REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive Containing 6-Phytase (Ronozyme® HiPhos) Used as a Feed Additive for Poultry, Weaned Piglets, Pigs for Fattening and Sows (RP1298)

Food Standards Agency[1], Food Standards Scotland[2]

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in October 2021 from DSM Nutritional Products Ltd. ('the applicant') for the renewal and modification of authorisation of an additive (Ronozyme® HiPhos) containing 6-phytase, under the category 'zootechnical' additive, functional group 'digestibility enhancer'. The modification is for a change in production strain from *Aspergillus oryzae* DSMZ 22594 to a new 'optimised' strain (DSMZ 33699). The additive, in both granular (GT) and liquid (L) form, is proposed to be used at an inclusion rate of 500 – 4,000 FYT/kg of feed in poultry, weaned piglets, pigs for fattening and sows.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly characterised, and that it is stable for 24 months with loss of activity compensated for through additive overfill. No further concerns were raised for the identity section of the additive.

The proposed level of inclusion of the additive in feed is safe for the target species, the consumer and the environment. The active substance is non-irritant to the skin and eyes; however, it is regarded as a potential respiratory sensitiser and, in the absence of skin sensitisation tests, a potential skin sensitiser.

Ronozyme® HiPhos is efficacious in poultry, weaned piglets, pigs for fattening and sows at the proposed dose range of 500 – 4,000 FYT/kg of feed.

The views of ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive containing 6-phytase (Ronozyme® HiPhos, DSM Nutritional Products Ltd in Switzerland, Wurmigsweg 576, 4303, Kaiseraugst, Switzerland) under assimilated Regulation (EC) No 1831/2003 (EC, 2003). The application sought a renewal authorisation in the category 'zootechnical additive', functional group 'digestibility enhancer', for its use in poultry, weaned piglets, pigs for fattening and sows. The applicant also sought a modification of the terms of the existing authorization, to include a change in production organism to an optimized strain from the same safe strain line but with a higher yield.

To support the safety assessment by FSA and FSS, the ACAF provided advice to the FSA and FSS outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered and concluded that the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by the Advisory Committee on Animal Feedingstuffs (ACAF) at its October 2023 meeting, after which a request for further information was communicated to the applicant. The applicant's response to this request and subsequent requests were evaluated by ACAF at its July 2024 meeting.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Animal species
6-phytase produced by *Aspergillus oryzae* DSMZ 33699	Feed additive	Zootechnical additive	All poultry, weaned piglets, pigs for fattening and sows

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

The active substance of the additive Ronozyme® HiPhos is a 6-phytase enzyme derived from the genetically modified fungus *Aspergillus oryzae* DSMZ 33699. The additive is formulated as both a liquid (Ronozyme® HiPhos (L)) and a solid (Ronozyme® HiPhos (GT)). The applicant provided data from five batches supporting the identification values outlined below (Table 2):

Table 2. Identity table Ronozyme® HiPhos

Composition		
	Liquid (L)	Solid (GT)
Phytase	4.0 %	2.0 %
Cellulose	-	4.0 %
Dextrin	-	7.0 %
Sodium Sulfate	-	86.0 %
Sodium Benzoate	0.15 %	-
Potassium Sorbate	0.05 %	-
Sorbitol	49.8 %	-
Water	46.0 %	1.0 %
Appearance		
	Transparent yellow to light brown liquid	Off white granulated product
Phytase Activity		
	20,000 FYT/g	10,000 FYT/g
Chemical-physical specifications		
Density	1.24 g/mL	-
Viscosity	40 mPas	-
pH	4.6	-
Bulking Density	-	1.15 g/mL
Dusting Potential	-	9.9 mg/m^3
Average Particle Size	-	557 µm (no particles less than 150 µm diameter)
Impurities		
Heavy Metals		

Composition	
Arsenic	≤ 3.0 mg/kg
Lead	≤ 0.5 mg/kg
Cadmium	≤ 5.0 mg/kg
Mercury	≤ 0.5 mg/kg
Microbial Count	
Total Viable Count	≤ 50,000 CFU/g
Total Coliforms /g (GT form)	≤ 30 CFU/g
Total Coliforms /g (L form)	≤ 30 CFU/g
E. coli	Absent in 25 g
Salmonella spp.	Absent in 25 g
Yeast and Filamentous Fungi	< 10 CFU/g
B. cereus	< 10 CFU/g

During the first evaluation, the ACAF identified several items of information that were necessary to inform assessment of the additive's identity. This included: yeast, filamentous fungi and *Bacillus cereus* microbial data, clarification on the relationship between the original production strain (DSMZ 22594) and the new 'optimised' strain (DSMZ 33699), and the 24-month stability data. This information was requested from the applicant, and all aspects were satisfactorily addressed.

The additive was shown to be stable for 24 months with residual activity of the granular and liquid product at 25°C being 45 % and 63 %, respectively. The applicant stated that the loss of activity will be compensated by overdosing to a level necessary to meet the declared minimum activity until the product expiry date.

The proposed conditions of use of the additive are described in Table 3:

Table 3. Conditions of use of Ronozyme® HiPhos proposed by the applicant

Conditions of use proposed by applicant				
Species or category of animal	Min-max Age	Min. content	Max. content	Withdrawal period
		Units of phytase enzyme activity per kg of compound complete feedingstuff (FYT/kg feed)		
Poultry Swine	- -	500 500	4000 4000	- -
Other provisions				
Inclusion method	The liquid product form Ronozyme® HiPhos (L) is designed to be sprayed directly onto the compound feed using appropriate spraying equipment. The granular product form Ronozyme® HiPhos (GT) is designed to be used in mash feed.			
Specific conditions or	For user safety – in case of insufficient ventilation wear an approved mask with a particle filter type P3 used according to the manufacturer instructions. Protective			

Conditions of use proposed by applicant	
restrictions for handling	gloves, skin should be washed after contact. Safety glasses with side shields (or goggles). Wear long sleeved clothing.

2.1.1. Conclusions on Section II

The ACAF concluded that both the liquid and granular form of the additive were correctly characterised. Both forms of the additive are stable for 24 months at 25°C provided overdosing of the additive to ensure minimum activity levels are met until the product's expiry date. No further concerns were raised for Section II of the dossier.

2.2. Section III: Safety

The Committee were satisfied that the extensive literature review identified no relevant papers that described adverse effects of the additive on target species, consumers or the environment. Furthermore, the applicant provided a signed quality statement by manufacturers and distributors of Ronozyme® HiPhos noting the absence of reports of adverse effects on target animals, users or the environment.

The ACAF were satisfied with the safety of the 'optimised' production strain termed 33693. The applicant provided a history of safe use of the *A. oryzae* strains and demonstrated an absence of known toxins.

2.2.1. Safety for the target species

The applicant provided new calculations to establish the maximum safe concentration of the additive in feed for the target species. This was derived by applying a 100-fold uncertainty factor to the NOAEL from the 90-day repeat-dose oral toxicity study performed in rats. The ACAF agree with the applicant that the calculations confirm the absence of safety concerns when supplementing with the additive at the maximum recommended dose of 4,000 FYT/kg feed. The calculated values are reported in Table 4, which shows that for all classes of target species tested the estimated maximum safe concentration (as enzyme activity) of the additive was much greater than the recommended dose.

Table 4. Safe feed concentration for the enzyme in pig and poultry, as derived from the NOAEL

NOAEL	
90-day rat study	523710 FYT/kg bodyweight/day
Uncertainty factor	100
Safety daily intake for target species	5237 FYT/kg bodyweight/day
Weaned piglets	
Default feed intake level for weaned piglets	44 g DM/kg bodyweight
Max safe concentration in feed weaned piglets	104742 FYT/kg feed
Pigs for fattening	

NOAEL	
Default feed intake for pigs for fattening	37 g DM/kg bodyweight
Max safe concentration in feed for pigs for fattening	124558 FYT/kg feed
Lactating Sows	
Default feed intake for lactating sows	30 g DM/kg bodyweight
Max safe concentration in feed for lactating sows	153622 FYT/kg feed
Chickens for fattening	
Default feed intake for chickens	79 g DM/kg bodyweight
Max safe concentration in feed for chickens	58337 FYT/kg feed
Laying Hens	
Default feed intake for layers	53 g DM/kg bodyweight
Max safe concentration in feed for layer	86956 FYT/kg feed

2.2.2. Safety for the consumer

The applicant presented the following tests on the active ingredient to evaluate the safety of the additive for the consumer:

- Sub-chronic oral toxicity study (OECD 408)
- Bacterial reverse mutation (Ames) test (OECD 471)
- *In vitro* micronucleus test in human lymphocytes (OECD 487)

No adverse effects were found in the toxicity study up to the highest dose tested, 523710 FYT/kg body weight/day, which was taken as the NOAEL. The negative results from the two genotoxicity studies indicated that the active ingredient was not mutagenic. Ingredients other than the active ingredient were commonly used substances that are known to be safe at the levels present in the additive.

The Committee were satisfied with the evidence provided and concluded that the additive showed no adverse effect which could give rise to concern of the additive for the consumer.

2.2.3. Safety for the user

Two irritation studies performed with the active ingredient (optimised production strain DSMZ 33699) were presented to evaluate the safety of the additive for the user/worker:

- Bovine Corneal Opacity Permeability (BCOP) test performed following OECD 437 to assess eye irritation
- *In vitro* EpiSkin™ reconstructed human epidermis model test performed following OECD 439 to assess skin irritation

The Committee concluded that the active substance is non-irritant to the skin and eyes; however, because the additive is an enzyme it is considered to be a respiratory sensitiser, and in the absence of skin sensitisation tests, a potential skin sensitiser.

2.2.4. Safety for the environment

No studies were provided as the active substance is an enzyme and the other ingredients are commonly used substances that are not expected to harm the environment.

2.2.5. Conclusions on safety

The ACAF concluded that there were no safety concerns to the target species when supplementing with the additive at the maximum recommended dose of 4,000 FYT/kg feed.

The additive showed no adverse effect which could give rise to concern of the additive for target animals or the consumer.

The active substance is non-irritant to the skin and eyes; however, because the additive is an enzyme it is considered to be a respiratory sensitiser, and in the absence of skin sensitisation tests, a potential skin sensitiser.

The additive poses no risk to the environment.

2.3. Section IV: Efficacy

The Committee questioned the potential difference between the original production strain and the new 'optimised' strain. Upon accepting the similarities between strains, the ACAF noted that the phytase product obtained from the 'optimised' strain shares the same composition, manufacturing process and phytase. The ACAF deemed the efficacy studies submitted to be acceptable for the application. The applicant submitted the efficacy trials previously submitted to FEEDAP Panel (FEEDAP, 2012b, 2012a, 2016). The Committee concluded that the additive demonstrated efficacy in poultry, weaned piglets, pigs for fattening and sows at the minimum recommended dose of 500 FYT/kg feed.

2.3.1. Conclusions on efficacy

The ACAF concluded that the additive was demonstrated to be efficacious.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory (EURL) for Feed Additives on the Method(s) of the Analysis for Ronozyme HiPhos (EURL, 2018):

> "For the quantification of the phytase activity in feedingstuffs the Applicant submitted the ring-trial validated colorimetric standard method EN ISO 30024 and demonstrated the suitability of the method at 200 FYT/kg feedingstuffs. In addition, the Applicant applied this method with minor experimental modifications to analyse the feed additive (Ronozyme® HiPhos) and premixtures and obtained similar method performance characteristics.
>
> Based on the performance characteristics available the EURL recommends for official control the colorimetric methods mentioned above for the quantification of the phytase activity in the feed additive, premixtures and feedingstuffs".

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical methods proposed as appropriate for official controls for this feed additive. The analytical methods were approved in 2018, at the time in which the UK was still an EU member.

4. Conclusions

The ACAF concluded the additive was correctly characterised, and that it is stable for 24 months at 25°C, with overfilling of the additive to compensate for residual loss of activity. No further concerns were raised for Section II of the dossier.

The active substance is non-irritant to the skin or eyes; however, because the additive is an enzyme it is considered a respiratory sensitiser, and in the absence of skin sensitisation tests, a potential skin sensitiser.

The additive poses no risk to the environment.

The ACAF concluded that the additive is efficacious in poultry, weaned piglets, pigs for fattening and sows at the dose range of 500 – 4000 FYT/kg feed.

Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CFU	Colony forming units
EC	European Commission
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances Used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland

Acronym	Definition
FYT	Phytase activity
NOAEL	No observed adverse effect level

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