Safety and efficacy of RONOZYME® HiPhos (6-phytase) as a feed additive for sows and fish

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

RONOZYME® HiPhos is a preparation of 6-phytase which is authorised in the EU for use in poultry and pigs. The applicant requested to lower the authorised dose in sows and for a new use of the product as a feed additive for fish. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered that the conclusions reached in previous opinions regarding the safety for the consumer, user and environment would not be affected by the current application. The Panel concluded that the additive is safe for the consumer and the environment; it is not an irritant to skin or eye but it should be treated as a skin sensitisier. The Panel considered that the current request would not affect previous conclusions in this regard. Considering the tolerance for target species, the FEEDAP Panel also evaluated in a previous opinion the safety for the target species, including a tolerance trial carried out with sows and concluded that the additive is safe at the dose of 4,000 FYT (phytase units)/kg. The proposed reduction in the minimum recommended dose would not affect that conclusion. On the basis of results obtained in a tolerance trial carried out with rainbow trout, the Panel concluded that the additive is safe for fish at the dose of 2,000 FYT/kg feed. The demonstration of the efficacy of phytases can be supported by three short-term studies provided that digestibility of phytate/total P and partial or total P retention is measured. In the case of sows and considering welfare aspects, study of the digestibility may suffice. Four short-term trials in sows showed that the supplementation of the diet with RONOZYME® HiPhos increases apparent faecal digestibility of phosphorus at 500 FYT/kg feed; the Panel concluded that the additive is efficacious at that dose. Five short-term trials were submitted to support the efficacy in fish; however, none of the studies provided evidence on the partial or total retention of phosphorus and consequently the Panel could not conclude on the efficacy of RONOZYME® HiPhos as a feed additive for fish.

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Keywords: zootechnical additive, 6-phytase, safety efficacy, sows, fish

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Correspondence: feedap@efsa.europa.eu
**Panel members:** Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Koubia, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester

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Summary

Following a request from the European Commission (EU), the 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 RONOZYME® HiPhos (6-phytase) as a feed additive for sows and fish. This feed additive, available in three forms (one liquid and two solid), is authorised in the EU for use in poultry species and pigs. In 2012, the FEEDAP Panel issued an opinion on the safety and efficacy of RONOZYME® HiPhos as a feed additive for poultry and pigs which considered the safety and efficacy aspects of the additive regarding the target species (poultry and pigs), the safety for the consumer, the user, the environment and the genetic modification of the production strain. A second opinion on another formulation of the additive was also adopted.

The applicant has requested to modify the terms of authorisation of the product regarding the use in sows and to extend the authorisation for the use of the additive in fish (salmonids, sea bream and tilapia). The additive is to be used in feed for sows (for reproduction and in order to have benefit in piglets) at a dose range from 500 to 4,000 FYT (phytase units)/kg feed and in feed for fish (salmonids, sea bream and tilapia) at a dose range from 500 to 2,000 FYT/kg feed.

Safety aspects regarding the use of this additive in feed including the safety of the genetic modification of the production strain, the safety for the consumers, for the users and for the environment were established in 2012. The Panel considered that the current request would not affect previous conclusions in this regard.

Regarding the tolerance for target species, in 2012, the FEEDAP Panel evaluated a tolerance trial carried out with sows and concluded that the additive is safe at the dose of 4,000 FYT/kg. As this is the maximum recommended dose, the conclusion is still valid. On the basis of results obtained in a tolerance trial carried out with rainbow trout, the Panel concludes that the additive is safe for fish reared for flesh production at the maximum dose.

The demonstration of the efficacy of phytases can be supported by three short-term studies provided that digestibility of phytate/total P and partial (e.g. bone ash/P) or total P retention are included as end points. Considering welfare aspects in the case of sows (gestating or lactating), study of the digestibility may suffice. Four short-term trials carried out in sows and five carried out in fish were submitted in order to support the efficacy. In the four trials carried out in sows, the supplementation of the diet with RONOZYME® HiPhos resulted in a higher apparent faecal digestibility of phosphorus at the dose of 500 FYT/kg feed. Therefore, the Panel concluded that the additive is efficacious for sows at that dose. The five trials carried out in fish studied the apparent faecal digestibility of phosphorus but did not provide evidence on the partial or total retention of phosphorus and consequently cannot support the efficacy of the product. The Panel cannot conclude on the efficacy of RONOZYME® HiPhos as a feed additive for fish.
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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 a feed additive shall submit an application in accordance with Article 7. Also, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of 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.

The European Commission received a request from DSM Nutritional Products Ltd (Switzerland)\(^2\) for a modification of the terms of authorisation when used as a feed additive for sows (for reproduction and in order to have benefits in piglets) and a new use in fish (salmonids, tilapia, sea bream and shrimps) of the product RONOZYME® HiPhos, 6-phytase (category: zootechnical additive; functional group: digestibility enhancers). During the course of the assessment the applicant informed EFSA on its intention to withdraw the application on shrimps.

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) and under Article 13(3) (modification of the authorisation of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.\(^3\) The particulars and documents in support of the application were considered valid by EFSA as of 1 August 2014.

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 RONOZYME® HiPhos (6-phytase) when used under the newly proposed conditions of use for sows and when used in the new target species (see Section 3.1.2).

1.2. Additional information

RONOZYME® HiPhos is a preparation of 6-phytase produced by a genetically modified strain of *Aspergillus oryzae* (DSM 22594). This feed additive, available in three forms (one liquid (L) and two solid (M and GT)), is authorised in the EU for use in poultry, weaned piglets, pigs for fattening and sows.\(^4,5\) The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of RONOZYME® HiPhos (M and L) as a feed additive for poultry, weaned piglets, pigs for fattening and sows (EFSA FEEDAP Panel, 2012a). This opinion considered the safety and efficacy aspects of the additive regarding the target species, the safety for the consumer, the user, the environment and the genetic modification of the production strain. A second opinion on another form of the additive (GT) was also adopted in 2012 (EFSA FEEDAP Panel, 2012c).

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\(^{2}\) DSM Nutritional Products Ltd (Switzerland) represented by DSM Nutritional Products Sp. z o.o. Poland, Tarczynska 113, PL 96-320 Mszczonow, Poland.

\(^{3}\) EFSA Dossier reference: FAD-2014-0008.


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\(^6\) in support of the request on the modification of the authorisation and new use of RONOZYME\(^{\circledR}\) HiPhos as a feed additive for sows and fish. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008 and the applicable EFSA guidance documents.

The European Union Reference Laboratory considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.\(^7\)

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy RONOZYME\(^{\circledR}\) HiPhos is in line with the principles laid down in Regulation (EC) No 429/2008\(^8\) and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012b) and technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

3. Assessment

3.1. Characterisation

3.1.1. Shelf-life

The applicant has submitted new data on the shelf-life of the different formulations at different temperatures and longer periods of storage than the ones previously reported.

The shelf-life was measured in three batches of each formulation.\(^10\) The initial mean enzyme activity was 13,000 FYT/g for the GT formulation, 62,000 FYT/g for the M formulation and 25,000 FYT/g for the L formulation. Samples were stored in sealed containers at 10, 25, or 35°C for up to 24 months or at 40 and 50°C for up to 12 months. Samples of the two solid formulations were also kept in open containers at 40°C/60% RH for 3 or 1 month for GT and M forms, respectively. Enzyme activity recoveries for the GT/M/L formulations after 18 months stored at 10, 25 and 35°C were 103/97/97%, 100/90/79% and 72/61/51%, respectively. The corresponding figures after 24 months were 93/99/100%, 86/84/69% and 54/57/35%, respectively. The recoveries for the samples stored at 40/50°C were, as expected, lower than the ones obtained at lower temperatures. The GT and M formulations would comply with the minimum specifications of 10,000 and 50,000 FYT/g, respectively, after 24 months when stored at temperatures of up to 25°C. The L formulation would comply with the minimum specifications of 20,000 FYT/g after 24 months when stored at 10°C.

3.1.2. Conditions of use

The additive is to be used in feed for sows at a dose range of 500–4,000 FYT/kg feed and in fish (salmonids, sea bream and tilapia) at a dose range of 500–2,000 FYT/kg feed.

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\(^{\circledR}\) FEED dossier reference: FAD-2014-0008.

\(^7\) The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0019.pdf


\(^9\) FYT: phytase units, 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.

\(^{10}\) Technical dossier/Section II/Annexes II.31, 32 and 33.
3.2. Safety

Safety aspects regarding the use of this additive in feed including the safety of the genetic modification of the production strain, the safety for the consumers, for the users and for the environment have been previously evaluated (EFSA FEEDAP Panel, 2012a,c). The FEEDAP Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected. Considering the safety for the user, it was concluded that the additive is not an irritant to skin or eye but should be treated as a skin sensitiser.

The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously. Moreover, the FEEDAP Panel considers that the new use and the proposed modifications in the minimum recommended dose for sows requested by the applicant would not modify the above conclusions.

In 2012, the FEEDAP Panel also evaluated the safety for the target species, including a tolerance trial carried out with sows and concluded that the additive is safe at the dose of 4,000 FYT/kg. The proposed reduction in the minimum recommended dose would not affect that conclusion.

As the application covers the use of the additive in fish, the applicant provided one tolerance study in rainbow trout.

3.2.1. Safety for fish

A total of 525 female juvenile rainbow trout (Onchorhyncus mykiss, generation E10SPR; initial body weight 42 ± 1.1 g) were distributed in groups of 35 fish to 15 tanks and allocated to three dietary treatments (five replicate tanks per treatment).11 A basal diet based on fish meal, fish and rapeseed oil and wheat meal (total P content 11.0 g/kg feed) was either not supplemented (control) or supplemented with RONOZYME® HiPhos (L) to provide phytase at 2,000 (1 × maximum recommended dose) or 200,000 (100 ×) FYT/kg feed. Phytase activity was confirmed by analysis. Feed in pelleted form was provided twice daily for 58 days. Feed was offered restrictively according to the body weight of the fish, from 1.52% of the body weight at the start of the experiment to 1.35% at the very end of the experimental feeding period. Fish were weighed individually on days 0, 29, and 58 of the experiment, and weight gain, specific growth rate (% body weight per day) and feed to gain ratio were calculated. Survival of fish was monitored. At day 58 (end) of the experiment, eight fish per tank (40 fish per treatment) were individually weighed, length measured, externally examined and then dissected for gross pathology examination of the following: ‘global viscera’, gut, liver, bile gladder and spleen. Liver was weighed and condition factor (100 × body weight/length3) and liver-somatic index (100 × liver weight/body weight) were determined. The liver of one fish from every tank (five liver samples per treatment) was processed for histopathological analysis. An analysis of variance (ANOVA) was performed on the data obtained, and mean values were compared with the Newman–Keuls test. For data not normally distributed, a Kruskal–Wallis test was performed. Differences were considered significant at a level of at least p < 0.05.

The temperature of the water was 14.7°C and no mortality was recorded during the study. There were no feed refusals during the experimental period. Phytase addition at any dose increased significantly body weight (136, 139 and 144 g for control, 1 × and 100 ×, respectively), specific growth rate (2.0, 2.1 and 2.1% body weight per day), and reduced feed to gain ratio (0.72, 0.70 and 0.67). The results of the 100 × showed an improvement in these parameters as compared with the 1 ×. Macroscopic and histopathological (liver only) observation showed no abnormalities related to the dietary treatments. The supplementation of the experimental diets with RONOZYME® HiPhos (L) at up to 100 × the maximum recommended dose did not have a negative effect on the performance and health of juvenile rainbow trout. Therefore, the Panel concludes that the additive is safe for young fish for flesh production at the maximum recommended dose of 2,000 FYT/kg feed.

3.3. Efficacy

The demonstration of the efficacy of phytases can be supported by three short-term studies provided that digestibility of phytate/total P and partial (e.g. bone ash/P) or total P retention are

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11 Technical dossier/Section III/Annex III.2 and Supplementary information March 2015.
included as end points (EFSA FEEDAP Panel, 2012b). Considering welfare aspects in the case of sows (gestating or lactating), study of digestibility may suffice.¹²

3.3.1. Efficacy for sows

Four short-term trials were submitted, two carried out in the gestation phase and two carried out during lactation.

3.3.1.1. Gestating sows

One of the trials performed in the gestation phase had been evaluated by EFSA in 2012 (EFSA FEEDAP Panel, 2012a) and the Panel concluded that the phytase increased significantly the apparent faecal digestibility of phosphorus at the dose of 500 FYT/kg feed.¹³

In the second trial performed in gestating sows, a total of 24 sows (hybrid line EUROC, parity number 2 or 3), individually kept, were allocated to two dietary treatments (representing 12 replicates per treatment).¹⁴ Prior to the start of the study, sows received a diet with a low phosphorus content (2.4 g/kg feed) from day 56 to day 67 of gestation. The dietary treatments were offered from day 68 to day 79 of gestation. A basal diet based on maize and soybean meal with a total phosphorus content of 2.4 g/kg feed (calcium 7.1 g/kg feed) was either not supplemented (control) or supplemented with RONOZYME® HiPhos (GT) to provide phytase at 500 FYT/kg feed (confirmed by analysis). A total of 2.5 kg feed per day was offered to sows in pelleted form. The feeds contained chromium oxide as external marker. An adaptation period to the diet of 7 days was followed by 5 days of collection of faeces (days 75–79 of gestation). Faeces were obtained twice daily by rectal grab sampling. Feed and faeces were analysed for the marker, ash, calcium and phosphorus in order to determine the digestibility (data shown for phosphorus only). Feed intake of the sows, body weight and consistency of the faeces were measured during the trial (data not shown). An ANOVA was performed with the data obtained. Phytase addition increased significantly the apparent faecal digestibility of phosphorus (p < 0.001; 31.0% vs 42.5%).

3.3.1.2. Lactating sows

In the first trial in lactating sows, 32 lactating sows (Duroc × Landrace, parity 2–5) were housed individually and allocated to two dietary treatments (representing 16 replicates per treatment).¹⁵ The trial started on day 7 of lactation and ended on day 21. A basal diet based on maize, full-fat extruded soybean and soybean meal with a total phosphorus content of 5.3 g/kg (calcium 9.5 g/kg feed) was either not supplemented (control) or supplemented with RONOZYME® HiPhos (GT) to provide 500 FYT/kg feed (confirmed by analysis). Feed was offered ad libitum in pelleted form and contained titanium oxide as external marker. An adaptation period of 11 days was followed by 3 days of spot collection of faeces (day 19 until day 21 of lactation). Feed and faeces were analysed for the marker, dry matter, ash, calcium and phosphorus (data shown for phosphorus only). Feed intake of the sows was monitored throughout the study. Initial and final body weight of the sows (weight loss was calculated) and the number of piglets at the beginning of the study were reported. An ANOVA was performed with the data obtained. Phytase addition increased significantly the apparent faecal digestibility of phosphorus (p < 0.001; 27.5% vs 38.7%). There were no significant treatment-related effects on body weight loss (30 kg on average) and daily feed intake (6 kg/day on average) of sows.

In the second study in lactating sows, 45 sows (Large White × Landrace, parity 1–6) were housed individually and allocated to three dietary treatments (representing 15 replicates per treatment).¹⁶ The trial started on day 2 of lactation and ended on day 15. A basal diet based on wheat and soybean meal with a total phosphorus content of 5.9 g/kg feed (calcium 11.6 g/kg) was either not supplemented (control) or supplemented with RONOZYME® HiPhos (GT) to provide 500 FYT/kg feed (confirmed by analysis). A positive control diet with a total phosphorus content of 8.6 g/kg (calcium 12.9 g/kg) was also considered. Feed was offered ad libitum in pelleted form and contained titanium dioxide as external marker. Faecal samples were collected on days 8 and 15, once per day and by spot sampling, and analysed separately. Feed and faeces were analysed for the external marker, gross

¹³  Technical dossier/Section IV/Annex IV.2.
¹⁴  Technical dossier/Section IV/Annex IV.4 and Supplementary information March 2015.
¹⁵  Technical dossier/Section IV/Annex IV.1 and Supplementary information March 2015.
¹⁶  Technical dossier/Section IV/Annex IV.3 and Supplementary information March 2015.
energy, dry matter, crude protein, ash, calcium and phosphorus (data shown for phosphorus only). Feed intake of the sows was recorded daily. Sows’ body weight and back-fat thickness were measured on days 1 and 15 of lactation. On the same days, blood samples were collected to determine plasma metabolites, including plasma phosphorus. Litter weight was measured on days 1, 8 and 15 of age. Weight gain of piglets was used to estimate sow’s milk production. An ANOVA was performed with the data obtained. Phytase addition increased significantly the apparent faecal digestibility of phosphorus on day 8 and 15 as compared to the control diet (day 8 (p = 0.044): 36.0% vs 42.1% and day 15 (p < 0.001): 33.9% vs 46.0%). There were no significant treatment-related effects (p > 0.05) on sows’ body weight loss (~10 kg), daily feed intake (5 kg/day), daily milk production (10 kg/day), back-fat thickness loss (~2 mm loss), litter weight gain from day 1 to day 15 (32 kg on average).

3.3.1.3. Conclusion on efficacy for sows

The FEEDAP Panel concludes that RONOZYME® HiPhos is efficacious at the dose of 500 FYT/kg feed in improving the faecal apparent phosphorus digestibility in the category of sows as currently authorised.

3.3.2. Efficacy for fish

Five trials were submitted with young fish reared for flesh production, three carried out with rainbow trout (O. mykiss), one with Nile tilapia (Oreochromis niloticus) and one with gilthead seabream (Sparus aurata). The five trials provided studied the apparent faecal digestibility of phosphorus but did not provide evidence on the partial or total retention of phosphorus and consequently cannot support the efficacy of the product. The Panel cannot conclude on the efficacy of RONOZYME® HiPhos as a feed additive for fish.

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 and Good Manufacturing Practice.

4. Conclusions

The reduction in the dose proposed for sows would not change the previous conclusions regarding the safety for sows, consumer, user and environment. The Panel concluded that the additive is safe for sows, consumer and the environment; it is not an irritant to skin or eye but it should be treated as a skin sensitiser.

The additive is safe for fish at the maximum recommended dose. The Panel considers that the extension of use to fish would not introduce any safety concerns for consumers, users and the environment not already considered before.

The additive is efficacious in increasing the apparent faecal phosphorus digestibility in sows at the minimum recommended dose (500 FYT/kg feed).

The Panel cannot conclude on the efficacy of RONOZYME® HiPhos as a feed additive for fish.

5. Documentation provided to EFSA

1) RONOZYME® HiPhos for fish and sows. February 2014. Submitted by DSM Nutritional Products Ltd.
2) RONOZYME® HiPhos for fish and sows. Supplementary information. March 2015. Submitted by DSM Nutritional Products Ltd.
3) RONOZYME® HiPhos for fish and sows. Supplementary information. December 2015. Submitted by DSM Nutritional Products Ltd.
4) Comments from Member States.

17 Technical dossier/Section IV/Annexes 5–9.
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