daily gain, and lower feed to gain ratio) and from 500 U/kg feed in trial 2 (higher final body weight and average daily gain, and lower feed to gain ratio) in comparison with the control diet (see Table 6). In trial 2, higher P retention was also observed at the minimum use level and above in comparison with the control.

[47] Technical dossier/FAD-2021-0067/Section IV/Annex IV.3.1.
[48] Technical dossier/FAD-2021-0067/Section IV/Annex IV.3.2.

Table 6: Effects of VTR-phytase on the zootechnical performance, phosphorus retention and bone mineralisation of weaned piglets

Trial	Groups (U/kg feed)	ADFI (g)	Final body weight (kg)	Average daily gain (g)	Feed to gain ratio	P retention (%)	Bone P (g/kg DM)
1	0	550	22.2^{b}	349^{c}	1.58^{a}	n/a	17.3^{b}
	500	569	23.6^{ab}	383^{bc}	1.52^{ab}		17.9^{a}
	750	582	24.3^{ab}	398^{ab}	1.46^{bc}		17.8^{ab}
	1,000	597	25.6^{a}	429^{a}	1.40^{c}		17.8^{ab}
2	0	731	28.6^{b}	493^{b}	1.49^{a}	37.7^{c}	n/a
	500	743	29.5^{a}	513^{a}	1.45^{b}	44.6^{b}	
	750	751	30.1^{a}	528^{a}	1.42^{bc}	51.4^{a}	
	1,000	741	29.9^{a}	523^{a}	1.42^{c}	53.6^{a}	

^{a,b,c}: Mean values within a trial and within a column with a different superscript are significantly different $P < 0.05$.
n/a = not analysed.

3.3.2.2. Efficacy in sows

The applicant submitted three trials to support the efficacy of the additive in sows. Two of them were short-term trials focused on the phosphorus utilisation either during the gestation[49] or the lactation[50] phase. In both cases, the collection period of faecal samples was shorter than the durations stipulated in the guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018; 2 days vs. 4–6 days), and no considerations were given to required endpoints (litter and sows' body weight). Therefore, none of these trials was considered further in the assessment.

The long-term trial aimed at assessing the zootechnical performance and phosphorus utilisation in both gestating and lactating sows.[51] In total, 80 Danbred sows (parity –5) were randomly allocated into four treatment groups balanced by parity and body weight (20 replicates/treatment). The trial lasted from approximately day 71 of gestation until weaning (day 26 post farrowing). From day 71 to 107 of gestation, sows from the same treatment were distributed in collective pens with automatic feeders. From day 108 of gestation until the end of the experiment, the sows were moved to individual farrowing crates. The gestation (from day 71 to 107 of gestation) and lactation (from day 108 of gestation until weaning) basal diets composed of barley, Optigrain,[52] rye and soya bean meal were either not supplemented (control diet) or supplemented with VTR-phytase powder to provide 500, 750 and 1,000 U/kg complete feed. The enzyme activity and the Ca/P content of the feeds were analysed for each experimental diet.[53] Overall, the experimental diets were provided in mash form for 70 days, on a predefined feeding regime based on the parity number, body weight, litter weight gain and physiological stage of the animals. All diets included an external marker for digestibility analysis. General health status of the sows and their litters was monitored daily, and the mortality (including culls) was recorded as it occurred, including the most likely cause of death. The body weight, back fat thickness and body condition score of the sows were measured at days 71 and 107 of gestation, and at days 1 (only body weight) and 26 post-farrowing, while individual feed intake was recorded daily during the whole experimental period. Regarding the reproductive parameters, the number and weight of piglets born alive, after cross-fostering and at weaning were recorded, as well as the creep feed intake (from day 9 to 26 of lactation). Cross fostering to sows within the same treatment was applied within the 24 h of birth. From day 32 to 35 (gestation phase) and from day 63 to 66 (lactation), faecal samples were collected and pooled per sow. Feed and faecal samples were analysed for dry matter, external marker and P, and the apparent total tract digestibility (ATTD) of P calculated. The experimental data were analysed with ANOVA, including the treatment as fixed effect. Mean groups were compared with Tukey test. Significance was set at 0.05. No sow was found dead or was removed during the trial. The mortality rate in piglets was 8.9, 7.3, 5.4 and 5.8% for the control, 500, 750

[49] Technical dossier/FAD-2021-0067/Section IV/Annex IV.2.2.
[50] Technical dossier/FAD-2021-0067/Section IV/Annex IV.2.3.
[51] Technical dossier/FAD-2021-0067/Section IV/Annex IV.3.1.
[52] 50% corn & 25% barley & 25% wheat, purified & heat treated (80°C).
[53] Gestation showing 274, 779, 1,180 and 1,270 U/kg feed, and lactation 192, 776, 1,130, 1,290 U/kg for the control, 500, 750 and 1,000 U/kg groups. P concentration was 0.37% in the gestation diets and 0.48 in the lactation diets. Ca concentration was 0.43–0.44% in the gestation diets and 0.59–0.63% in the lactation diets.

and 1,000 U/kg groups, respectively. The sows receiving the 6-phytase contained in the additive at 500 U/kg feed showed higher apparent total tract digestibility (ATTD) of P in comparison with the control diet both during the gestation and the lactation phase (see Table 7). No difference was observed in the zootechnical performance of sows and piglets in the treated groups in comparison with the control, except from a positive effect on litter weight at weaning in the group receiving 750 U/kg (see Table 8).

Table 7: Effects of VTR-phytase on the zootechnical performance of sows and piglets and the apparent total tract digestibility of sows during gestation and lactation

Groups	Total feed intake gestation	Total feed intake lactation	BW loss post farrowing	ATTD P gestation	ATTD P lactation	Litter weight at birth/after cross-fostering	Litter weight at weaning	Litter weight gain
U/kg feed	(Kg)	(kg)	(kg)	(%)	(%)	(kg)	(Kg)	(Kg)
0	144.9	175.5	13.6	44.6^{c}	41.8^{c}	20.9/17.6	80.4^{b}	62.7^{b}
500	144.8	176.3	14.1	49.5^{b}	46.0^{b}	20.6/17.3	84.2^{ab}	66.9^{ab}
750	145.0	177.3	11.8	53.2^{a}	48.8^{a}	21.3/17.6	92.4^{a}	74.8^{a}
1,000	145.0	178.8	13.2	53.9^{a}	49.3^{a}	20.3/17.4	86.3^{ab}	68.8^{ab}

^{a,b,c}: Mean values within a trial and within a column with a different superscript are significantly different $P < 0.05$.

3.3.2.3. Conclusions on efficacy

The Panel concludes that the additive has the potential to be efficacious in laying hens at 1,000 U/kg. Considering that the mode of action is well known and is expected to be the same between poultry species, the conclusions on laying hens are extrapolated to other birds for egg production or breeding. Due to the lack of sufficient data, the Panel is not in a position to conclude on the efficacy of the additive in weaned piglets, sows and chickens for fattening, and consequently, no conclusion can be reached in the efficacy of all pig species or growing poultry species.

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 Regulation[54] and Good Manufacturing Practice.

4. Conclusions

No viable cells and/or DNA of the production strain were detected in the final products. VTR phytase (liquid/solid) produced by *Komagataella phaffii* CGMCC 7.370 does not pose any safety concern regarding the production strain.

VTR phytase (liquid/solid) produced by *Komagataella phaffii* CGMCC 7.370 is safe for all Suidae and all avian species at the proposed conditions of use.

The use of both forms of the additive under assessment in animal nutrition under the conditions of use proposed is of no concern for consumer safety or for the environment.

The liquid VTR phytase and powder VTR phytase are considered to be non-irritant to skin or eyes but should be considered skin and respiratory sensitisers.

The additive has the potential to be efficacious in laying hens at 1,000 U phytase/kg complete feed. The conclusion can be extrapolated to other birds for egg production or breeding. The FEEDAP Panel cannot conclude on the efficacy of all pigs or growing poultry species.

[54] 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.

5. Documentation provided to EFSA/chronology

5.1. FAD-2021-0066

Date	Event
25/03/2021	Dossier received by EFSA. VTR-phytase liquid, VTR phytase powder (6-phytase) for all avian species, including ornamental, exotic and game birds. Submitted by Victory Enzymes GmbH.
21/04/2021	Reception mandate from the European Commission
16/07/2021	Application validated by EFSA – Start of the scientific assessment
18/10/2021	Comments received from Member States
21/10/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
16/11/2021	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 production strain/manufacturing process/ characterisation of the additive/safety for the user/efficacy*
06/07/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
23/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

5.2. FAD-2021-0067

Date	Event
26/03/2021	Dossier received by EFSA. VTR-phytase liquid, VTR-phytase powder (6-phytase) for all pigs. Submitted by Victory Enzymes GmbH
19/04/2021	Reception mandate from the European Commission
04/08/2021	Application validated by EFSA – Start of the scientific assessment
21/10/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
05/11/2021	Comments received from Member States
21/12/2021	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 production strain/ manufacturing process/ characterisation of the additive/safety for the user/efficacy*
06/07/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
23/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADG	average daily gain
ADI	acceptable daily intake
BW	body weight
CAS	Chemical Abstracts Service
CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
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
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development

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

In the current applications, authorisations 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 yeast *Komagataella phaffii*. Other preparations of 6-phytase from different *Komagataella phaffii* strains are currently authorised as feed additives.

The activity of 6-phytase is expressed in phytase units (U) where, according to the Applicant 'one U is the amount of enzyme needed to release one micromole of inorganic phosphorous per minute from 5.0 mmol/l sodium phytate solution at 37 °C and pH 5.5'. This definition is in agreement with the phytase activity unit as described in EN ISO 30024.

The product is marketed as two different formulations namely VTR-phytase powder and VTR-phytase liquid with a guaranteed minimum 6-phytase activity of 50000 U/g and 5000 U/g for the powder and liquid formulations, respectively. VTR-phytase is intended to be included through premixtures or directly in feedingstuffs to obtain a minimum recommended activity of 500 U/kg feedingstuffs for all the target species.

The Applicant proposed the ring-trial validated VDLUFA 27.1.4 and VDLUFA 27.1.3 methods for the quantification of the phytase activity, respectively, in the product (VTR-phytase) and premixtures and the ring-trial validated EN ISO 30024 method for the quantification of the phytase activity in feedingstuffs.

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.