REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive of Endo-1,4-Beta-Xylanase, Alpha-Amylase and Subtilisin Protease (Avizyme® 1505) as a Feed Additive for All Avian species. (RP1341)

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 November 2021 from Genencor International B.V ('the applicant') for the modification and new authorisation of an additive (Avizyme® 1505) containing endo-1,4-beta-xylanase produced by *Trichoderma reesei* (ATCC PTA 5588), subtilisin protease produced by *Bacillus subtilis* (ATCC 2107) and alpha-amylase produced by *Bacillus amyloliquefaciens* (ATCC 3978), under the category 'zootechnical' additive, functional group 'digestibility enhancer'. The additive is proposed to be used in all avian species for fattening, reared for laying, and reared for breeding (except ducks) at an inclusion rate of 0.125 kg/tonne, in all avian species for laying (except ducks) at an inclusion rate of 0.2 kg/tonne, and for all ducks at an inclusion rate of 0.05 kg/tonne.

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 the additive is stable in premixtures and feedingstuffs for 16 months at 20 °C. The additive is stable during pelleting at 90 °C for 30 seconds. Homogeneity in feed was also demonstrated.

The additive can be considered safe for the target animal, the consumer and the environment. The additive is a mild irritant to the skin and eyes and is not a dermal sensitiser. The additive contains enzymes that are potential respiratory sensitisers, and measures should be taken to avoid contact through inhalation.

It was concluded that Avizyme® 1505 is efficacious for all avian species for fattening, reared for breeding and reared for laying (except ducks), and for all avian species for laying (except ducks) at the proposed inclusion rates detailed under the conditions of use of the additive. Efficacy in ducks at the proposed inclusion rate was only demonstrated for the first 42 days.

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.

Introduction

The FSA and FSS have undertaken an assessment for a feed additive containing endo-1,4-beta-xylanase produced by *Trichoderma reesei* ATCC PTA 5588, subtilisin protease produced by *Bacillus subtilis* ATCC 2107 and alpha-amylase produced by *Bacillus amyloliquefaciens* ATCC 3978 (Avizyme® 1505, Genencor International B.V, Willem Einthovenstraat 4, 2343 BH, Oegstgeest, The Netherlands) under assimilated Regulation (EC) No 1831/2003 (EC, 2003), in the category 'zootechnical additive', functional group 'digestibility enhancer', for its use in all avian species (except ducks) for fattening, reared for laying, reared for breeding and for laying. The applicant also sought renewal of authorisation for ducks.

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 ACAF at its October 2023 meeting, and further complimentary information was evaluated at its January 2024 meeting. Following evaluation, a request for further information was

communicated to the applicant. The applicant's response to this request and subsequent requests were evaluated at its April 2024 and July 2024 meetings.

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, alpha-amylase and subtilisin protease	Feed Additive	Zootechnical additive	All avian species

2. Assessment

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

The additive is a preparation of endo-1,4-beta-xylanase produced by *Trichoderma reesei* (ATCC PTC 5588), subtilisin protease produced by *Bacillus subtilis* (ATCC 2107) and alpha-amylase produced by *Bacillus amyloliquefaciens* (ATCC 3978). All three production strains are genetically modified organisms. The applicant provided data from several batches supporting the identification values outlined below (Table 2).

Table 2. Identity table Avizyme® 1505

Active constituents	
Endo-1,4-beta-xylanase	1500 U/g
Subtilisin (protease)	20000 U/g
Alpha-amylase	2000 U/g
Appearance	
Light brown fine granular powder	
Chemical-physical specifications	
Bulk density	0.847 g/mL
Particle size distribution	< 100 µm – 11.9 % < 50 µm - 7.6 % < 10 µm - 1.5 %
Dusting potential	0.0 mg/m^3
Impurities	
Arsenic	< 0.1 mg/kg
Lead	< 0.05 mg/kg
Mercury	< 0.005 mg/kg
Escherichia coli	Absent in 25g
Coliforms	< 100 CFU/g
Salmonella spp.	Absent in 25g

The ACAF discussed the characterisation of the production microorganisms, concluding that further information would be required to allow a comprehensive assessment. The applicant provided further information for species identity, functional traits and antimicrobial production allowing the ACAF to conclude that the additive was correctly characterised. Absence of the production strain and recombinant DNA from the final product was demonstrated and the genetic modification of the production strain did not raise any further concerns for the safety of the additive.

The ACAF reviewed the changes made to the manufacturing process since the original authorisation and concluded that they do not significantly impact the additive and do not raise any additional safety concerns.

The ACAF reviewed the stability data provided, concluding that the additive is stable for 16 months at 20 °C. In premixtures and feedingstuffs, the additive is stable for 6 months at 20 °C. Stability during pelleting was confirmed at 90 °C for 30 seconds. Homogeneity of the additive was also demonstrated.

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

Table 3. Proposed conditions of use by the applicant for Avizyme® 1505

Conditions of use proposed by the applicant				
Species or category of animal	Min-max Age	Min. content	Max. content	Withdrawal period
		kg/tonne of complete feedingstuffs		
All avian species for fattening (except ducks)	To slaughter age and weight	0.125	Not applicable	Not applicable
All avian species reared for laying (except ducks)	To point of lay			
All avian species reared for breeding (except ducks)	No maximum age			
All avian species for laying (except ducks)	No maximum age	0.2		
Ducks	Throughout whole production cycle	0.05		

The ACAF were only able to conclude on the efficacy of the additive in ducks for the first 42 days and not for the whole production cycle. This is discussed further in Section IV.

2.1.1. Conclusions on Section II

The ACAF concluded that the additive was correctly characterised and is stable in premixtures and feedingstuffs for 16 months at 20 °C and is stable during pelleting at 90 °C for 30 seconds. Homogeneity in feed was also demonstrated. 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 ACAF reviewed the studies submitted for the original authorisation and the conclusions of FEEDAP from their original assessment. The ACAF agreed with FEEDAP's conclusion that a 15-fold overdose was well tolerated by chickens for fattening, female turkeys for fattening and laying hens. The ACAF concluded that the results can be extrapolated to all avian species for laying, for fattening, reared for breeding and reared for laying without the requirement of further studies. A literature review provided by the applicant did not reveal any new relevant studies that would affect safety for the target species. The Committee concluded that the additive can be considered safe for the target species.

2.2.2. Safety for the consumer

The ACAF concluded that the proposed modifications to the authorisation do not impact safety for the consumer. The literature review provided did not reveal any new relevant studies that would impact safety for the consumer. The ACAF concluded that the additive can be considered 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:

- Acute inhalation toxicity study (OECD 403)
- Acute dermal irritation/corrosion study (OECD 404)
- Acute eye irritation/corrosion (OECD 405)
- Skin sensitisation: Local lymph node assay (OECD 429)

The ACAF reviewed the studies provided, concluding that the additive is a mild irritant to the skin and eyes. The additive is not a dermal sensitiser. The enzymes in the additive are potential respiratory sensitiser as a result of their high protein content.

2.2.4. Safety for the environment

The ACAF concluded that the proposed modification to authorisation does not impact the additive's safety with regards to the environment. The literature review provided did not provided any further studies that would impact safety to the environment. The additive can be considered safe for the environment owing to the degradation of the enzyme in the target species.

2.2.5. Conclusions on safety

The literature review provided covering the period since the original authorisation did not raise any further concerns regarding the safety of the additive for the target species, the consumer, or the environment. The proposed modification to the authorisation did not raise any further safety concerns.

The additive is a mild irritant to the skin and eyes. The additive is not a dermal sensitiser. The additive contains potential respiratory sensitisers and measures should be taken to avoid contact through inhalation.

2.3. Section IV: Efficacy

The ACAF reviewed the EFSA opinion for the original authorisation, agreeing that the conclusions can be extrapolated to all avian species for fattening, reared for breeding and reared for laying (except ducks), and for all avian species for laying (except ducks) without the need for additional efficacy studies. Owing to the data presented, efficacy for ducks could only be concluded upon for 42 days. The additive is efficacious in all growing poultry species under the proposed conditions of use and is efficacious in ducks at the proposed inclusion rate for the first 42 days.

2.3.1. Conclusions on efficacy

The ACAF concluded that Avizyme® 1505 is efficacious for all avian species for fattening, reared for breeding and reared for laying (except ducks), and for all avian species for laying (except ducks) at the inclusion rates detailed in the proposed conditions of use of the additive. The additive is efficacious at the proposed inclusion rate for ducks, however, efficacy in ducks was only demonstrated for the first 42 days.

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 on the Method(s) of the Analysis for Avizyme® 1505 (CRL, 2010):

> "For the determination of endo-1,4-β-xylanase, α-amylase and subtilisin, the applicant proposed single-laboratory validated methods, further verified by a second independent laboratory. For the determination of the activity of endo-1,4-β-xylanase in the feed additive, premixtures and feedingstuffs, the applicant proposed colorimetric methods based on the quantification of water-soluble dyed fragments produced by the action of endo-1,4β-xylanase on commercially available azurine cross-linked wheat

arabinoxylan substrates. The analysis is carried out at pH 4.2 and 50 °C. The rate of dye release is measured on a spectrophotometer at 590 nm and quantified against a reference enzyme standard. The following method performance characteristics for feedingstuffs were recalculated by the CRL: - a relative standard deviation for repeatability (RSDr) ranging from 3.2 to 4.2 %, - a relative standard deviation for intermediate precision (RSDip) ranging from 4.2 to 5.3 %, - a recovery rate (RRec) of 97 %, and a limit of quantification (LOQ) of 133 U/kg which is well below the minimum activity proposed by the applicant. For the determination of the activity of α-amylase in the feed additive, premixtures and feedingstuffs, the applicant proposed colorimetric methods based on the quantification of water-soluble dyed fragments produced by the action of α-amylase on commercially available azurine cross-linked starch polymer substrates. The analysis of feed additive is carried out at the pH 7.0 and 40 °C, whereas the conditions of the analysis in premixtures and feedingstuffs are pH 6.3 and 37 °C. The rate of dye release is measured on a spectrophotometer at 590 nm and quantified against a reference enzyme standard. The following method performance characteristics for feedingstuffs were recalculated by the CRL: -RSDr ranging from 5.1 to 5.6 %, - RSDip ranging from 6.6 to 8 %, - RRec = 98 %, and LOQ = 189 U/kg, which is well below the minimum activity proposed by the applicant.

For the determination of the activity of subtilisin in the feed additive, premixtures and feedingstuffs, the applicant proposed colorimetric methods based on the quantification of water-soluble dyed fragments (azurine) produced by the action of subtilisin on commercially available cross-linked casein substrates. The analysis is carried out at pH 10.0 and 50 °C; the rate of dye release is measured on a spectrophotometer at 590 nm and quantified against a reference enzyme standard. The following method performance characteristics for feedingstuffs were recalculated by the CRL: - RSDr ranging from 5.4 to 6.5 %, - RSD ip ranging from 6.3 to 19 %, - R Rec = 93 %, and LOQ 1920 U/kg, which is well below the minimum activity proposed by the applicant. Based on the satisfactory performance characteristics mentioned above, the CRL recommends for official control the methods submitted by the applicant for the determination of endo-1,4-β-xylanase, α-amylase and subtilisin 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."

FSA/FSS accepts the CRL 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 2010, at the time in which the UK was still an EU member.

4. Conclusions

The ACAF concluded that the additive was correctly characterised and is stable for 16 months at 20 °C and during pelleting at 90 °C for 30 seconds. Homogeneity in feed was also demonstrated. No further concerns were raised for Section II of the dossier.

The ACAF concluded that the proposed level of inclusion in feed is safe for the target species and the consumer. The additive is a mild irritant to the skin and eyes and is not a dermal sensitiser. The additive is a potential respiratory sensitiser. Measures should be taken to avoid contact through inhalation. The additive does not pose a risk to the environment.

The ACAF concluded that Avizyme® 1505 is efficacious for all avian species for fattening, reared for breeding and reared for laying (except ducks), and for all avian species for laying (except ducks) at the inclusion rates detailed under the proposed conditions of use of the additive. The additive was only demonstrated to be efficacious in ducks for the first 42 days.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CFU	Colony forming units
CRL	Community Reference Laboratory for Feed Additives
FSA	Food Standards Agency
FSS	Food Standards Scotland
EC	European Commission
GMO	Genetically modified organism
LOQ	Limit of quantification
Rrec	Recovery rate
RSDip	Relative standard deviation for intermediate precision
RSDr	Relative standard deviation for repeatability
OECD	Organisation for Economic Co-operation and Development
U	Enzyme units

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