Assessment of the safety and efficacy of endo-1,4-betaxylanase and endo-1,3(4)-beta-glucanase (Axtra® XB) as a feed additive for all avian species reared for fattening, reared for breeding, reared for laying, laying hens, lactating sows, weaned piglets, pigs for fattening and minor porcine species

Reference number RP416

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

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Regulated Product Dossier Assessment
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Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
CFU	Colony forming units
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
LOQ	Limit of quantification
MSDS	Material Safety Data Sheet
ppm	Parts-per million
OECD	Organisation for Economic Cooperation and Development
RSD_r	Standard deviation for repeatability
RSD_{int}	Standard deviation for intermediate precision
R_{Rec}	Recovery rate

Summary

An application was submitted to the Food Standards Agency in February 2021 by Genencor International B.V. ("the applicant") for the authorisation of an additive (endo-1,4-betaxylanase and endo-1,3(4)-beta-glucanase - Axtra® XB), under retained regulation No 1831/2003[1]. The application seeks authorisation under the category 'zootechnical additives', functional group 'digestibility enhancers' for two conditions:

- A renewal of authorisation for use in all avian species reared for fattening, reared for breeding, and reared for laying, laying hens, weaned piglets and pigs for fattening, lactating sows, and growing and lactating minor porcine species.
- An extension of use for suckling piglets.
- A modification of authorisation for the reduction of the minimum dose for turkeys for fattening and breeding (610 U xylanase /kg of complete feed and 76 U glucanase /kg of complete feed), by extrapolation of safety and efficacy data from chickens for fattening.

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 Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) concluded that the additive was correctly identified and characterised, and that the additive can be considered safe for the target species, the consumer and the environment. The product should be considered a respiratory sensitiser and a potential eye and skin irritant. The product is not a skin sensitiser.

Based on data from a new trial in turkeys, the ACAF concluded that Axtra® XB, in both formulations (TPT/L), has the potential to be efficacious in turkeys for fattening and breeding at the proposed reduced minimum dose of 610 U/kg of complete feed (xylanase) and 76 U/kg of complete feed (glucanase). Previous conclusions on efficacy can be extrapolated to suckling piglets.

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 and endo-1,3(4)-beta-glucanase - Axtra® XB, Genencor International B.V., Willem Einthovenstraat 4, 2342 BH, Oegstgeest, The Netherlands) under retained regulation (EC) No 1831/2003[1] under the category 'zootechnical additives', functional group 'digestibility enhancers' and for two conditions of authorisation:

- A renewal of authorisation for use in all avian species reared for fattening, reared for breeding, and reared for laying, laying hens, weaned piglets and pigs for fattening, lactating sows, and minor growing and lactating porcine species.
- An extension of use for suckling piglets.
- A modification of authorisation for the reduction of the minimum dose for turkeys for fattening and breeding (610 U xylanase /kg of complete feed and 76 U glucanase /kg of complete feed), by extrapolation of safety and efficacy data from chickens for fattening.

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 2022, and by ACAF at their December 2022 and April 2023 meetings. Further information was provided by the applicant in August 2022, December 2022 and February 2023 responding to queries by the FSA.

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

2. Assessment

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

The additive is a preparation containing endo-1,4-betaxylanase, produced by a genetically modified strain of *Trichoderma reesei*. The additive comes in a solid form (TPT) and a liquid form (L). The applicant claims the product guarantees a minimum activity of 12,000 U/g xylanase and 1,520 U/g glucanase. The applicant provided data from several batches supporting the identity values outlined below.

Table 1: Identity table Axtra® XB 201 TPT

Composition	
Endo-1,4-betaxylanase	Minimum activity of 12,000 U/g
Endo-1,3(4)-beta-glucanase	Minimum activity of 1,520 U/g
Appearance	
Off-white, fine granular product	
Chemical-physical characteristics	
Dusting potential	Average of 8.3 mg/m^3 of air
Particle size distribution	Average diameter of 478 µm; 0.1% of particles with diameter < 200 µm; absence of particles <10 µm
Microbiological profile	
Salmonella	Absent in 25 g of sample
E. coli	Absent in 25 g of sample
Total coliforms	< 30 CFU/g
Antimicrobial activity	Negative
Heavy metals	
Arsenic	0.1 - 0.2 mg/kg
Lead	0.32 - 0.38 mg/kg
Cadmium	0.04 mg/kg
Mercury	< 0.005 mg/kg
Mycotoxins	

Aflatoxin B1	< 0.5 µg/kg
Deoxynivalenol	< 20 µg/kg
Fumonisin B1+B2	< 40 µg/kg
Ochratoxin AT-2 & HT-2	< 10 µg/kg
Zearalenone	< 10 µg/kg

Table 2: Identity table Axtra® XB 201 L

Composition	
Endo-1,4-betaxylanase	Minimum activity of 12,000 U/g
Endo-1,3(4)-beta-glucanase	Minimum activity of 1,520 U/g
Appearance	
Light brown liquid preparation	
Chemical-physical characteristics	
Viscosity	12.5-16.4 mPa*s at 20°C
Microbiological profile (CFU/g)	
Salmonella	Absent in 25 g of sample
E. coli	Absent in 25 g of sample
Total coliforms	< 30 CFU/g
Antimicrobial activity	Negative
Heavy metals	
Arsenic	< 0.1 mg/kg
Lead	< 0.05 mg/kg
Cadmium	< 0.01 mg/kg
Mercury	< 0.005 mg/kg
Mycotoxins	
Aflatoxin B1	< 1 µg/kg
Deoxynivalenol	< 20 µg/kg
Fumonisin B1+B2	< 40 µg/kg
Ochratoxin AT-2 & HT-2	< 2 µg/kg
Zearalenone	< 10 µg/kg

After the first evaluation of the application, the AFFAJEG identified several items of information that the applicant would have to provide in order to inform the assessment of the product, including: testing for absence of the production strain in the final product, updated certificates, clarification on the composition of the additive and provision of material safety data sheets (MSDS) for all substances used in the production process. These queries were satisfactorily addressed by the applicant. It was also confirmed by the Committee that the product under renewal is the same as that of the original application.

The stability of the additive under conditions of high heat for several minutes was questioned by the Group, in answer to which the applicant presented further evidence, showing the additive is stable for up to 2 minutes at 82°C. The product's homogeneity when mixed was also shown after a request by the Group.

The applicant proposes that the additive is included in animal feed as shown in Table 3:

Table 3: Proposed mode of use of Axtra® XB 201 (TPT/L)

Proposed mode of use in animal nutrition			
Additive		12,000 U/g xylanase and 1,520 U/g glucanase	
Registration number		4a15	
Category and functional group		Zootechnical additive, digestibility enhancer	
Description			
Composition, description		**Formula**	**Method of analysis**
Preparation of xylanase and glucanase produced by *Trichoderma Reesei*		Minimum xylanase activity: 12,000 U/g Minimum glucanase activity: 1,520 U/g	Colourimetric method
Trade name			Axtra® XB 201 (TPT/L)
Name of the holder of authorisation			Genencor International B.V.
Conditions of use			
Species or category of animal	**Maximum Age**	**Minimum enzyme content in complete feed**	**Withdrawal period**
All avian species for fattening	To slaughter age and weight	610 U/kg xylanase 76 U/kg glucanase	N/A
All avian species reared for breeding	No maximum age		
All avian species reared for laying	To point of lay		
Piglets (weaned and suckling)	Up to 35 kg		
Pigs for fattening	To slaughter age and weight		
Minor growing porcine species	To slaughter age and weight		

Laying hens	No maximum age	1220 U/kg xylanase 152 U/kg glucanase	
Lactating sows	No maximum age		
Minor lactating porcine species	No maximum age		

2.1.1. Conclusions on Section II

The AFFAJEG/ACAF concluded that the additive was correctly identified and characterised.

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

2.2. Section III: Safety

2.2.1. Safety for the target species

The applicant presented previous conclusions by EFSA[2,3] and a comprehensive literature review. Since the application included a request for modifying the authorisation and extrapolation of data and given that the EFSA conclusions were based on an outdated maximum recommended dose, the Group requested the applicant to provide the original toxicological study reports. After re-evaluating the data, the Committee concluded that the additive can be considered safe for the proposed target species at the suggested dose.

2.2.2. Safety for the consumer

The AFFAJEG evaluated the literature review provided by the applicant as well as and the toxicological studies on which previous EFSA conclusions were based. The Group concluded that the additive remains safe for consumers.

2.2.3. Safety for the user

The additive is proteinaceous in nature and is, therefore, regarded as a respiratory sensitiser by default. Measures should be taken to minimise worker exposure by inhalation. Based on previous tests presented, the AFFAJEG concluded that the additive is a potential eye irritant and a potential skin irritant. Two new skin sensitisation tests (local lymph node assays in mice) were evaluated by the Group, who concluded that the additive is not a dermal sensitiser.

2.2.4. Safety for the environment

The active substances of the additive are proteins and are expected to be degraded in the digestive tract of the target animals. The AFFAJEG concluded that the additive can be considered safe for the environment.

2.2.5. Conclusions on safety

The AFFAJEG/ACAF concluded that the additive can be considered safe for the target species, the consumer and the environment.

The additive should be considered a respiratory sensitiser and a potential eye irritant. The product is not a skin sensitiser.

2.3. Section IV: Efficacy

The efficacy of the additive for the target species listed in the renewal of authorisation did not require evaluation. Conclusions on efficacy can be extrapolated to suckling piglets.

The AFFAJEG evaluated the initial efficacy evidence presented for a proposed reduced minimum dose in turkeys for fattening and breeding of 610 U/kg of complete feed (xylanase) and 76 U/kg of complete feed (glucanase), largely based on EFSA's previous opinion, which concluded favourably in two out of three efficacy trials. Two inconsistencies were noted: a disparity in the weight of the study animals and the fact that the minimum recommended dose was higher in the EFSA evaluation than for this application. The request to modify the authorisation to lower the minimum dose in turkeys was not supported by the evidence provided, as no difference in output was shown between control and treatment groups for the proposed dose of 610 U/kg xylanase and 76 U/kg glucanase. The Group requested the applicant to provide the original efficacy studies and further evidence of efficacy in turkeys at the lower dose.

The applicant provided the information requested, including a new efficacy report in turkeys. The AFFAJEG re-evaluated the studies and concluded that the additive has the potential to be efficacious in turkeys for fattening and breeding at the proposed reduced minimum dose of 610 U/kg of complete feed (xylanase) and 76 U/kg of complete feed (glucanase).

2.3.1. Section IV: Conclusions on Efficacy

The efficacy of the additive for the target species listed in the renewal of authorisation did not require evaluation. Previous conclusions on efficacy can be extrapolated to suckling piglets.

The additive has the potential to be efficacious in turkeys for fattening and breeding at the proposed reduced minimum dose of 610 U/kg of complete feed (xylanase) and 76 U/kg of complete feed (glucanase).

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the Community Reference Laboratory for Feed Additives for the Methods(s) of Analysis for endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase[4]:

“For the determination of the activity of endo-1,4-β-xylanase in the feed additive, premixtures and feedingstuffs, the applicant proposes colorimetric methods based on the quantification of water soluble dyed fragments produced by the action of endo-1,4-β-xylanase on commercially available azurine cross-linked wheat arabinoxylan substrates. Enzymatic activity of the sample is calculated using a reference enzyme standard. These methods were single laboratory validated and further verified by a second independent laboratory. 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 6.6 to 9.5 %, - a relative standard deviation for intermediate precision (RSD_{int}) ranging from 7.2 to 11 %, and – a recovery rate (R_{Rec}) ranging from 103 to 112 %,

- for premixtures: - RSD_r ranging from 2.3 to 8.0 %, - RSD_{int} ranging from 6.6 to 6.9 %, and - R_{Rec} ranging from 93 to 95 %, and

- for feedingstuffs: - RSD_r ranging from 2.3 to 5.8 %, - RSD_{int} ranging from 4.0 to 6.9 %, - R_{Rec} ranging from 93 to 97 %, and a limit of quantification (LOQ) of 79 U/kg well below the minimum activity proposed by the applicant.

For the determination of the activity of endo-1,3(4)-β-glucanase in the feed additive, premixtures and feedingstuffs, the applicant proposes colorimetric methods based on the quantification of water soluble dyed fragments produced by the action of endo-1,3(4)-β-glucanase on commercially available cross-linked barley beta-glucan substrates. The enzymatic activity of the sample is calculated using reference enzyme standard. These methods were single laboratory validated and further verified by a second independent laboratory. The following method performance characteristics were derived from the validation and verification studies:

- for the feed additive: - RSD_r ranging from 2.5 to 3.2 %, - RSD_{int} ranging from 3.0 to 11.9 %, and - R_{Rec} ranging from 88 to 109 %,
- for premixtures: - RSD_r ranging from 4.3 to 8.5 %, - RSD_{int} ranging from 6.8 to 14 %, and - R_{Rec} ranging from 92 to 97 %, and
- for feedingstuffs: RSD_r ranging from 7.3 to 8.5 %, - RSD_{int} ranging from 8.5 to 14.0 %, - R_{Rec} ranging from 92 to 94 %, and an acceptable LOQ = 51 U/kg.

Based on the satisfactory performance characteristics mentioned above, the CRL recommends for official control the single laboratory validated and further verified analytical methods submitted by the applicant for the determination of the activity of endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase 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

The AFFAJEG/ACAF concluded that the additive was correctly identified and characterised.

The AFFAJEG/ACAF concluded that the additive can be considered safe for the target species, the consumer and the environment.

The additive should be considered a respiratory sensitiser and a potential eye and skin irritant. The product is not a skin sensitiser.

The additive has the potential to be efficacious in turkeys for fattening and breeding at the proposed reduced minimum dose of 610 U/kg of complete feed (xylanase) and 76 U/kg of complete feed (glucanase). Previous efficacy conclusions can be extrapolated to suckling piglets.

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. Available at https://www.legislation.gov.uk/eur/2003/1831/contents

2. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in animal feed), 2015. Scientific Opinion on the safety and efficacy of Danisco® Glycosidase TPT/L (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) as feed additive for poultry, piglets and pigs for fattening. EFSA Journal 2010;8(12):1916, 22 pp. https://doi.org/10.2903/j.efsa.2010.1916

3. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of Axtra® XB 201 (endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase) as a feed additive for lactating sows and minor porcine species. EFSA Journal 2016;14(1):4350, 11 pp. https://doi.org/10.2903/j.efsa.2016.4350

4. 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. Danisco Glycosidase TPT and L. Available at: FAD-2010-0007 (europa.eu)

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