REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment of the Safety and Efficacy of an Additive Consisting of Endo-1,4-Beta-Xylanase (Produced by *Aspergillus Oryzae* DSM 33700) for All Poultry Species and All Pigs (Suidae) (DSM Nutritional Products Ltd). (RP1393)

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

[1] Regulated Products Risk Assessment, Food Standards Agency, UK, [2] Risk Assessment, Food Standards Scotland, UK

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in December 2021 from DSM nutritional products Ltd ('the applicant') for the renewal, extension of use and new use of an additive (RONOZYME® WX) containing endo-1,4-beta-xylanase (produced by *Aspergillus oryzae* DSM 33700), under the category 'zootechnical' additive, functional group 'digestibility enhancer'. The applicant seeks renewal of the additive for use in poultry for fattening, piglets (weaned), pigs for fattening, lactating sows and laying hens, as well as an extension of use of the additive in all poultry species and all pigs (Suidae). In addition, the applicant seeks authorisation for the new use concerning use of an improved production strain, substituting strain *A. oryzae* DSM 26372 with *A. oryzae* DSM 33700.

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 fully identified and characterised. It was concluded that RONOZYME® WX (CT and L), manufactured with the *A. oryzae* DSM 33700 strain, did not raise any safety concerns regarding the genetic modification of the production strain. Members concluded positively on the stability and homogeneity of the additive. However, the pelleting stability for RONOZYME® WX (CT) was only tested for 20 seconds, and therefore this should be made clear on the label as some of the uses may require longer conditioning times.

The additive is considered safe for the target species, the consumer and the environment. The additive RONOZYME® WX (CT and L) is not a skin irritant and RONOZYME® WX (L) is not an eye irritant. RONOZYME® WX (CT) is a potential eye irritant. No information on skin sensitisation was provided; therefore, the additive is regarded as a potential skin sensitiser. The additive should be classified as a respiratory sensitiser due to the proteinaceous nature of its active ingredient (enzyme).

It was concluded that the additive is efficacious in all poultry species and in all pigs (Suidae) at a minimum recommended level of 100 Fungal Xylanase Units (FXU)/kg and 200 FXU/kg complete feed, respectively, with the exception to gestating sows, as no data was provided.

The views of ACAF have been considered 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 endo-1,4-beta-xylanase (RONOZYME® WX, DSM Nutritional Products Ltd, Wurmisweg 576 CH-4303 Kaiseraugst, Switzerland) under Assimilated Regulation (EC) No 1831/2003 (EC, 2003), in category 'zootechnical' additive, functional group 'digestibility enhancer'. The applicant seeks renewal of the additive for use in poultry for fattening, piglets (weaned), pigs for fattening, lactating sows and laying hens, as well as an extension of use of the additive in all poultry species and all pigs (Suidae). In addition, the applicant seeks authorisation for the new use of an improved production strain, substituting strain *A. oryzae* DSM 26372 with *A. oryzae* DSM 33700.

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 Assimilated Regulation (EC) 1831/2003 (EC, 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 December 2023 meeting, after which a request for further information was communicated to the applicant. The dossiers and the applicants' responses were further reviewed by ACAF at their July 2024 meetings.

The views of ACAF have been considered 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	Intended species or categories of animals
RONOZYME® WX (CT and L)	Feed Additive	Zootechnical additive / digestibility enhancer	All poultry species and all pigs (Suidae)

2. Assessment

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

The active ingredient of RONOZYME® WX is endo-1,4-beta-xylanase produced by an *Aspergillus oryzae* strain (DSM 26372) available in the following physical formulations:

- RONOZYME® WX (CT): Coated thermo-stable and solid formulation with a minimum activity of 1,000 FXU/g;
- RONOZYME® WX (L): A liquid formulation with a minimum activity of 650 FXU/mL.

The applicant provided data from several batches supporting the identification values outlined in Table 2 for the solid formulation and Table 3 for the liquid formulation. The applicant clarified that the *Bacillus cereus* testing used batches of enzyme concentrates, which are used as ingoing material to produce RONOZYME® WX (L) and RONOZYME® WX (CT), instead of the batches of the product.

The Committee agreed that the strain had been fully characterised using whole genome sequencing (WGS) and were able to conclude on the identity and characterisation of the additive after the applicant provided additional data demonstrating batch-to-batch variation and impurity testing. Substituting strain *A. oryzae* DSM 26372 with *A. oryzae* DSM 33700 did not raise any safety concerns regarding the genetic modification of the production strain. Supporting documentation and certification relating to quality assurance and further information on Hazard Analysis and Critical Control Point (HACCP) principles, including critical control points and safety data sheets, were also provided upon request.

Members noted that the extent to which spent growth medium is incorporated into the final product was not clearly indicated. Clarification provided by the applicant regarding spent growth medium was deemed satisfactory.

Table 2. Identity table Ronozyme® WX (CT).

Composition	
Xylanase (endo-1,4-)	1 %(w/w)
Enzyme activity	1,040 - 1,203 FXU(W)/g
Sodium sulphate	56 %(w/w)
Total organic solids (kaolin, dextrin, calcium carbonate, palm oil hydrogenated, cellulose)	42 %(w/w)
Water	1 %(w/w)
Chemical-physical properties	
Dusting on Heubach filter	0.7 (0 - 1) mg
Particle size distribution	> 850 µm – 21 % < 850 µm – 79 % < 420 µm – 14 % < 150 µm – <1 %
Loose density	1,160 g/L
Tapped density	1,310 g/L
Impurities	
Arsenic	0.82 mg/kg
Lead	1.17 mg/kg
Cadmium	0.11 mg/kg
Mercury	≤ 0.05 mg/kg
Total viable count	250 (<100 – 400) /g
E. coli	Neg/25 g
Salmonella spp.	Neg/25 g
Enterobacteriacea	<10 CFU/g
Coliforms	11 /g
Yeasts and filamentous fungi	≤ 10 CFU/g
Bacillus cereus[a]	< 10 CFU/g
Alfatoxin B1[a]	< 0.0003 mg/kg

[a] Analysis was carried out on enzyme concentrate.

The average residual activity of RONOZYME® WX (CT) was 94% (ranging from 89% to 105%) after 24 months of storage at 25°C, and 86% (ranging from 84% to 86%) after 12 months of storage at 40°C. The average measured residual activity of RONOZYME® WX (L) was 85% (ranging from 79% to 88%) after 24 months of storage at 25°C, and 63% (ranging from 62% to 63%) after 12 months of storage at 40°C. The study was completed in 2013, and the batches used were produced with the currently authorised production strain (DSM 26372). In 2021, a new study was conducted, showing an average measured residual activity of RONOZYME® WX (CT) of approximately 100% (ranging from 101% to 104%) after 12 months at 25°C, and 83% (ranging from 73% to 92%) after 12 months at 40°C. The average measured residual activity of RONOZYME® WX (L) was 96% (ranging from 87% to 105%) after 24 months of storage at 25°C, and 58% (ranging from 56% to 60%) after 12 months of storage at 40°C. The applicant stated that values above 100% are due to analytical variations.

Table 3. Identity table Ronozyme® WX (L)

Composition	
Xylanase (endo-1,4-)	0.5 %(w/w)
Enzyme activity	675- 685 FXU(W)/g
Water	52.3 %(w/w)
Total organic solids (glycerol, sorbitol potassium sorbate)	47.2 %(w/w)
Appearance	
Light yellow	
Chemical-physical properties	
Density	1.17 g/mL
Viscosity at 25°C	12 mPa.s
Impurities	
Arsenic	≤ 0.3 mg/kg
Lead	≤ 0.5 mg/kg
Cadmium	≤ 0.05 mg/kg
Mercury	≤ 0.05 mg/kg
Total viable count	140 (<100 – 200) /g
E. coli	Neg/25 g
Salmonella spp.	Neg/25 g
Enterobacteriacea	< 10 CFU/g
Coliforms	≤ 4 /g
Yeasts and filamentous fungi	< 10 CFU/g
3-nitropropionic (BNP)	≤ 0 mg/kg
Cyclopiazonic acid (CPA)	≤ 0 mg/kg
Alfatoxin B1[a]	< 0.0003 mg/kg

[a] Analysis was carried out on enzyme concentrate.

Members noted that the pelleting stability for RONOZYME® WX (CT) was only tested for 20 seconds, and therefore this should be made clear on the label as some of the uses may require longer conditioning times. Homogeneity of RONOZYME® WX (CT) and RONOZYME® WX (L) in feed was also demonstrated.

The proposed conditions of use of the additive are described in Table 4.

2.1.1. Conclusions on Section II: Identity, characterisation and condition of use

The ACAF concludes that the additive was fully identified and characterised. It was concluded that RONOZYME® WX (CT and L), manufactured with the *A. oryzae* DSM 33700 strain, did not raise any safety concerns regarding the genetic modification of the production strain. Members were able to conclude positively on the stability and homogeneity of the additive. It was noted that the pelleting stability for

Table 4. Conditions of use of RONOZYME® WX (CT) and RONOZYME® WX (L) proposed by the applicant.

Species or category of animal	Min. content in feed	Max. content in feed	Withdrawal period
Poultry	100 FXU/kg compound feed	-	-
Pigs (Suidae)	200 FXU/kg compound feed	-	-
Other provisions and additional requirements for the labelling			
Specific conditions or restrictions for use	For use in compound feed rich in non-starch polysaccharides (mainly arabinoxylans), e.g. containing at least 15% wheat, barley, rye and/or triticale. The dry form RONOZYME® WX (CT) is mixed into feedingstuffs directly with the other feed materials or after incorporation in a premixture. The liquid product form RONOZYME® WX (L) is designed to be sprayed directly onto the compound feedingstuffs using appropriate spraying equipment. Avoid temperature in excess of 85 °C during pelleting. Store closed and dry below 25°C. Once opened, use contents quickly.		
Specific conditions or restrictions for handling	For user safety – May cause allergy or asthma symptoms or breathing difficulties if inhaled. May form combustible dust concentrations in air. Avoid breathing dust/fume/gas/mist/vapours/spray. Wear respiratory protection. If inhaled, remove person to fresh air and keep comfortable for breathing. If experiencing respiratory symptoms: Call a POISON CENTER/doctor. Dispose of contents/ container to an approved waste disposal plant. Contains: xylanase, endo-1,4- (9025-57-4).		

RONOZYME® WX (CT) was only tested for 20 seconds, and therefore this should be made clear on the label as some of the uses may require longer conditioning times. No further concerns were raised for Identity, characterisation and condition of use section of the dossier.

2.2. Section III: Safety

2.2.1. Safety for the target species

Based on the 90-day toxicity study carried out in a total of 45 male and 45 female rats and using liquid concentrate intermediate used to formulate the additive, the Committee agreed with the proposed no observed adverse effect level (NOAEL) value of 1,010 mg TOS (total organic solids)/kg bw/day. Depending on the formulation, TOS forms between 1 and 2% of the actual product. The target dietary inclusion of RONOZYME® WX (CT) and RONOZYME® WX (L) in feed is shown in Table 5 and Table 6, respectively. The maximum safe concentrations of an additive in feed were calculated in accordance with the algorithm presented by EFSA using NOAEL value derived from the 90-day toxicity study, an uncertainty factor of 100 and default feed intakes provided in the guidance for relevant species.

Table 5. The target dietary inclusion of RONOZYME® WX (CT) in feed where the inclusion level of TOS is 2% in 1 g product.

Species	Recommended min. inclusion (FXU)	Inclusion level of product in feed (mg/kg)	Inclusion level of TOS in feed (mg/kg)	Max safe dose (mg of TOS in 1 kg feed)
Poultry for fattening	100	100	2	112
Piglets (weaned)	200	200	4	202
Pigs for fattening	200	200	4	240
Lactating sows	200	200	4	296
Laying hens	100	100	2	167

Table 6. The target dietary inclusion of RONOZYME® WX (L) in feed where the inclusion level of TOS is 1% in 1 mL product.

Species	Recommended min. inclusion (FXU)	Inclusion level of product in feed (mg/kg or mL/kg)	Inclusion level of TOS in feed (mg/kg or mL/kg)	Max safe dose (mg of TOS in 1 kg feed)
Poultry for fattening	100	154	1.54	112
Piglets (weaned)	200	308	3.08	202
Pigs for fattening	200	308	3.08	240
Lactating sows	200	308	3.08	296
Laying hens	100	154	1.54	167

Members noted that the EFSA opinion for sows referred to a repeated dose toxicity study with the reproduction/developmental toxicity screening test (OCED 422) and requested that this study be provided by the applicant. Upon receival of this study, the Committee concluded that the study did not raise any concerns.

Based on the data provided, the Committee concludes that the additive is safe for poultry for fattening and laying hens at the recommended dose of 100 FXU/kg feed and for piglets (weaned), pigs for fattening and sows at the recommended dose of 200 FXU/kg feed. The Committee concludes that the safety findings for poultry for fattening, laying hens, piglets (weaned), pigs for fattening and sows can be extended to all poultry species and all pigs (Suidae). Therefore, the additive is considered safe for all poultry species and all pigs (Suidae) under the authorised and proposed conditions of use.

2.2.2. Safety for the consumer

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

- Bacterial Reverse Mutation Assay test carried out using xylanase produced by the optimized production strain DSM 33700;
- *In Vitro* Human Lymphocyte Micronucleus test carried out using xylanase produced by the optimized production strain DSM 33700;
- 90-day rat gavage study carried out using liquid concentrate intermediate used to formulate the additive produced by the production strain DSM 26372.

The Committee agrees with the conclusions of these studies, which do not indicate any concerns regarding consumer safety from the use of the product as a feed additive in all poultry species and all pigs (Suidae).

2.2.3. Safety for the user

The applicant provided no new studies, highlighting that zero incidents have been reported since the introduction of the additive to the market more than 2 decades ago. The applicant presented the following tests to evaluate the safety of the additive for the user/worker:

- *In vitro* EpiSkin™ skin irritation test, following OECD guideline No. 439, using both forms of the additive RONOZYME® WX (CT and L);
- Bovine corneal opacity permeability (BCOP) assay for eye irritancy, following OECD guideline No. 437 using both forms of the additive RONOZYME® WX (CT and L).

The results of the studies showed that both formulations of the RONOZYME® WX (C and L) are not skin irritants. According to the provided studies, RONOZYME® WX (L) is not an eye irritant. However, the *In Vitro* Irritancy Score (IVIS) was measured to be 6.7 for RONOZYME® WX (CT), meaning that 'No stand-alone prediction' of eye irritation can be made under the conditions of the test.

The Committee agreed with the conclusions of the authors of the *in vitro* EpiSkin™ Skin Irritation Test and Bovine Corneal Opacity Permeability (BCOP) assay that the additive RONOZYME® WX (C and L) is not a skin irritant and RONOZYME® WX (L) is not an eye irritant. RONOZYME® WX (CT) is a potential eye irritant. No information on skin sensitisation was

provided; therefore, the additive is regarded as a potential skin sensitiser. The Committee concluded that the additive should be classified as a respiratory sensitiser due to the proteinaceous nature of its active ingredient (enzyme).

2.2.4. Safety for the environment

The applicant proposed that as the additive is a feed enzyme, the xylanase it contains will be degraded in the gastrointestinal tract of the animals to amino acids; therefore, the risk of release of the additive to the environment is considered extremely low. To further assess safety for environment, Members requested a Phase I assessment for the additive (as sold) according to EFSA guidance. In their response, the applicant justified the biodegradability of the enzyme, and that bioaccumulation is not expected in aquatic or terrestrial organisms. The Committee concluded that the additive, as well as the genetic modification of the production strain, is unlikely to present a safety concern for the environment.

2.2.5. Conclusions on safety

The Committee concluded that RONOZYME® WX (CT and L), manufactured with the *A. oryzae* DSM 33700 strain, did not raise any safety concerns regarding the genetic modification of the production strain. The additive is considered safe for all poultry species and all pigs (Suidae) under the authorised and proposed conditions of use. The use of RONOZYME® WX (CT and L) manufactured with the production strain *A. oryzae* DSM 33700 raises no concerns for safety of consumers and environment. In the absence of testing, the additive is regarded as a potential skin sensitiser. The Committee concludes that the additive RONOZYME® WX (CT and L) is not a skin irritant and RONOZYME® WX (L) is not an eye irritant. RONOZYME® WX (CT) is a potential eye irritant. The additive should be classified as a respiratory sensitiser due to the proteinaceous nature of its active ingredient (enzyme).

2.3. Section IV: Efficacy

RONOZYME® WX is currently authorised for use in poultry for fattening, piglets (weaned), pigs for fattening, lactating sows and laying hens. The applicant requested an extension of use to include all poultry species and all pigs (Suidae). According to the EFSA guidance for efficacy, efficacy data cannot be extrapolated between categories of the same species at different production stages. Members discussed the extrapolation, noting that gestating sows are at a physiologically distinct stage of production; therefore, the applicant was asked to provide reproductive and developmental toxicity studies and efficacy studies on gestating sows. The applicant could not provide these studies; therefore, the Committee could not conclude on efficacy for gestating sows and so extrapolation to all

stages of a pig's life is not possible. The Committee concludes that the additive is efficacious in all poultry species and in all pigs (Suidae) at a minimum recommended level of 100 FXU/kg and 200 FXU/kg complete feed, respectively, with the exception of gestating sows, as no data was provided.

2.3.1. Conclusions on efficacy

The Committee concludes that the additive is efficacious in all poultry species and in all pigs (Suidae) at a minimum recommended level of 100 FXU/kg and 200 FXU/kg complete feed, respectively, with the exception to gestating sows, as no data was provided.

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 for Feed Additives on the Method(s) of the Analysis for endo-1,4-b-xylanase (EURL, 2015):

> "The product is intended to be marketed as solid and liquid formulations having a guaranteed minimum xylanase activity of 1000 FXU/g and 650 FXU/ml respectively. The feed additive formulations are intended to be included through premixtures (solid) or directly in feedingstuffs (solid and liquid) to obtain a minimum activity of 100 or 200 FXU/kg feedingstuffs, depending on the target species.
>
> For the quantification of the xylanase activity in the feed additive the applicant proposed a single-laboratory validated and further verified colorimetric method that requires the use of an automatic device (Konelab Analyzer). Upon request of the EURL of a manual method, the applicant proposed to apply a ring-trial validated colorimetric method based on the quantification of the coloured compounds produced by the dinitro salicylic acid (DNSA) and the xylosylic moieties released by the action of endo-1,4-b-xylanase on arabinoxylan. Furthermore, for the quantification of the xylanase activity in premixtures and feedingstuffs the applicant proposes a single-laboratory validated and further verified colorimetric method based on the quantification of the water soluble dyed fragments produced by the action of endo-1,4-b-xylanase on oat spelt azo-xylan. In both methods the calibration is conducted using a reference standard with a known enzyme activity expressed in FXU.

> Based on the satisfactory performance characteristics, recalculated from the experimental data available, the EURL recommends for official control the colorimetric methods mentioned above for the quantification of the xylanase activity in the feed additive, premixtures and feedingstuffs.
>
> Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary."

The 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.

4. Conclusions

The ACAF concludes that the additive was fully identified and characterised. It was concluded that RONOZYME® WX (CT and L), manufactured with the *A. oryzae* DSM 33700 strain, did not raise any safety concerns regarding the genetic modification of the production strain. Members concluded positively on the stability and homogeneity of the additive. It was noted that the pelleting stability for RONOZYME® WX (CT) was only tested for 20 seconds, and therefore this should be made clear on the label as some of the uses may require longer conditioning times.

The additive is considered safe for all poultry species and all pigs (Suidae), the consumer and the environment, under the authorised and proposed conditions of use. In the absence of testing, the additive is regarded as a potential skin sensitiser. The Committee concludes that the additive RONOZYME® WX (CT and L) is not a skin irritant and RONOZYME® WX (L) is not an eye irritant. RONOZYME® WX (CT) is a potential eye irritant. The additive should be classified as a respiratory sensitiser due to the proteinaceous nature of its active ingredient (enzyme).

The Committee concludes that the additive is efficacious in all poultry species and in all pigs (Suidae) at a minimum recommended level of 100 FXU/kg and 200 FXU/kg complete feed, respectively, with the exception of gestating sows, as no data was provided.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
BCOP	Bovine Corneal Opacity Permeability
BNP	3-nitropropionic
CFU	Colony forming units

Abbreviation	Definition
CPA	Cyclopiazonic acid
DNSA	Dinitro salicylic acid
EC	European Commission
EFSA	European Food Safety Authority
EURL-FA	European Reference Laboratory for Feed Additives
FXU	Fungal Xylanase Units
HACCP	Hazard Analysis and Critical Control Point
LOQ	Limit of quantification
NOAEL	No observed adverse effect level
TOS	Total organic solids
WGS	Whole genome sequence

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