Assessment on the safety and efficacy of endo-1,4-betaxylanase (Hostazym® X) as a feed additive for use in chickens for fattening, laying hens, turkeys for fattening, minor poultry species for fattening, minor poultry species for laying, weaned piglets, pigs for fattening, chickens reared for laying, carp, breeding hens, turkeys reared for breeding, ornamental birds, sucking piglets, minor pig species for fattening and minor poultry species reared for breeding.

Reference number RP309

Regulated Products Risk Assessment Unit
Science, Evidence and Research Division, FSA

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Regulated Product Dossier Assessment
Assessment finalised: 04/12/2023

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Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
LOQ	Limit of quantification
RSD_{ip}	Standard deviation for intermediate precision
RSD_{int}	Standard deviation for intermediate precision
RDS_{R}	Standard deviation for reproducibility
R_{Rec}	Recovery rate

Summary

An application was submitted to the Food Standards Agency in February 2021 from Huvepharma NV ("the applicant") for the authorisation of an additive (endo-1,4-betaxylanase - Hostazym® X), under the category 'zootechnical additives', functional group 'digestibility enhancers' for two uses:

- A renewal of authorisation for its use in chickens for fattening, laying hens, turkeys for fattening, minor poultry species for fattening, minor poultry species for laying, weaned piglets, pigs for fattening, chickens reared for laying and carp.
- A new use in breeding hens, turkeys reared for breeding, ornamental birds, suckling piglets, minor pig species for fattening and minor poultry species reared for breeding.

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

The Joint Expert Group on Animal Feed and Feed Additives (AFFAJEG) concluded that both solid formulations of the additive had a considerable percentage of respirable particles to which workers could be exposed by inhalation.

The additive can be considered safe for the target species, consumers and the environment, based on the literature review presented by the applicant. Regarding user safety, the Group agreed with the applicant that the additive should be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser.

The data that demonstrated efficacy in the target species covered by the existing authorisation could be used as evidence of efficacy also in the new species proposed for the extension of authorisation.

The views of AFFAJEG and 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 a risk assessment for a feed additive (endo-1,4-betaxylanase - Hostazym® X, Huvepharma NV., Uitbreidingstraat 80, 2600 Antwerp, Belgium) under retained regulation No 1831/2003[1] under the category 'zootechnical additives', functional group 'digestibility enhancers' for two uses:

- A renewal of authorisation for its use in chickens for fattening, laying hens, turkeys for fattening, minor poultry species for fattening, minor poultry species for laying, weaned piglets, pigs for fattening, chickens reared for laying and carp.
- A new use in breeding hens, turkeys reared for breeding, ornamental birds, suckling piglets, minor pig species for fattening and minor poultry species reared for breeding.

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

The dossier was evaluated on behalf of the FSA and FSS by the AFFAJEG. 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.

With thanks to the members of the AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Professor Katrina Campbell, Susan MacDonald, Professor Matthew Fisher, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Dr. Helen Warren and Dr. Nick Wheelhouse.

The dossier was evaluated by the AFFAJEG at their April 2021 meeting and by ACAF at their October 2022 meeting. Further information was provided by the applicant in August 2022, responding to queries by the FSA. The conclusions by the AFFAJEG were reviewed and approved by the ACAF at their October 2023 meeting.

This document outlines the discussion and conclusions of the AFFAJEG's assessment on the safety and efficacy of Hostazym® X as a feed additive.

2. Assessment

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

The active substance of Hostazym® X is endo-1,4-betaxylanase, produced by the fungus *Trichoderma citrinoviride*. The concentration of active substance present depends on the formulation of product: Hostazym® X 6000 MicroGranulate (0.65%), Hostazym® X 30000 MicroGranulate (3.28%), Hostazym® X 6000 Liquid (0.69%) and Hostazym® X 15000 Liquid (1.72%). Compositions of batches of different product forms are shown in Table 1 and Table 2.

Table 1: Composition of Hostazym® X 6000 MicroGranulate and Hostazym® 30000 MicroGranulate.

Name of ingredients	**Content, % w/w**	
	Hostazym® X 6000	Hostazym® X 30000
Active substance:		
Endo-1,4-betaxylanase purified fermentation product with activity 1 000 000 EPU/g	0.65	3.28
Other components:		
Pregelatinised starch	0.68	0.68
Wheat meal	Up to 100	Up to 100

Table 2: Composition of Hostazym® X 6000 Liquid and Hostazym® X 15000 Liquid.

Name of ingredients	**Content, % w/w**	
	Hostazym® X 6000	Hostazym® X 15000
Active substance:		
Endo-1,4-betaxylanase purified fermentation product with activity 1 000 000 EPU/g	0.69	1.72
Other components:		
Sorbitol, liquid	40.5	54.2
Propylene glycol	11.0	11.0
Sodium benzoate	0.3	0.3
Potassium sorbate	0.1	0.1
Water, purified	Up to 100	Up to 100

The applicant provided data from five batches supporting the specification values given below (Tables 3 and 4).

Table 3: Identity table for Hostazym® X 6000 and 30000 MicroGranulate.

Test characteristics	Requirements
Appearance	Granules
Colour	Beige
Loss on drying, %	Not more than 10.0
Dusting potential	< 00.00 g/m^3
Endo-1,4-betaxylanase content, EPU/g: Hostazym® X 6000 MicroGranulate Hostazym® X 30000 MicroGranulate	 Not less than 6000 Not less than 30000
Microbial contamination: *Salmonella*, CFU/25 g	 Absent
Heavy metals: Arsenic, mg/kg Lead, mg/kg Cadmium, mg/kg Mercury, mg/kg	 Not more than 4 Not more than 10 Not more than 0.5 Not more than 0.2

Table 4: Identity table for Hostazym® X 6000 and 15000 Liquid.

Test characteristics	Requirements
Appearance	Liquid
Colour	Yellowish to brown
Loss on drying, %	From 1.10 to 1.30
Endo-1,4-betaxylanase content, EPU/g: Hostazym® X 6000 Liquid Hostazym® X 15000 Liquid	 Not less than 6000 Not less than 15000
Microbial contamination: *Salmonella*, CFU/25 g	 Absent
Heavy metals: Arsenic, mg/kg Lead, mg/kg Cadmium, mg/kg Mercury, mg/kg	 Not more than 4 Not more than 10 Not more than 0.5 Not more than 0.2

The Group evaluated the identity and characterisation of the additive and no issues were raised. Members noted that a high proportion of the particles had diameters of less than 100 µm and so the applicant was asked to measure the particle size distribution of the additive by laser diffraction to help inform the safety of the additive for users. The results of this showed that both solid formulations had more than 1% by weight consisting of particles with a diameter of 50 micrometres or less, indicating a potential inhalation hazard for workers. The product does not generate dust, as showed by a dusting potential of 0 g/m^3 in the Stauber-Heubach test.

In the characterisation section, the additive is described as being “readily soluble in water”, leading members to raise concerns regarding the use of the additive in carp feed. Therefore, the applicant was asked to provide further information on the formulation of the product and how it remains efficacious in water. In their response,

the applicant stated that carp immediately consume feed that floats on the water after it is spread in ponds or cages. Extruded feed containing the additive is also expected to float on water, facilitating rapid consumption by carp before the additive dissolves in water.

The manufacturing process was well detailed with no concerns raised by members for this section.

The proposed conditions of use of the additive are described in Table 5.

Table 5. Proposed conditions of use of additive.

Proposed mode of use in animal nutrition				
Additive			Hostazym® X (endo-1,4-betaxylanase)	
Registration N°			4a1617	
Category of additive			Zootechnical feed additive	
Functional group(s) of additive			Digestibility enhancer	
Description				
Composition, description		**Purity criteria**		**Method of analysis**
Endo-1,4-betaxylanase purified fermentation product		Not less than 1 000 000 EPU/g		In-house validated spectrophotometric method
Trade name (if appropriate)			Hostazym® X	
Name of the holder of authorisation (if appropriate)			Huvepharma	
Species or category of animal	**Maximum age**	**EPU of active substance per kg of complete feedingstuffs**		**Other provisions**
		Minimum content	**Maximum content**	
All turkey species Minor poultry species for fattening Carp	-	1 050 EPU	-	1. In the directions for use of the additive and premixture, the storage conditions and stability to heat treatment shall be indicated. 2. For use in feed rich in starch and non-starch polysaccharides (mainly beta-arabinoxylans). 3. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from their use. Where those risks cannot be eliminated or reduced to a minimum by such
Chickens for fattening Chickens reared for laying Laying hens Breeding hens Minor poultry species reared for laying or breeding Minor poultry species for laying or breeding Ornamental birds Piglets (suckling and weaned) Pigs for fattening	-	1 500 EPU	-	

				procedures and measures, the additive and premixtures shall be used with personal protective equipment, including skin, eyes and breathing protections. 4. For use in suckling piglets for the period when solid feed is given.

2.1.1. Conclusions on Section II

The AFFAJEG concluded that the additives were correctly identified and characterised.

No further concerns were raised for Section II of this dossier.

2.2. Section III: Safety

The applicant provided a literature review to support the safety evaluation of the additive for target species, consumers, users and the environment. In this literature review, the applicant considered the safety of the production strain *Trichoderma citrinoviride* and any potential adverse events found with this strain or xylanases in general. The Group considered the review to be of a high standard and its conclusions did not report any adverse reactions or incidences of concern.

2.2.1. Safety for the target species

For the renewal of authorisation, the applicant proposes a tolerance level of up to 300,000 EPU/kg and claims that this safety level can be extrapolated to breeding hens, turkeys for breeding purposes, minor poultry species, ornamental birds, suckling piglets and minor pig species for fattening. The Group were satisfied with this extrapolation.

2.2.2. Safety for the consumer

No further data was provided by the applicant as the literature search did not reveal any issues with the safety of the additive.

2.2.3. Safety for the user

The Group agreed with the applicant's proposal that the additive be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser. Particle size distribution data indicated that both solid formulations of the additive had a considerable percentage of respirable particles to which workers could be exposed through inhalation. No acute inhalation toxicity studies would be needed as the results would not change the need to regard the additive as potentially hazardous by inhalation and appropriate protection used.

2.2.4. Safety for the environment

The proteinaceous nature of the additive means the majority will be degraded in the gastro-intestinal tract, therefore, accumulation in the environment was not a concern to the Group.

2.2.5. Conclusions on safety

The AFFAJEG concluded that the additive can be considered safe for the target species, consumer and the environment. The additive should be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser.

2.3. Section IV: Efficacy

Efficacy studies are not required for the renewal of authorisations of enzymes, therefore, no new efficacy data are provided for turkeys for fattening and minor poultry species for fattening, chickens for fattening, minor poultry species for laying, weaned piglets, pigs for fattening, chickens reared for laying, minor poultry reared for laying and carp. Members concluded that the data that demonstrated efficacy in the target species covered by the original authorisation can be used as evidence of efficacy also in the new species, including breeding hens, turkeys for breeding purposes, minor poultry species for breeding purposes, turkeys reared for breeding, minor poultry species reared for breeding, ornamental birds, suckling piglets for the period when solid feed is given and minor pig species for fattening.

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®[4]:

All species other than carp:

"For the determination of the activity of endo-1,4-b-xylanase in the feed additive, premixtures and feedingstuffs, the applicant proposes a single laboratory validated and further verified colorimetric methods based on the quantification of water soluble dyed fragments produced by the action of endo-1,4-beta-xylanase on commercially available azurine cross-linked wheat arabinoxylan substrates. Enzymatic activity of the sample is calculated using a reference enzyme standard, available from the applicant upon request. The following method performance characteristics were derived from the validation and verification studies: for the feed additive: - a relative standard deviation for repeatability (RSD_r) ranging from 1.9 to 3.3 %; - a relative standard deviation for intermediate precision (RSD_{int}) ranging from 1.9 to 3.3 %; and - a recovery rate (R_{Rec}) ranging from 101 to 104 %; for premixtures: - RSD_r ranging from 3.2 to 8.2 %; - RSD_{int} = 3.2 %, and - R_{Rec} ranging from 96 to 103 %; for feedingstuffs: - RSD_r ranging from 7.6 to 16 %; - RSD_{int} ranging from 8.9 to 16 %; - R_{Rec} ranging from 93 to 112 %; - a limit of detection (LOD) and quantification (LOQ) of 107 and 358 EPU/kg, respectively.

Based on the satisfactory performance characteristics mentioned above, the CRL recommends for official control the single laboratory validated and further verified colorimetric methods submitted by the applicant for the determination of the activity of endo-1,4-beta-xylanase in the feed additive, premixtures and feedingstuffs."

Carp:

"For the quantification of the endo-1,4-β-xylanase activity in the feed additive, premixtures and feedingstuffs, the Applicant submitted spectrophotometric methods based on the quantification of water soluble dyed fragments produced at pH 4.7 and 50 °C by the action of endo-1,4-β-xylanase on commercially available azurine cross-linked wheat arabinoxylan substrates from Megazyme. The analytical methods

presented were already evaluated in the frame of the authorised dossier FAD-2010-0001. Furthermore, the Applicant submitted a verification study demonstrating the fitness for purpose of the analytical method for the quantification of endo-1,4-β-xylanase in fish feed. The following performance characteristics were derived from the data presented: a relative standard deviation for repeatability (RSD_r) and for intermediate precision (RSD_{ip}) ranging from 1.9 to 16 %; and a recovery rate (R_{Rec}) ranging from 93 to 116 %. Based on the satisfactory performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified colorimetric methods submitted by the Applicant for the quantification of endo-1,4-beta-xylanase in the feed additive, premixtures and feedingstuffs."

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

After the applicant had provided further information on the particle size distribution and the formulation of the product used in carp feed, no further causes for concern were identified by the AFFAJEG in the identity, characterisation and manufacturing sections.

The AFFAJEG concluded that the additive can be considered safe for the target animal species, the consumer and the environment. Due to the proteinaceous nature of the additive, it should be assumed to be a potential respiratory sensitiser. Additionally, the additive should be considered a potential skin and eye irritant, and a potential skin sensitiser.

Re-evaluation of efficacy was not required for the renewal of authorisation and the AFFAJEG was able to conclude that the additive was expected to be also efficacious in the new species including breeding hens, turkeys for breeding purposes, minor poultry species for breeding purposes, turkeys reared for breeding, minor poultry species reared for breeding, ornamental birds, suckling piglets for the period when solid feed is given and minor pig species for fattening.

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

5. References

1. EC (European Commission), 2003. Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition.

2. EURL-FA (European Reference Laboratory for Feed Additives), 2016. 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. Available at: FAD-2010-0001 (europa.eu)

3. EURL-FA (European Reference Laboratory for Feed Additives), 2017. 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. Available at: FAD-2017-0010 (europa.eu)

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