REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive of Endo-B-1,4-Xylanase Produced by *Komagataella phaffii* CGMCC 7.371 (VTR-xylanase) as a Feed Additive for Weaned Piglets, Suckling Piglets, Minor Growing Porcine and All Avian Species (RP1039/1040)

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 April 2021 from Victory Enzymes GmbH ('the applicant') for the new of authorisation of an additive (VTR-xylanase) containing endo-β-1,4-xylanase, under the category of 'zootechnical additives' and functional group 'digestibility enhancers'. The additive is proposed to be used at an inclusion rate of 2000 U/kg for weaned piglets, suckling piglets, minor growing porcine species, laying hens and other laying birds, and at an inclusion rate of 1,000 U/kg for chickens for fattening and minor avian species.

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 12 months in its powder form when stored at 25°C, and for 18 months in its liquid form when stored at 5°C. The additive is stable at 70°C for 15 seconds during pelleting. Homogeneity in feed was also demonstrated. No further concerns were raised for section II of the dossier.

The additive can be considered safe for the target species, consumers and the environment. The additive should be regarded as a skin sensitiser in both its powder and liquid form. The liquid form of the additive is not a skin irritant. In its powder form, the additive should be considered a potential skin irritant. The additive should be considered an irritant to the eyes in both forms. As an enzyme preparation, the additive should be considered a potential respiratory sensitiser.

VTR-xylanase is efficacious for weaned piglets, suckling piglets, minor growing porcine species, laying hens and other laying birds at 2,000 U/kg, and in chickens for fattening and minor avian species at 1,000 U/kg.

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

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive [endo-β-1,4-xylanase (VTR-xylanase), Victory Enzymes GmbH, Fürschlag 3, D-91564, Neuendettelsau, Germany] under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) under the category of 'zootechnical additives' and functional group 'digestibility enhancers', for its use in weaned piglets, suckling piglets, minor growing porcine and all avian species.

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 whether 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 ACAF at their April 2023 meeting, after which a request for information was communicated to the applicant. The response to this request was provided in September 2023 and was reviewed by the ACAF at their December 2023 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	Intended species or categories of animals
Endo-1,4-beta-xylanase produced by Komagataella phaffii CGMCC 7.371	Feed additive	Zootechnical additive	Weaned piglets, suckling piglets, minor growing porcine and all avian species

2. Assessment

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

The additive's active substance is endo-β-1,4-xylanase and is produced in two formulations, powder (VTR-xylanase powder) and liquid (VTR-xylanase liquid). In its powder form the active substance has a minimum guaranteed activity of 100,000 U/g, and in its liquid form has a minimum guaranteed activity of 30,000 U/g. The applicant provided data from five batches for each form to support the identification values outlined below (Table 2 and Table 3):

Table 2. Identity table VTR-xylanase powder

Composition	
Activity	≥ 100,000 U/g
Moisture	≤ 10%
pH	-
Appearance	
White to light grey powder	
Chemical-physical specifications	
Particle size distribution	<100 µm – 35.56% <10 µm – 0.06%
Dusting potential	0.00 – 35.00 mg/m^3
Bulk density	0.527 – 0.538 g/cm
Impurities	
Enterobacteriaceae	<10 CFU/g
Yeasts	<100 CFU/g
Molds	<100 CFU/g
Salmonella	Absent in 25g
Arsenic	0.021 – 0.029 mg/kg
Lead	0.017 – 0.025 mg/kg
Cadmium	<0.0004 mg/kg
Mercury	<0.003 mg/kg
PCDD/Fs	0.0855 pg WHO-TEQ/g OS
DLPCBs	0.0132 – 0.0142 pg WHO-TEQ/g OS
PCDD/Fs and DLPCBs	0.0986 – 0.0997 pg WHO-TEQ/g OS

Table 3. Identity table VTR-xylanase liquid

Composition	
Activity	≥ 30,000 U/g
Moisture	-
pH	4 - 6
Appearance	
Yellowish to light brown	
Chemical-physical specifications	
Vapour pressure	2.20 – 2.22 kPa
Viscosity	1.99 – 2.03 mPas
Specific weight	1.10 g/cm^3
Impurities	
Enterobacteriaceae	<10 CFU/g
Yeasts	<100 CFU/g
Molds	<100 CFU/g
Salmonella	Absent in 25g
Arsenic	<0.005 mg/kg
Lead	0.018 – 0.025 mg/kg
Cadmium	<0.0004 mg/kg
Mercury	<0.002 mg/kg
PCDD/Fs	0.0855 pg WHO-TEQ/g OS
DLPCBs	0.0132 pg WHO-TEQ/g OS
PCDD/Fs and DLPCBs	0.0986 pg WHO-TEQ/g OS

The Committee reviewed the data presented for the genetic modification of the production strain *K. phaffii*, concluding that a comprehensive description had been provided with no concerns identified. The characterisation data presented demonstrated the production strain to be suitable for the qualified presumption of safety (QPS) assessment route, with absence of the production strain and DNA from the additive confirmed. Members noted that pelleting stability and homogeneity of the additive could not be concluded upon from the data presented. The applicant was issued a request for information and supplied an appropriate response. The Committee reviewed the additional data and concluded that the additive is stable for 12 months in its powder form when stored at 25°C, and for 18 months in its liquid form when stored at 5°C. The additives stability during pelleting could only be concluded upon at 70°C for 15 seconds. Homogeneity in feed was also demonstrated.

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

Table 4. Proposed conditions of use of VTR-xylanase

Proposed mode of use in animal nutrition				
Additive	Endo-β-1,4-xylanase			
Registration number/ EC No/No	4axx			
Category(-ies) of additive	4. Zootechnical feed additive			
Functional group(s) of additive	a. Digestibility enhancer			
Description				
Description	**Purity criteria**	**Method of analysis**		
Preparation of Endo-β-1,4-xylanase	Liquid form: min. activity: 30,000 U/g Powder form: min. activity: 100,000 U/g	Validated colorimetric method (Megazyme T-XAX:2014-11)		
Trade name (if appropriate)	VTR-xylanase liquid VTR-xylanase powder			
Name of the holder of authorisation (if appropriate)	Victory Enzymes GmbH			
Conditions of use				
Species or category of animal	**Max Age**	**Min. content**	**Max. content**	**Withdrawal period**
		U/kg of complete feedingstuffs		
Suckling piglets	Until weaning*	2,000 U/kg	--	--
Weaned piglets	120 days	2,000 U/kg	--	--
Minor growing porcine species	No max. age	2,000 U/kg	--	--
Chickens for fattening and minor avian species	Slaughter age and weight	1,000 U/g	--	--
Laying hens and other laying birds	No max. age	2,000 U/g	--	--

*Only during the period when solid feed is given.

2.1.1. Conclusions on Section II

The ACAF concluded the additive was correctly characterised, and that it is stable for 12 months in its powder form when stored at 25°C, and for 18 months in its liquid form when stored at 5°C, the additive is stable at 70°C for 15 seconds during pelleting. Homogeneity in feed was also demonstrated.

No further concerns were raised for Section II of these dossiers.

2.2. Section III: Safety

2.2.1. Safety for the target species

The applicant provided several studies performed using the VTR-xylanase liquid concentrate to demonstrate safety for the target species:

- Sub-chronic oral toxicity study (OECD 408)
- Bacterial reverse mutation assay (OECD 471)

- *In vitro* mammalian cell (human lymphocyte) micronucleus test (OECD 487)

No treatment-related adverse effects were seen in the oral toxicity study and a NOAEL of 52,000 U/kg has been established. The additive was demonstrated to not have genotoxic potential.

2.2.2. Safety for the consumer

As an enzyme produced by an organism suitable for the qualified presumption of safety assessment approach, the additive is presumed safe for the consumer.

2.2.3. Safety for the user

The applicant presented the following tests to evaluate the safety of the additive for the user/worker:

- *In vitro* skin irritation test (EPISKIN reconstructed human epidermis model – OECD 439)
- *In vitro* eye irritation test (reconstructed human cornea-like epithelium model – OECD 492)
- *In vitro* skin sensitisation test (Kerstinosens Assay – OECD 442D)
- *In vitro* skin sensitisation test (Human cell line activation test – OECD 442E)

The Committee concluded that the additive should be regarded as a skin sensitiser in both its powder and liquid form. The liquid form of the additive is not a skin irritant, however, owing to the absence of data, the powdered form should be considered a potential skin irritant. The additive should be considered a potential irritant to the eyes in both forms. As an enzyme, the additive should be considered as a potential respiratory sensitiser.

2.2.4. Safety for the environment

As an enzyme produced by an organism suitable for the qualified presumption of safety assessment approach, the additive is presumed safe for the environment.

2.2.5. Conclusions on safety

The additive can be considered safe for the target species, consumers and the environment. The additive should be regarded as a skin sensitiser in both its powder and liquid form. The liquid form of the additive is not

a skin irritant, in its powder form, the additive should be considered a potential skin irritant. The additive should be considered an irritant to the eyes in both forms. As an enzyme preparation, the additive should be considered a potential respiratory sensitiser. The Committee considered that user exposure by inhalation to liquid and solid forms of the additive would be low, noting the low dusting potential of the solid form.

2.3. Section IV: Efficacy

The applicant presented three short-term efficacy studies in laying hens and three long-term efficacy studies in chickens for fattening to demonstrate efficacy in all avian species. For pigs, one short term efficacy study in weaned piglets and two long term studies in weaned piglets were presented. An overview of the efficacy trials is shown in Table 5:

Table 5. Overview of efficacy trials for VTR-xylanase

Laying hens			
Study	Replicates/treatments	VTR-xylanase (U/kg feed)	Duration (days)
1	8	0, 1000, 1500, 2000	28
2	10	0, 1000, 1500, 2000	63
3	10	0, 1000, 1500, 2000	63
Chickens for fattening			
Study	Replicates/treatments	VTR-xylanase (U/kg feed)	Duration (days)
1	52 (days 0-21) 26 (days 21-35)	0, 1000, 1500, 2000	35
2	9	0, 1000, 1500, 2000	35
3	9	0, 1000, 1500, 2000	35
Weaned piglets			
Study	Replicates/treatments	VTR-xylanase (U/kg feed)	Duration (days)
1	8	0, 1000, 1500, 2000	17
2	10	0, 1000, 1500, 2000	42
3	10	0, 1000, 1500, 2000	42

The Committee queried the use of only the powdered form of the additive in the efficacy trials and the comparability of these result for the liquid form of the additive. In response to a request for information the applicant clarified the equivalence of the powder and liquid form, both being derived from the same concentrate. The Committee reviewed the data presented and concluded that the additive VTR-xylanase can be considered efficacious in all avian species, weaned piglets, suckling piglets and minor growing porcine species under proposed conditions of use.

2.3.1. Conclusions on efficacy

The ACAF concluded that VTR-xylanase is efficacious for weaned piglets, suckling piglets, minor growing porcine species, laying hens and other laying birds at 2,000 U/kg, and in chickens for fattening and minor avian species at 1,000 U/kg.

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-beta-xylanase (EURL-FA, 2023):

> "For the determination of endo-1,4-beta-xylanase (xylanase) in the feed additive, premixtures and compound feed the Applicant submitted a colorimetric method, based on the quantification of water-soluble dyed fragments produced by the action of xylanase on a commercially available (Xylazyme AX®, Megazyme) azurine cross-linked wheat arabinoxylan substrate. A sample of the feed additive or compound feed is suspended in phosphate buffer (pH 6.5) shaking it gently at room temperature for 15 min. The obtained extract is then centrifuged and appropriately diluted. An aliquot of the obtained supernatant is then placed into a test tube. One Xylazyme AX® tablet is added to the tube and incubated at 37 °C. The reaction is then stopped by adding TRIS buffer into the tube, mixing vigorously and letting rest at room temperature. Then the solution is shaken again and filtered through a syringe membrane filter. The content of xylanase is finally determined by colorimetry at 590 nm using a calibration curve prepared with a reference xylanase enzyme in the buffer solution (for the feed additive) or in a blank feed extract (for the compound feed). The xylanase enzyme of known activity is available from the Applicant upon request. The Applicant, upon request of the EURL single-laboratory validated and further verified the proposed method for the feed additive formulation and for compound feed. A summary of the relevant performance characteristics as provided by the Applicant is shown in Table 1. Furthermore, the Applicant reported a limit of quantification (LOQ) of 0.57 U / g compound feed. For the determination of endo-1,4-beta-xylanase (xylanase) in premixtures the Applicant proposed a solid dilution of the premixtures with blank feed followed by the analysis of the diluted samples according to the method for compound feed described above. Furthermore, the

> Applicant, in the frame of the stability studies, provided experimental evidence demonstrating the suitability of this approach. Based on the performance characteristics available, the EURL recommends for official control the described above single-laboratory validated and further verified colorimetric methods for the quantification of xylanase activity in the feed additive, premixtures and compound feed."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The ACAF concluded that the additive was correctly characterised, and that it is stable for 12 months in its powder form when stored at 25°C, and for 18 months in its liquid form when stored at 5°C. The additive is stable at 70°C for 15 seconds during pelleting. Homogeneity in feed was also demonstrated. No further concerns were raised for section II of the dossier.

The additive can be considered safe for the target species, consumers and the environment. The additive should be regarded as a skin sensitiser in both its powder and liquid form. The liquid form of the additive is not a skin irritant, in its powder form, the additive should be considered a potential skin irritant. The additive should be considered an irritant to the eyes in both forms. As an enzyme preparation, the additive should be considered a potential respiratory sensitiser.

The ACAF concluded that VTR-xylanase is efficacious for weaned piglets, suckling piglets, minor growing porcine species, laying hens and other laying birds at 2,000 U/kg, and in chickens for fattening and minor avian species at 1,000 U/kg.

The FSA/FSS agree with the conclusions reached by the ACAF. FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
DLPCBs	Dioxin-like polychlorinated biphenyls

Acronym	Definition
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
EURL-FA	European Union Reference Laboratory for Feed Additives
FSA	Food Standards Agency
FSS	Food Standards Scotland
LOQ	Limit of Quantification
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCDD/Fs	Polychlorinated dibenzo-p-dioxins and dibenzofurans
QPS	Qualified Presumption of Safety
U	Enzyme units

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References

EC (European Commission). (2003). *Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2003/1831/contents

EURL-FA (European Reference Laboratory for Feed Additives). (2023). *Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Endo-1,4-beta-xylanase (EC 3.2.1.8)*. FAD-2021-0068/FAD-2021-0080 – European Commission (europa.eu).