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Safety and efficacy of the feed additive consisting of 6-phytase (produced by *Aspergillus oryzae* DSM 33699) (RONOZYME® Hiphos GT/L) for poultry, pigs for fattening, weaned piglets and sows (DSM Nutritional Products Ltd)

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Abstract

The additive RONOZYME® Hiphos (GT/L) contains 6-phytase produced with a genetically modified strain of the filamentous fungus *Aspergillus oryzae*, it is currently authorised for poultry, pigs for fattening, weaned piglets and sows. The applicant has requested to change the production strain, substituting strain *A. oryzae* DSM 22594 for *A. oryzae* DSM 33699. RONOZYME® Hiphos (GT/L), manufactured with the production strain *A. oryzae* DSM 33699, did not give rise to safety concerns with regard to the genetic modification of the production strain. No viable cells of the production strain nor its recombinant DNA were detected in an intermediate product representative of both final forms of the additive. RONOZYME® Hiphos (GT/L) was considered safe for poultry, pigs for fattening, weaned piglets and sows at the recommended inclusion levels of 500–4,000 FYT/kg complete feed. The use of RONOZYME® Hiphos GT and L manufactured with the production strain *A. oryzae* DSM 33699 raised no concerns for consumers. In the absence of data on the final formulations, the Panel could not conclude on the potential of the additive to be irritant to eyes or skin, or a skin sensitiser. Due to the proteinaceous nature of the active substance, the additive was considered a respiratory sensitiser. The additive manufactured by *A. oryzae* DSM 33699 raises no safety concerns for the environment. The additive has the potential to be efficacious in poultry, pigs for fattening, weaned piglets and sows at 500 FYT/kg complete feed.

Keywords: zootechnical additives, digestibility enhancers, 6-phytase, RONOZYME® Hiphos GT/L, safety, efficacy

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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 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States. In addition, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from DSM Nutritional Products Ltd represented in the EU by DSM Nutritional Products Sp. z o.o[2] for the modification of the current authorisation and renewal of the authorisation of the additive consisting of 6-phytase (produced by *Aspergillus oryzae* DSM 33699) (RONOZYME® Hiphos GT/L), when used as a feed additive for poultry, pigs for fattening, weaned piglets and sows (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 13(3) (modification of the authorisation of a feed additive) and under Article 14(1) (renewal of the authorisation). 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 10 November 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 feed additive consisting of 6-phytase (produced by *A. oryzae* DSM 33699) (RONOZYME® Hiphos GT/L), when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

The EFSA Panel on Additives and Products or Substances used in Animal feed (FEEDAP) issued three opinions on the safety and efficacy of RONOZYME® HiPhos produced by *A. oryzae* DSM 22594 when used as a feed additive for poultry, weaned piglets, pigs for fattening and sows (EFSA FEEDAP Panel, 2012a, 2012c) and for sows and fish (EFSA FEEDAP Panel, 2016).

The additive (4a18) is currently authorised for use in poultry, weaned piglets, pigs for fattening and sows.[3] The applicant is changing the production strain (substituting strain DSM 22594 of *A. oryzae* for strain DSM 33699 of the same species of filamentous fungi) with no other modification on the authorisation.

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[4] in support of the authorisation request for the use of 6-phytase (produced by *A. oryzae* DSM 33699) (RONOZYME® Hiphos GT/L) 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, peer-reviewed scientific papers, other scientific reports, to deliver the present output.

[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] DSM Nutritional Products Ltd, Wurmisweg 5,764,303 Kaiseraugst Switzerland, represented in the EU by DSM Nutritional Products Sp. z o.o., Tarczyńska 113 96–320 Mszczonów Poland.
[3] Commission Implementing Regulation (EU) No 837/2012 of 18 September 2012 concerning the authorisation of 6-phytase (EC 3.1.3.26) produced by *Aspergillus oryzae* (DSM 22594) as feed additive for poultry, weaned piglets, pigs for fattening and sows (holder of authorisation DSM Nutritional Products) and amendments. OJ L 252, 19.09.2012, p. 7.
[4] FEED dossier reference: FAD-2021-0039.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance in animal feed are valid and applicable for the current application.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of 6-phytase (produced by *A. oryzae* DSM 33699) (RONOZYME® Hiphos GT/L) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013), 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 FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (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), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

This assessment regards the renewal of the authorisation and a change in the production strain (substituting strain DSM 22594 of *A. oryzae* for strain DSM 33699 of the same species of filamentous fungi) for the product RONOZYME® Hiphos (6-phytase) when used as a zootechnical additive (functional group of digestibility enhancers) in poultry, weaned piglets, pigs for fattening and sows. The additive under assessment will be hereafter referred to as RONOZYME® Hiphos GT or RONOZYME® Hiphos L.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The 6-phytase present in the additive is obtained by fermentation with a genetically modified strain of *A. oryzae* which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH with the deposition number DSM 33699.[7] [redacted] (EFSA FEEDAP Panel, 2012a).

The taxonomic identification of the production strain as *A. oryzae* was achieved [redacted]

3.1.1.1. Information related to the genetically modified microorganism[8]

Characterisation of the recipient or parental microorganism

[redacted]

Characterisation of the donor organisms

[redacted]

[5] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2017-0021_en
[6] 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.
[7] Technical dossier/Section II/Annexes/Annex II-25.
[8] Technical dossier/Section II/Annexes/Annex II-27.

[redacted]

Description of the genetic modification

[redacted]

[9]

3.1.2. Manufacturing process

According to the applicant, the manufacturing process has not been substantially modified as compared to the one previously reported for the additive, with the exception of the change in the production strain (see above).

[redacted]

[9] Technical dossier/Supplementary information June 2022/DSM HiPhos SIn 2022–2 - Data package/DSM HiPhos SIn 2022–2 – Annex, reply to question 2.

[redacted][10]

3.1.3. Characterisation of the additive

The additive is currently authorised in two different formulations, solid and liquid, ensuring 10,000 and 20,000 FYT[11]/g additive, respectively. In previous applications, three formulations were described: RONOZYME® Hiphos M and GT (solid forms) and RONOZYME® Hiphos L (liquid form). The applicant provided data to characterise the solid form RONOZYME® Hiphos GT and the liquid form RONOZYME® Hiphos L obtained with the new strain. No data on the RONOZYME® Hiphos M formulation was provided in the context of the current assessment.

RONOZYME® Hiphos GT is an off-white granulated product which ensures a minimum activity of phytase of 10,000 FYT/g additive. The batch-to-batch variation of the enzyme activity was studied in five batches and the mean value was 10,760 FYT/g, ranging from 10,100 to 11,200 FYT/g.[12] This formulation contains 6-phytase concentrate (2%), cellulose (4%), dextrin (7%), sodium sulfate (86%) and water (1%).

RONOZYME® Hiphos L is a transparent yellow to light brown liquid form which ensures a minimum activity of phytase of 20,000 FYT/g additive. The batch-to-batch variation of the enzyme activity was studied in five batches and the mean value was 25,920 FYT/g, ranging from 24,900 to 27,100 FYT/g. It contains the liquid 6-phytase concentrate (4%), sodium benzoate (0.15%), potassium sorbate (0.05%), sorbitol (49.8%) and water (46%).

The applicant set specifications for chemical and microbiological contamination which include arsenic (< 3 mg/kg), cadmium (< 0.5 mg/kg), lead (< 5 mg/kg), mercury (< 0.5 mg/kg), total viable count (< 5×10^4 colony forming units (CFU)/g), total coliforms (< 30 CFU/g), *Escherichia coli* (not detected in 25 g) and *Salmonella* spp. (not detected in 25 g). Analytical data to confirm the specifications were provided for five batches of both forms of the additive, showing the following values:[14] arsenic, cadmium, mercury and lead were below their corresponding limit of detection (LOD); total viable count <100–200 and < 100–1.3×10^3 CFU/g for the solid and liquid form, respectively; total coliforms < 10 and < 4 CFU/g for the solid and liquid form, respectively; *E. coli* and *Salmonella* spp. were not detected in 25 g both for the solid and liquid form. Enterobacteriaceae were also measured and showed results < 10 CFU/g for the solid form and < 10–20 CFU/g for the liquid form.

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

The capacity of the production strain to produce substances with antimicrobial activity was not investigated. However, the potential presence of antimicrobial activity was tested in five batches of the final product formulations (GT and L) using a disk diffusion method and the following indicator strains: *Staphylococcus aureus* ATCC 6538, *E. coli* ATCC 11229, *Bacillus cereus* ATCC 2, *Bacillus circulans* ATCC 4516, *Streptococcus pyogenes* ATCC 12344 and *Serratia marcescens* ATCC 14041.[15] No antimicrobial activity was detected.

Some *Aspergillus* species are known to be capable of producing mycotoxins and other secondary metabolites. However, during the development of the production strain *A. oryzae* DSM 33699, [redacted], thereby eliminating the potential of the strain to produce them. Analysis of five batches of RONOZYME® Hiphos L and three batches of the 6-phytase liquid concentrate for the presence and quantification of β-nitropropionic acid (BNP) and kojic acid from *A. oryzae* were performed. Results showed values for BNP of < 0.28 and < 0.58 mg/kg, and for kojic acid of < 0.025 and < 0.032 mg/kg, for RONOZYME® Hiphos L and the 6-phytase liquid concentrate, respectively.[16]

[10] Technical dossier/Section II/Annexes/Annex II-27.
[11] One Phytase Unit (FYT) is defined as the amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute under reaction conditions with a phytate concentration of 5.0 mM at pH 5.5 and temperature 37°C.
[12] Technical dossier/Section II/Annexes/Annex II-14.
[13] Technical dossier/Section II/Annexes/Annex II-15.
[14] Technical dossier/Section II/Annexes/Annex II-14 and Annex II-15 and Supplementary information June 2022/DSM HiPhos SIn 2022–1 - Data package/FAD-2021-0039_Sin_130622/Appendix B. LOD in mg/kg were 0.3 for arsenic, 0.05 for cadmium and mercury, 0.5 for lead.
[15] Technical dossier/Section II/Annexes/Annex II-14 and Annex II-15 and Supplementary information September 2022/ Appendix A.
[16] Technical dossier/Section II/Annexes/Annex II-15 and Annex II-24 and Supplementary information June 2022/DSM HiPhos SIn 2022–1 - Data package/Appendix E. [redacted].

The presence of viable cells of the production strain was investigated in three batches of the liquid concentrate ( ) that is used to formulate the two final formulations.[8] No growth was detected.

The applicant provided two sets of data regarding the possible presence of recombinant DNA from the production strain in the additive. Both analyses were conducted using the liquid concentrate batches analysed for the presence of viable cells. No recombinant DNA was detected in the samples.

3.1.4. Physical properties of the additive

RONOZYME® Hiphos GT has a density of 1,170 g/m^3 (1,160–1,190 g/m^3). The dusting potential of five batches of the additive was determined using the Heubach I method and showed an average value of 9.9 mg/m^3 (range 5.5–18 mg/m^3) (mg airborne dust per m^3 of air).[17] The particle size distribution of the product was analysed in the same five batches by laser-diffraction method and showed that particles have a diameter in the range of 180–500 μm.

RONOZYME® Hiphos L has a density of 1.24 g/ml (1.24–1.25 g/ml), a pH of 4.6, and a viscosity of 36.6 cP (34–45 cP), measured in five batches.[13]

3.1.5. Stability and homogeneity

The enzyme itself (same 6-phytase), the manufacturing process and the composition of the two forms of the additive have not been modified. Therefore, the change in the production strain is not expected to have an impact on the shelf life and the stability and homogeneous capacity to distribute of the 6-phytase. Stability and homogeneity of both forms of the additive were addressed in previous opinions (EFSA FEEDAP Panel 2012a, 2012c and 2016). However, data on the shelf-life up to 3 months for the two forms of the additive obtained with *A. oryzae* DSM 33699 were submitted and are reported below.

The shelf-life of RONOZYME® Hiphos GT was evaluated in three batches when stored in glass vials for up to 8 weeks at 10°C, and up to 3 months at different temperatures: −18, 25, 30, 40°C. Recoveries were expressed as % of the activity of the sample kept at −18°C at the same time point. No losses were observed after 3 weeks at 10°C or after 3 months at 25°C. Recoveries were 95% and 69% for the samples kept at 30 and 40°C after 3 months, respectively.

Regarding RONOZYME® Hiphos L, three batches were stored in glass vials up to 3 months at −18, 25, 30 and 40°C. Recoveries (expressed as % of the activity of the sample kept at −18°C at the same time point) were 95, 88 and 68% for the samples kept at 25, 30 and 40°C after 3 months, respectively.

3.1.6. Conditions of use

RONOZYME® Hiphos (GT/L) produced by *A. oryzae* DSM 22594 is currently authorised for use in feed as a zootechnical additive for poultry, pigs for fattening, weaned piglets and sows at the recommended inclusion levels of 500 to 4,000 FYT/kg complete feed.

The applicant has not asked to modify these conditions of use but requested to substitute the current production strain (*A. oryzae* DSM 22594) with a new strain of the same species of filamentous fungi (DSM 33699).

The current authorisation foresees under other provisions:

- In the directions for use of the additive and premixture, indicate the storage temperature, storage life, and stability to pelleting.

[17] Technical dossier/Section II/Annexes/Annex II-14 and Supplementary information June 2022/DSM HiPhos SIn 2022–1 - Data package/Appendix F.

- Recommended dose per kilogram of complete feed for:
  - poultry, piglets (weaned) and pigs for fattening: 500–4,000 FYT,
  - sows: 500–4,000 FYT.
- For use in feed containing more than 0.23% phytin-bound phosphorus.
- For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from its use. Where those risks cannot be eliminated or reduced to a minimum by such procedures and measures, appropriate personal protective equipment should be used.
- For use in weaned piglets up to 35 kg.

3.2. Safety

The applicant performed a literature search in order to provide evidence that in the light of the current knowledge the additive remains safe under the approved conditions for the target species, consumers, users and the environment. The applicant searched in a total of three relevant databases (Web of Science Core Collection, Biosis Citation Index and Medline). The search was conducted without restrictions and the search terms and search strategy were provided.[18] The main search terms included the commercial name and the production organism in combination with the enzyme or terms relevant for consumers, users, target animals and the environment safety. The literature search retrieved 32 publications which were manually reviewed for their relevance for the safety for the target species, humans or the environment. Most of the publications (26) were excluded from the assessment because the safety of the product was not assessed. The other publications found did not report any safety issues. However, the outcome of this literature search is of limited relevance considering the modifications done in the production strain of the additive under assessment.

3.2.1. Safety of the production organism

The parental strain, [redacted] has a long history of use in enzyme production. The production strain *A. oryzae* DSM 33699 differed from the parental strain by expressing [redacted]. The introduced sequences raise no safety concern, [redacted]. No viable cells of the production strain nor its recombinant DNA were detected in an intermediate product representative of both final forms of the additive. The product RONOZYME® Hiphos (GT/L) manufactured with *A. oryzae* DSM 33699 does not give rise to safety concern with regard to the genetically modified production strain.

3.2.2. Toxicological studies

The applicant submitted genotoxicity studies and a subchronic oral toxicity study to support the safety of the additive, which are presented here below. All the toxicological studies were performed with 6-phytase produced by *A. oryzae* DSM 33699 (89,100 FYT/g) from which the two final RONOZYME® Hiphos formulations are obtained.[19] The test item is considered representative for the two final formulations of the additive.

3.2.2.1. Bacterial reverse mutation test

Test item was tested for the induction of reverse mutations in *Salmonella* Typhimurium tester strains TA98, TA100, TA1535 and TA1537 and in *Escherichia coli* WP2 *uvrA* (pKM101). The experimental protocol was in line with OECD testing guideline (TG) 471 and following the Good Laboratory Practice (GLP) principles. The test item was diluted in water purified by reverse osmosis and tested either in the presence or in absence of the exogenous metabolic activation system (S9 mix) based on microsomal fraction from the liver of rats treated with phenobarbital and 5,6-benzoflavone. The "treat and wash" (preincubation method), was performed. Bacterial cultures were exposed to the tested item for 90 min at 34 to 39°C with shaking. Seven concentrations of the test item expressed as TOS per mL of the bacterial culture were tested (5, 15, 50, 150, 500, 1,500 and 5,000 μg of TOS/mL)

[18] Technical dossier/Section III/Annexes/Annex III-7.
[19] Technical dossier/Supplementary information September 2022/DSM HiPhos SIn 2022–3 – Annex.

in two independent experiments. In the second experiment, the S9 mix content was increased from 10% (v/v) to 20% (v/v). Since 1 mL of the culture was exposed to any of the tested concentration of 6-phytase and plated, concentrations expressed in μg TOS/mL of culture may be considered as equals to μg TOS/plate. Appropriate positive controls were used for testing in the presence and in the absence of S9 mix, purified water was used as the solvent (negative) control. No precipitate or cytotoxicity towards the tester strains were observed at concentrations up to 5,000 μg of TOS/mL (i.e. 5,000 μg of TOS/plate) in either experiment. Positive controls induced significant increase in the number of revertant colonies regardless of the bacteria or strain, either in the presence or in the absence of S9 mix confirming the sensitivity of the tests and the efficacy of the S9 mix. No increase in the number of revertant colonies was observed in any strain and experimental condition. The Panel concluded that the test item did not induce gene mutations in bacteria under the experimental conditions applied in the study.

3.2.2.2. *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 whole blood human lymphocytes according to OECD TG 487 and following GLP principles.[20] In the report, test item concentrations were expressed in terms of total organic solids, i.e. correction was made for a TOS content of 11.7%. Based on a preliminary cytotoxicity test, the concentrations selected for the analysis of micronuclei were 1,000, 2,500 and 5,000 μg TOS/mL for the short exposure (3 h + 17 h of recovery) in the presence and absence of metabolic activation and 100, 1,000, 3,000 μg TOS/mL for the continuous exposure (20 h) in the absence of metabolic activation. Cytochalasin B was used to obtain binucleated cells. Cytotoxicity up to 55% compared to vehicle control values was induced by continuous treatment with 3,000 μg TOS/mL, with a concurrent statistically significant increase of micronuclei. Values were within the negative historical control range. These equivocal results were not confirmed by an additional analysis doubling the number of scored cells. Overall, no increase of the frequency of micronuclei was observed in binucleated cells in any experimental condition. The Panel concluded that the test item did not induce clastogenic and aneugenic effects in human lymphocytes under the experimental conditions employed in this study.

3.2.2.3. Subchronic oral toxicity study

RccHan™;WIST rats (10/sex/group) received the test item by oral gavage at dose levels of 10, 33, 100% of phytase giving doses of 0 (control), 122.0, 402.7 or 1,220.3 mg TOS/kg body weight (bw) per day (equivalent to 92,931, 306,673 or 929,313 FYT/kg bw per day) for 90 consecutive days.[21] The study was conducted in compliance with OECD TG 408. No treatment-related clinical signs were observed. All animals survived the treatment period. No effects were observed on survival, behaviour, body weight, feed intake, haematology, clinical parameters, gross pathology and histological examination. From this study, a no observed adverse effect level (NOAEL) of 1,220.3 mg TOS/kg bw/day (equivalent to 929,313 FYT/kg bw per day), the highest dose tested, was identified.

3.2.2.4. Conclusions on the toxicological studies

The FEEDAP Panel concludes that the 6-phytase used for the formulation of the additive showed no genotoxicity potential. Moreover, the results obtained in a subchronic oral toxicity study raised no concerns regarding the product and allowed to derive a NOAEL of 929,313 FYT/kg bw per day.

3.2.3. Safety for the target species

The safety of RONOZYME® Hiphos (GT/L) produced by *A. oryzae* DSM 22594 for the target species was evaluated in a previous opinion (EFSA FEEDAP Panel, 2012a). Based on the results obtained, the FEEDAP Panel concluded that the additive is safe for chickens and turkeys for fattening, laying hens, weaned piglets and sows at 4,000 FYT/kg complete feed. The conclusions were extended to all poultry species and pigs for fattening.

The product under assessment shares with the previous product the manufacturing process, the composition and the enzyme 6-phytase. The production strains *A. oryzae* DSM 33699 (new) and *A. oryzae* DSM 22594 (previous) derive from the same parental strain. In addition, the new production strain contains several deletions in genes and pathways of concern. Nevertheless, in order to support

[20] Technical dossier/Section III/Annexes/Annex III-18.
[21] Technical dossier/Supplementary information June 2022/DSM HiPhos SIn 2022–2 - Data package/Appendix A.

the safety of the additive for the target species, the applicant referred to the 90-day toxicity study that has been described above (see Section 3.2.2). The NOAEL identified (929,313 FYT/kg bw per day) was used to calculate the maximum safe level in feed for the different target species in accordance with the procedure described in the Guidance on the safety for the target species (EFSA FEEDAP Panel, 2017b). The calculated maximum safe concentrations in feed are presented in Table 1.

Table 1: Maximum safe concentration of the additive in feed

	Body weight (kg)	Feed intake (kg DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe concentration (FYT/kg feed)(1)
Chicken for fattening	2	0.158	79	103,518
Turkey for fattening	3	0.176	59	139,396
Laying hen	2	0.106	53	154,301
Pig for fattening	60	2.20	37	223,035
Piglet	20	0.88	44	185,863
Lactating sow	175	5.28	30	271,050

DM: dry matter; bw: body weight; FYT: One Phytase Unit.
(1): Complete feed containing 88% dry matter, milk replacer 94.5% dry matter.

The maximum safe levels obtained are higher than the maximum recommended use level of 4,000 FYT/kg complete feed for the intended target species. Therefore, the FEEDAP Panel concludes that RONOZYME® Hiphos (GT/L) produced by *A. oryzae* DSM 33699 is safe for poultry, pigs for fattening, weaned piglets and sows at the proposed conditions of use.

3.2.4. Safety for the consumer

The results obtained with the 6-phytase produced by *A. oryzae* DSM 33699, which is representative of the final formulations of the additive, in the genotoxicity studies and in the sub-chronic oral toxicity study do not indicate any reason for concern for consumer safety arising from the use of the product as feed additive. The Panel considers that the substances added during the formulation of the additive are not of concern for the consumer.

3.2.5. Safety for the user

The studies presented under this section were performed with the 6-phytase obtained with *A. oryzae* DSM 33699. The final formulations of the additive were not tested.

3.2.5.1. Effect on respiratory system

The dusting potential of the solid form of the additive was up to 0.018 g/m^3, therefore the exposure through inhalation is considered unlikely. However, based on 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 skin irritation potential of the 6-phytase was investigated using an *in vitro* skin irritation test performed according to OECD TG 439 and following the principles of GLP.[22] The results of the study demonstrated that the test item is not a skin irritant.

The eye irritation potential of the 6-phytase was investigated using an *in vitro* eye irritation test performed according to OECD TG 437 and following the principles of GLP.[23] The results of this test showed that the test item is not an eye irritant.

No information on skin sensitisation potential was provided, therefore the FEEDAP Panel cannot conclude on the skin sensitisation potential of the additive.

[22] Technical dossier/Section III/Annexes/Annex III-22.
[23] Technical dossier/Section III/Annexes/Annex III-21.

3.2.5.3. Conclusions on safety for the user

The Panel notes that the final formulations were not tested. Based on the studies provided, the test item (6-phytase) is not a skin or eye irritant. In the absence of data on the final formulations, the Panel cannot conclude on the potential of the additive to be 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.

3.2.6. Safety for the environment

Viable cells of the production strain and its recombinant DNA were not detected in an intermediate product representative of both final forms of the additive. The additive does not raise safety concerns for the environment with regard to the genetic modification of the production strain. The active substance of the additive is a protein, and as such will be degraded/inactivated during passage through the digestive tract of animals or in the environment. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy

The production strain DSM 33699 of the product under evaluation is different from the previous strain DSM 22594 of *A. oryzae* (see Section 3.1.1). However, the enzyme produced by the two strains shares the same amino acid sequence. Consequently, the efficacy results obtained testing the 6-phytase produced using *A. oryzae* DSM 22594 assessed in previous opinions (EFSA FEEDAP Panel, 2012a and 2016) are considered applicable to the efficacy of the 6-phytase produced with *A. oryzae* DSM 33699. The FEEDAP Panel concludes that the additive is efficacious in poultry, pigs for fattening, weaned piglets and sows at 500 FYT/kg complete feed.

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[24] and Good Manufacturing Practice.

4. Conclusions

RONOZYME® Hiphos (GT/L), manufactured with the production strain *A. oryzae* DSM 33699, does not give rise to safety concern with regard to the genetic modification of the production strain. No viable cells of the production strain nor its recombinant DNA were detected in an intermediate product representative of both final forms of the additive.

RONOZYME® Hiphos (GT/L) is considered safe for poultry, pigs for fattening, weaned piglets and sows at the recommended inclusion levels of 500–4,000 FYT/kg complete feed.

The use of RONOZYME® Hiphos GT and L manufactured with the production strain *A. oryzae* DSM 33699 raises no concerns for consumers.

In the absence of data on the final formulations, the Panel cannot conclude on the potential of the additive to be 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 additive raises no concerns to the environment.

The additive has the potential to be efficacious in poultry, pigs for fattening, weaned piglets and sows at 500 FYT/kg complete feed.

5. Documentation provided to EFSA/Chronology

Date	Event
19/03/2021	Dossier received by EFSA. RONOZYME® HiPhos (6-phytase) for poultry, pigs for fattening, piglets (weaned) and sows. Submitted by DSM Nutritional Products Ltd.
26/04/2021	Reception mandate from the European Commission
10/11/2021	Application validated by EFSA – Start of the scientific assessment

[24] 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.

Date	Event
10/02/2022	Comments received from Member States
10/03/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: general, characterisation, user safety, efficacy*
28/03/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: general, characterisation, consumer safety*
13/06/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
06/09/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: general, characterisation, consumer safety*
22/09/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
22/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

bw	body weight
CFU	colony forming unit
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal feed
LOD	limit of detection