Assessment of the safety and efficacy of endo-1,4-beta-xylanase (Xygest® HT) as a feed additive for all poultry

Reference number RP226

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
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
AME	Apparent metabolizable energy
CAS	Chemical Abstracts Service
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
NDF	Neutral detergent fibre
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
U	Units of enzymatic activity

Summary

An application was submitted to the Food Standards Agency in February 2021 from Kemin Europa N.V. (“the applicant”) for the authorisation of an additive (endo-1,4-beta-xylanase-Xygest® HT), under the category of ‘zootechnical’ additives, functional group ‘digestibility enhancers’ for all poultry.

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 ACAF concluded that the additive was correctly identified and characterised. No causes for concern were raised in this section of the application.

The ACAF concluded that the additive can be considered safe for the target species, with a margin of safety of 300 for growing poultry and 25 for laying poultry. The additive can be considered safe for consumers and the environment. The additive should be considered a respiratory sensitiser. The additive is not an eye irritant, skin irritant or skin sensitiser.

Based on data from four trials in growing poultry and five trials in laying poultry, the ACAF concluded that the additive Xygest® HT can be considered to be efficacious in laying poultry and growing poultry. Conclusions can be extrapolated to all poultry.

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-beta-xylanase-Xygest® HT, Kemin Europa N.V., Toekomstlaan 42, 2200 Herentals, Belgium) under regulation (EC) No 1831/2003[1] under the category of 'zootechnical' additives, functional group 'digestibility enhancers' for its use in all poultry. 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 and the ACAF. 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 July 2021, October 2021 and February 2022 meetings, and by the ACAF at their July 2022 and December 2022 meetings. Further information was provided by the applicant in December 2021, April 2022 and November 2022, 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 Xygest® HT as a feed additive.

2. Assessment

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

The additive is a preparation containing a minimum of 3,000,000 U/g of endo-1, 4-β-xylanase (active substance) produced by genetically modified *Komagataella phaffii*, as well as corn starch and calcium carbonate, used as carriers. The applicant provided data from eighteen batches supporting the specification values outlined below (Table 1).

Table 1: Identity table

Composition	**CFU/g**	**w/w%**
Endo-1, 4-β-xylanase (active substance)	Minimum 3,000,000 U/g	11-20%
Corn starch	-	78-87%
Calcium carbonate	-	0.5-2%
Physico-chemical specifications		
Dusting potential	185-340 mg/m^3	
Particle size distribution	30% < 100 µm / 0.15% < 1 µm	
Purity specifications		
Coliform bacteria	Negative in 10 g	
Staphylococcus aureus	Negative in 10 g	
Salmonella spp.	Negative in 25 g	
E. coli	Negative in 10 g	
Fungal contamination	<100 CFU/g	

The Committee evaluated the identity and characterisation of the additive, noting that a full description of the genetic modification to the QPS microorganism *Komagataella phaffii* had been provided. No concerns were raised regarding the genetic modifications. No traces of genetic material were found in the final product.

In their first evaluation, members observed that the pelleting stability trials had been performed at varying temperatures for 30 seconds which is less stringent than those commonly used. The Committee evaluated several responses from the applicant aimed at addressing stability testing. At the December 2022 meeting, the latest stability test presented by the applicant was evaluated by the ACAF, which concluded that the additive could be considered stable under pelleting conditions of 86°C for 6 minutes.

The additive aims to supply a minimum of 30,000 U/kg of completed feed for growing poultry, and a minimum of 45,000 U/kg of complete feed for laying poultry. Conditions of use of the additive are summarised in Table 2:

Table 2: Proposed mode of use of Xygest HT

Proposed mode of use in animal nutrition				
Additive	Endo-1, 4-β-xylanase			
CAS No	9025-57-4			
Category(-ies) of additive	Zootechnical feed additive			
Functional group(s) of additive	Digestibility enhancer			
Description				
Composition, description	**Purity criteria**	**Method of analysis**		
Preparation of endo-1, 4-β-xylanase, corn starch and calcium carbonate	Containing a minimum of: 3,000,000 U/g	Validated internal method		
Trade name (if appropriate)		Xygest HT		
Name of the holder of authorisation (if appropriate)		--		
Conditions of use				
Species or category of animal	**Min-max Age**	**Min. content**	**Max. content**	**Withdrawal period**
		U of endo-1, 4-β-xylanase per kg of complete feed at 12% moisture		
All growing poultry	-	30,000 U/kg	-	-
All laying and breeding poultry	-	45,000 U/kg	-	-

2.1.1. Conclusions on Section II

The 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 ACAF evaluated two tolerance studies presented in the application.

Study 1 was carried out on chickens for fattening for 84 days, using doses of 45,000 U/kg, 90,000 U/kg and 9,000,000 U/kg of Xygest HT. No adverse effects were reported in the study. The mortalities reported had no statistical correlation to the additive. The Committee evaluated the data and concluded that the study showed that the additive is safe for growing poultry with a margin of safety of 100 times the maximum supplied dose (9,000,000 U/kg) and 300 times the minimum proposed inclusion rate of 30,000 U/kg.

Study 2 was carried out on laying hens for 84 days, using doses of 45,000 U/kg, 90,000 U/kg and 1,125,000 U/kg of Xygest HT. No adverse effects were reported in the study. The Committee evaluated the data and concluded that the study showed that the additive is safe for laying poultry with a margin of safety of 12.5 times the maximum supplied dose (90,000 U/kg) and 25 times the minimum proposed inclusion rate of 45,000 U/kg.

2.2.2. Safety for the consumer

The additive is an enzyme obtained from the QPS microorganism *Komagataella phaffii.* No studies for consumer safety were required.

2.2.3. Safety for the user

The additive is proteinaceous in nature and is, therefore, regarded as a respiratory sensitiser by default. A large proportion of particles (30% by weight) consisted of particles of inhalable size (less than 100 µm), but the additive was of low dusting potential. Thus, worker exposure by inhalation is expected to be quite low. Nevertheless, measures should be taken to minimise inhalation exposure.

Three more tests were presented to study the effects on eyes and skin:

- Acute dermal irritation/corrosion study in rabbits following OECD 404.
- Acute eye irritation/corrosion study in rabbits following OECD 405.
- Skin sensitisation study in guinea pigs following OECD 406.

Based on the data presented, the ACAF concluded that the additive is not an irritant to skin or eyes, and that it should not be considered a skin sensitiser.

2.2.4. Safety for the environment

The additive is an enzyme obtained from the QPS microorganism *Komagataella phaffii.* No studies for environmental safety were required.

2.2.5. Conclusions on safety

The ACAF concluded that the additive can be considered safe for the target species, with an established margin of safety of 300 for growing poultry and 25 for laying poultry. The additive can be considered safe for consumers and the environment.

The additive should be considered a respiratory sensitiser. The additive is not an eye irritant, skin irritant or skin sensitiser.

2.3. Section IV: Efficacy

The Committee evaluated Section IV of the dossier, containing four efficacy trials in growing poultry, and five efficacy trials in laying poultry.

2.3.1. Efficacy studies in growing poultry

- Study 1 was carried out on 900 Ross chickens for fattening for 35 days, with inclusion rates of 30,000 U/kg, 45,000 U/kg, 90,000 U/kg and 9,000,000 U/kg into a wheat-based growing poultry diet.
- Study 2 was carried out on 1000 Ross chickens for fattening for 35 days, with inclusion rates of 30,000 U/kg, 45,000 U/kg and 90,000 U/kg into a corn and soybean-based growing poultry diet.
- Study 3 was carried out on 288 Ross chickens for fattening for 21 days, with inclusion rates of 30,000 U/kg, 45,000 U/kg and 90,000 U/kg into a corn and soybean-based growing poultry diet.
- Study 4 was carried out on 368 Ross chickens for fattening for 35 days, with an inclusion rate of 30,000 U/kg into a corn and soybean-based growing poultry diet.

The Committee evaluated the reports presented and noted that the trials were designed and carried out to a high standard. The results of the studies are summarised in Table 3. The ACAF concluded that the additive was efficacious in growing poultry when included in feed at a dose of 30,000 U/kg of complete feed.

Table 3: Effect of Xygest HT on chickens for fattening

		Final Body Weight (kg)	Daily Feed Intake (g)	Feed Conversion Ratio	NDF Digestibility (%)	AME (kcal/kg)
Trial I	Control	1.91^{b}	84.2	1.62^{a}		
	30,000 U/kg	2.04^{a}	85.7	1.54^{b}		
	45,000 U/kg	1.99^{ab}	84.7	1.53^{b}		
	90,000 U/kg	1.93^{b}	82.4	1.55^{b}		
	9,000,000 U/kg	1.20^{ab}	85.4	1.54^{b}		
Trial II	Positive Control (negative control feed + 100 Kcal AME)	2.26^{ab}	94.7^{b}	1.50^{bc}	17.1^{c}	
	Negative Control	2.02^{c}	98.7^{a}	1.76^{a}	12.5^{b}	
	30,000 U/kg	2.19^{b}	95.7^{b}	1.56^{b}	22.1^{a}	
	45,000 U/kg	2.23^{ab}	95.7^{b}	1.53^{bc}	23.9^{a}	
	90,000 U/kg	2.33^{a}	95.7^{b}	1.47^{c}	25.1^{a}	
Trial III	Control	1.06	64.1	1.27		3,047.7^{b}
	45,000 U/kg	1.05	62.2	1.24		3,238.8^{a}
	90,000 U/kg	1.07	61.3	1.21		3,236.4^{a}
Trial IV	Control	2.72	108.3	1.39^{a}		
	30,000 U/kg	2.73	106.6	1.35^{b}		

NDF: Neutral detergent fibre; AME: Apparent metabolizable energy; Values within the same column, within a trial, that differ significantly ($p<0.05$) are indicated by different letters.

2.3.2. Efficacy studies in laying poultry

- Study 1 was carried out on 128 Hisex laying hens for 84 days, with inclusion rates of 0 U/kg, 45,000 U/kg, 90,000 U/kg and 1,125,000 U/kg into a wheat, soybean and sunflower-based feed.

- Study 2 was carried out on 288 Hy Line laying hens for 84 days, with inclusion rates of 0 U/kg, 45,000 U/kg and 90,000 U/kg into a wheat, soybean and rye-based feed.

- Study 3 was carried out on 96 Isa Brown laying hens for 28 days, with inclusion rates of 0 U/kg, 30,000 U/kg and 45,000 U/kg into a wheat, wheat bran and soybean-based feed.

- Study 4 was carried out on 600 Lohmann laying hens for 84 days, with an inclusion rate of 0 U/kg and 45,000 U/kg into a corn and soybean-based feed.

- Study 5 was carried out on 128 Isa Brown laying hens for 84 days, with an inclusion rate of 0 U/kg, 45,000 U/kg and 90,000 U/kg into a wheat and soybean-based feed.

The Committee evaluated the reports presented and noted that the trials were designed and carried out to a high standard. The results of the studies are summarised in Table 4. The ACAF concluded that the additive was efficacious in laying poultry when included in feed at a dose of 45,000 U/kg of complete feed.

Table 4: Effect of Xygest HT on laying hens

		Average Egg Weight (g)	Laying rate (%)	Daily Feed Intake (g)	Feed efficiency (g feed / g egg)	AME (kcal/kg)	Energy Digestibility (%)	Yolk Intensity	Haugh Unit
Trial I	**Control**	55.6^{c}	88.2^{b}	110^{a}	2.26^{a}	2,586^{b}	70.20^{b}		83.1^{c}
	45,000 U/kg	56.8^{b}	90.0^{ab}	108^{ab}	2.14^{b}	2,665^{ab}	72.29^{ab}		85.7^{b}
	90,000 U/kg	58.0^{a}	90.7^{a}	106^{bc}	2.04^{c}	2,679^{a}	72.71^{a}		86.6^{a}
	9,000,000 U/kg	58.0^{a}	90.7^{a}	106^{c}	2.04^{c}	2,981^{a}	72.76^{a}		86.7^{a}
Trial II	**Control**	59.2	83.8	123^{a}	2.49^{a}	2,493^{a}	64.4^{a}		
	45,000 U/kg	58.6	83.6	116^{b}	2.37^{ab}	2,552^{b}	69.9^{b}		
	90,000 U/kg	59.2	84.6	117^{b}	2.33^{b}				
Trial III	**Control**	57.6	87.4	127	2.58	2,636^{b}	71.1^{b}	12.1^{a}	
	45,000 U/kg	58.9	86.3	124	2.50	2,725^{a}	73.6^{a}	12.6^{b}	
	90,000 U/kg	57.9	87.6	127	2.552	2,745^{a}	74.1^{a}	12.0^{a}	
Trial IV	**Positive Control**	60.9	90.7	102	1.86			10.5^{a}	87.3^{ab}
	Negative Control	60.52	90.5	103	1.89			10.2^{a}	84.4^{a}
	45,000 U/kg	60.0	92.6	103	1.87			10.9^{b}	88.7^{b}
Trial V	**Control**	56.7^{a}	79.5^{a}	120^{a}	2.13^{a}			5.7^{a}	87.9^{ab}
	30,000 U/kg	57.3^{ab}	79.9^{a}	118^{c}	2.06^{b}			6.6^{b}	85.1^{a}

	45,000 U/kg	58.2^{bc}	81.3^{ab}	119^{bc}	2.04^{b}			7.7^{c}	91.5^{b}
	90,000 U/kg	58.1^{c}	84.1^{b}	119^{b}	2.05^{b}			6.9^{bc}	92.1^{b}
AME: Apparent metabolizable energy; Values within the same column, within a trial, that differ significantly ($p<0.05$) are indicated by different letters.									

2.3.3. Conclusions on efficacy

Based on the information presented in nine efficacy trials, the ACAF concluded that the additive Xygest® HT can be considered to be efficacious in laying poultry and growing poultry. Conclusions can be extrapolated to all poultry.

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[2]:

For the quantification of the xylanase activity in the feed additive the Applicant submitted a single-laboratory validated and further verified method based on the enzymatic hydrolysis of xylanase on the beechwood xylan substrate and the colour formation of the released reducing sugar with arsenomolybdate.

Furthermore, for the quantification of endo-1,4-beta-xylanase in premixtures and feedingstuffs the Applicant submitted another single-laboratory validated and further verified colorimetric method, based on the measurement 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.

Based on the acceptable performance characteristics the EURL recommends for official control the single-laboratory validated and further verified colorimetric (arsenomolybdate) method for the determination of endo-1,4-beta-xylanase in the feed additive and (ii) the colorimetric (Megazyme) method for the determination of 1,4-beta-xylanase in 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 ACAF concluded that the additive was correctly identified and characterised. The Committee concluded that the additive can be considered safe for the target species, with a margin of safety of 300 for growing poultry and 25 for laying poultry. The additive can be considered safe for consumers and the environment. The additive should be considered a respiratory sensitiser. The additive is not an eye irritant, skin irritant or skin sensitiser.

Based on data from four trials in growing poultry and five trials in laying poultry, the ACAF concluded that the additive Xygest® HT can be considered to be efficacious in laying poultry and growing poultry. Conclusions can be extrapolated to all poultry.

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. EURL-FA (European Reference Laboratory for Feed Additives), 2021. 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: finrep_fad-2020-0110_xygest-ht.pdf (europa.eu)

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