SCIENTIFIC OPINION

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Safety and efficacy of RONOZYME® NP (6-phytase) as a feed additive for pigs for fattening

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

Abstract

The additive RONOZYME® NP is a preparation of 6-phytase produced by a genetically modified strain of *Aspergillus oryzae*. This product is authorised in the European Union as a feed additive for poultry, weaned piglets, pigs for fattening and sows. The authorisation of RONOZYME® NP for use in pigs for fattening is at a minimum dose of 1,500 FYT/kg feed (recommended range 1,500–3,000 FYT/kg feed). The applicant requested to modify the terms of the authorisation for this species/category of animals, by lowering the minimum recommended dose from 1,500 to 1,000 FYT/kg feed. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded in previous assessments that the additive is safe for the target species, the consumers and the environment, and that the solid forms of the additive are regarded as dermal and eye irritants and all forms are assumed to be respiratory sensitisers. The reduction in the dose proposed would not change the previous conclusions regarding the safety for the target animals, consumer, user and environment. Three trials carried out with growing pigs were submitted in order to support the efficacy at the new recommended dose. In all the trials faecal apparent digestibility of phosphorus was measured, retention was measured only in one trial. The demonstration of efficacy for a phytase requires three studies showing positive and significant effects on the retention of phosphorus. In the trials submitted, the phosphorus retention was studied only in one trial; consequently the efficacy at the newly recommended dose was not demonstrated.

Keywords: zootechnical additive, 6-phytase, efficacy, pigs for fattening

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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 Kouba, 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, 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 RONOZYME® NP (6-phytase) as a feed additive for pigs for fattening. The opinion should consider the modification of the terms of the authorisation proposed by the applicant.

RONOZYME® NP is authorised in the European Union as a feed additive for poultry, weaned piglets, pigs for fattening and sows. The authorisation of RONOZYME® NP for use in pigs for fattening is at a minimum dose of 1,500 FYT/kg feed (recommended range 1,500–3,000 FYT/kg feed). The applicant requested to modify the terms of the authorisation for this species/category of animals, by lowering the minimum recommended dose from 1,500 to 1,000 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 have been previously established. The Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected. Considering the safety for the user, the solid forms of the additive are regarded as dermal and eye irritants and all forms are assumed to be respiratory sensitisers. The reduction in the dose proposed would not change the previous conclusions regarding the safety for the consumer, user and environment.

In a previous opinion, the FEEDAP Panel evaluated also a tolerance trial carried out with weaned piglets. Considering the results of that trial and the well-established mode of action of phytases, the FEEDAP Panel concluded that RONOZYME® NP is safe for pigs for fattening at the dose of 3,000 FYT/kg feed. The proposed reduction in the minimum recommended dose would not affect that conclusion.

Three trials carried out with growing pigs were submitted in order to support the efficacy at the dose of 1,000 FYT/kg. In all the trials, faecal apparent digestibility of phosphorus was measured and retention was measured only in one trial. The demonstration of efficacy for a phytase requires three studies showing positive and significant effects on the retention of phosphorus. In the trials submitted, the phosphorus retention was studied only in one trial. The FEEDAP Panel considers that phosphorus retention is the key end-point for the efficacy assessment of phytases. In the absence of this parameter in two of the three efficacy studies assessed, the FEEDAP Panel cannot conclude on the efficacy of the product at the newly proposed minimum recommended dose.

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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.

The European Commission (EC) received a request from DSM Nutritional Products Ltd (Switzerland)[2] for a modification of the terms of the authorisation of the product RONOZYME® NP, 6-phytase, when used as a feed additive for pigs for fattening (category: zootechnical additive; functional group: digestibility enhancers and substances which favourably affect the environment). The modification of the conditions of use consists in lowering the minimum recommended dose from 1,500 to 1,000 FYT/kg feed. The maximum recommended dose is not modified 3,000 FYT/kg feed.

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). 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 30 July 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® NP (6-phytase) when used under the newly proposed conditions of use.

1.2. Additional information

The additive RONOZYME® NP is a preparation of 6-phytase (phytase, EC 3.1.3.26) produced by a genetically modified strain of *Aspergillus oryzae* (DSM 17594). EFSA delivered an opinion on the safety and efficacy of RONOZYME® NP when used as a feed additive for chickens for fattening (EFSA, 2008), which included the assessment of the safety for the consumer, the user and the environment as well as the safety aspects of the genetic modification of the production strain. EFSA also delivered opinions on the safety and efficacy of RONOZYME® NP when used as a feed additive for poultry, piglets and pigs for fattening (EFSA, 2009) and for sows (EFSA FEEDAP Panel, 2010). This product is authorised in the European Union as a feed additive for poultry, weaned piglets, pigs for fattening[3] and sows.[4] The authorisation of RONOZYME® NP for use in pigs for fattening is at a minimum dose of 1,500 FYT/kg feed (recommended range 1,500-3,000 FYT/kg feed).

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[5] in support of the request on the modification of the authorisation of RONOZYME® NP as a

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] DSM Nutritional Products Ltd (Switzerland) represented by DSM Nutritional Products Sp. z o.o. Poland, Tarczynska 113, PL 96-320 Mszczonow, Poland.
[3] Commission Regulation (EC) No 1088/2009 of 12 November 2009 concerning the authorisation of a new use of an enzyme preparation of 6-phytase produced by *Aspergillus oryzae* (DSM 17594) as a feed additive for weaned piglets, pigs for fattening, poultry for fattening and poultry for laying (holder of authorisation DSM Nutritional Products Ltd., represented by DSM Nutritional Products SP. Z.o.o.). OJ L 297, 13.11.2009, p. 6.
[4] Commission Regulation (EU) No 999/2010 of 5 November 2010 concerning the authorisation of 6-phytase (EC 3.1.3.26) produced by *Aspergillus oryzae* (DSM 17594) as a feed additive for sows (holder of authorisation DSM Nutritional Products Ltd). OJ L 290, 6.11.2010, p. 24.
[5] FEED dossier reference: FAD-2014-0017.

feed additive for pigs for fattening. 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.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy RONOZYME® NP is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012) and technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

3. Assessment

3.1. Characterisation

The additive is available in three forms, two solid (CT and M) and one liquid (L). These formulations were characterised in the previous opinions (EFSA, 2008 and 2009). The minimum phytase activity for CT, M and L forms is 10,000, 50,000 and 20,000 FYT[8]/g, respectively.

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 claimed by the applicant for the solid formulations is of 24 months at room temperature and for the liquid is of 18 months at room temperature. The shelf-life was measured in three batches of each formulation. The initial mean enzyme activity was 13,200 FYT/g for the CT formulation, 67,333 FYT/g for the M formulation and 28,718 FYT/g for the L formulation. Samples were kept in sealed containers at 10, 25 or 35°C for 24 months or at 40 and 50°C up to 12 months.[9] Samples of the two solid formulations were kept also in open containers at 40°C/60% RH for 3 or 1 months for CT and M forms, respectively. Enzyme activity recoveries for the CT/M/L formulations after 18 months kept at 10, 25 and 35°C were 98/97/99, 90/84/77 and 80/66/40%, respectively. The corresponding figures after 24 months were 99/100/96, 90/89/69 and 78/66/35%, respectively. The recoveries for samples stored at 40°C were, as expected; lower than the one obtained at lower temperatures. The CT formulation would comply with the minimum specification of 10,000 FYT/g after 24 months when kept at temperatures up to 35°C. The M and the L formulation would comply with the minimum specifications of 50,000 and 20,000 FYT/g, respectively, when kept at up to 25°C for 24 or 18 months, respectively.

3.1.2. Conditions of use

The applicant has requested to modify the terms of the authorisation for this species/category of animals, by lowering the minimum recommended dose from 1,500 FYT/kg to 1,000 FYT/kg.

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 established (EFSA, 2008 and 2009). The Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected. Considering

[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2009-0003.pdf
[7] 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.
[8] FYT: phytase unit is the quantity of enzyme which liberates 1 micromole of inorganic phosphate per minute from sodium phytate at pH = 5.5 and 37°C.
[9] Technical dossier/Section II/Annexes II. 25, 26 and 27.

the safety for the user, the solid forms of the additive are regarded as dermal and eye irritants and all forms are assumed to be respiratory sensitisers. The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously.

In 2009, the FEEDAP Panel also evaluated a tolerance trial carried out with weaned piglets. Considering the results of that trial and the well-established mode of action of phytases, the FEEDAP Panel concluded that RONOZYME® NP is safe for pigs for fattening at the dose of 3,000 FYT/kg feed. The proposed reduction in the minimum recommended dose would not affect that conclusion.

3.3. Efficacy

Three trials were submitted, a summary of the design and results is presented in Table 1.

The first two trials, trial 1[10] and 2[11], are digestibility trials that were evaluated in a previous assessment (EFSA, 2009). The text describing these two trials is reproduced below.

'The first trial was carried out with six Large White ((ileal cannulated gilts (31.6 kg initial weight). All animals were fed the four diets following a cross-over design, in four experimental periods. Each experimental period lasted for 7 days; faeces were collected on days 6 and 7. The animals were fed in two equal meals at a daily rate of about 80 g $kg^{-0.75}$. Digestible P in the basal diet amounted to 1.10 g/kg'. (calculated; total P in the basal diet amounted to 3.3 g/kg) 'Apparent digestibility of P and Ca was evaluated in the faeces collected. Also, blood samples were obtained from the animals to study the concentration of inorganic P'.

In the second trial, 'a total of thirty-two cross-bred (Large White x (German) Landrace) growing pigs with an initial body weight of 47.8 kg were penned in groups of four animals. The pigs were fed for 30 days the unsupplemented diet containing 1.10 g digestible P/kg'. (calculated; 4.7 g total P/kg) 'After this period the animals received one of the four experimental diets for 13 days. During the last 3 days of administration, faeces were collected individually'.

Table 1: Summary of the design and results of the three short-term trials with RONOZYME® NP in pigs for fattening

Trial	Duration (days)	Diet (form)	RONOZYME® NP (FYT/kg feed)	Total Dietary P (g/kg)	Apparent faecal P digestibility (%)	P retention (g/day)
1	28 (four periods of 7 days)	Maize, barley, soya bean meal (Mash)	0	3.3	41.1[a]	–
			1,000	3.3	54.4[b]	–
			1,500	3.3	55.3[b]	–
			3,000	3.3	65.7[b]	–
2	13	Maize, barley, soya bean meal (Pellets)	0	4.7	22.8[a]	–
			1,000	4.7	51.0[b]	–
			1,500	4.7	56.7[b]	–
			3,000	4.7	62.1[b]	–
3	38 (two periods of 19 days)	Maize, barley, soya bean meal, rapeseed meal (Mash)	0	4.2	35.4[a]	2.39[a]
			1,000	4.2	49.7[b]	3.39[b]

a,b: Values on the apparent faecal phosphorus digestibility or phosphorus retention for a given trial without the same superscript are statistically different, $P < 0.05$ for digestibility values or $P < 0.001$ for retention values.

The third trial, newly submitted, followed a cross-over design and was performed with 10 female pigs ((Piétrain × (German Landrace × Large White)) initial weight of 39 kg), using two diets and two experimental periods (five pigs per diet and period).[12] A basal diet based on maize, barley, soya bean meal and rapeseed meal (with an analysed total phosphorus content of 4.2 g/kg feed; phytate-P

[10] Technical dossier/Section IV/Annex IV.2.
[11] Technical dossier/Section IV/Annex IV.3.
[12] Technical dossier/Section IV/Annex IV.1.

content of 3.6 g/kg feed and calcium 5.2 g/kg) was either not supplemented or supplemented with RONOZYME® NP to provide 1,000 FYT/kg feed. Phytase activity was confirmed by analysis. The trial was divided into two experimental periods each of which consisted of 14 days of adaptation and 5 days of collection. The pigs switched diets between periods. During the collection period, the animals were kept in metabolism cages and fed the diets in mash form restrictively twice daily. Urine (collected using urine bladder catheters) and faeces were collected quantitatively. Samples collected and feed were analysed for phosphorus, calcium, sodium and nitrogen. Retention of phosphorus was calculated. An analysis of variance was performed with the data obtained.

The results in the three trials showed a significant improvement of the apparent faecal digestibility of phosphorus when the pigs were fed RONOZYME® NP at 1,000 FYT/kg feed (or higher) as compared to the non-supplemented diet. The study of the phosphorus retention in trial 3 showed a higher daily phosphorus retention in the pigs fed the phytase at 1,000 FYT/kg.

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 (EFSA FEEDAP Panel, 2012). Apparent faecal digestibility of phosphorus was studied in all trials submitted, but phosphorus retention was studied only in one trial, which showed positive and significant effects at the dose of 1,000 FYT/kg feed. The FEEDAP Panel considers that phosphorus retention is the key end-point for the efficacy assessment of phytases. In the absence of this parameter in two of the three efficacy studies assessed, the FEEDAP Panel cannot conclude on the efficacy of the product at the newly proposed minimum recommended dose.

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[13] and Good Manufacturing Practice.

4. Conclusions

The reduction in the dose proposed would not change the previous conclusions regarding the safety for the target animals, consumer, user and environment. The Panel concluded that the additive is safe for pigs for fattening, consumer and the environment, the solid forms of the additive are regarded as dermal and eye irritants and all forms are assumed to be respiratory sensitisers.

The efficacy of the additive in pigs for fattening at 1,000 FYT/kg complete feed was not demonstrated.

[13] 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

1. RONOZYME® NP for pigs for fattening. May 2014. Submitted by DSM Nutritional Products Ltd.
2. RONOZYME® NP for pigs for fattening. Supplementary information. March 2015. Submitted by DSM Nutritional Products Ltd.
3. RONOZYME® NP for pigs for fattening. Supplementary information. December 2015. Submitted by DSM Nutritional Products Ltd.
4. Comments from Member States.

References

EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed and the Scientific Panel on Genetically Modified Organisms on the safety and efficacy of the product Ronozyme® NP (6-phytase) for chickens for fattening. The EFSA Journal, 871, 1–16.

EFSA (European Food Safety Authority), 2009. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the safety and efficacy of the product Ronozyme® NP (6-phytase) for poultry, weaned piglets and pigs for fattening. The EFSA Journal, 1097, 1–20.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010. Scientific Opinion on the safety and efficacy of Ronzoyme® NP (6-phytase) as a feed additive for sows. EFSA Journal 2010;8(6):1634. 10 pp. doi:10.2903/j.efsa.2010.1634

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. doi:10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. doi:10.2903/j.efsa.2012.2536

Abbreviations

EC	European Commission
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed