Assessment of the safety and efficacy of an additive of alpha-galactosidase and endo-1,4-betaglucanase (Agal-Pro BL and Agal-Pro BL L®) as a feed additive for chickens for fattening, minor poultry species for fattening and chickens reared for laying

Reference number RP746

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

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Regulated Product Dossier Assessment
Assessment finalised: 15/03/2024

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Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CRL	Central Reference Laboratory
DNA	Desoxyribonucleic acid
DNS	Dinitro salicylic acid
EC	European Commission
EFSA	European Food Safety Authority
FSA	Food Standards Agency
FSS	Food Standards Scotland
LOD	Limit of detection
LOQ	Limit of quantification
pNP	p-nitrophenol
pNPG	p-nitrophenyl-α-galactopyranoside
RRec	Recovery rates
RSDr	Relative standard deviations for repeatability
RSDR	Relative standard deviations for reproducibility

Summary

An application was submitted to the Food Standards Agency (FSA) in March 2021 from Kerry Ingredients and Flavours ("the applicant") for the renewal of authorisation of an additive (alpha-galactosidase and endo-1,4-betaglucanase-AGal-Pro BL and AGal-Pro BL L), under the category of 'zootechnical additive' and functional group 'digestibility enhancer'. The additive is intended for use at 50-100 mg/kg of complete feed for chickens for fattening, minor poultry species for fattening and chickens reared for laying.

The Advisory Committee on Animal Feedingstuffs (ACAF) was asked to review the dossier and the supplementary information submitted by the Applicant, and to advise the Food Standards Agency and Food Standards Scotland (FSA/FSS) in evaluating the dossier.

Based on ACAF's advice, the FSA/FSS concluded on a recommended dose at 50-100 mg/kg of complete feed.

The initial EFSA evaluation in 2011,[1] 2013[2] and 2014[3] confirmed that the additive is safe for the target species, consumers and the environment at the proposed conditions of use. The applicant conducted a literature review covering the period 2009 to 2020 and found no reported negative effects in this time. Regarding user/worker safety, the additive is considered a potential respiratory and skin sensitiser, as well as a skin and eye irritant.

The efficacy of the additive was not evaluated, as this was a renewal of authorisation.

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

1. Introduction

The FSA/FSS have undertaken a risk assessment for a feed additive (alpha-galactosidase and endo-1,4-betaglucanase-AGal-Pro BL and AGal-Pro BL L, Kerry Ingredients and Flavours, Kilnagleary, Carrigaline, P43a597, Cork, Ireland) under Assimilated Regulation (EC) No 1831/2003[4] for the renewal of authorisation under the category of "zootechnical additives" and functional group "digestibility enhancer" for its use in chickens for fattening, minor poultry species for fattening and chickens reared for laying. To support the safety assessment, the ACAF provided advice to the FSA/FSS as 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 the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

With thanks to the members of the ACAF during the course of the assessment, who were: Professor Nicholas Jonsson, Martin Briggs, Professor Emily Burton, Professor Katrina Campbell, Professor Matthew Fisher, Hannah Kane, Christine McAlinden, Susan MacDonald, Dr. Donald Morrison, Derek Renshaw, Dr. Oonagh Markey, Dr. Michael Salter, Dr. Adam Smith, Dr. Helen Warren and Dr. Nick Wheelhouse. Dr. Adam Smith declared an indirect conflict of interest and was allowed to take part in the assessment.

The dossier was evaluated by the ACAF at its December 2022 meeting, after which a request for further information was communicated to the applicant. The applicant's response to this request and subsequent requests were evaluated at the ACAF June 2023 and September 2023 meetings.

This document sets out the findings of the Committee's assessment on the safety of the feed additive, on which the FSA/FSS have made their opinion for the request of a new authorisation.

2. Assessment

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

The additive is a preparation containing the active agents alpha-galactosidase and endo-1,4-betaglucanase in two final forms, a microgranular solid product and a liquid product. The applicant provided data from several batches supporting the composition, physico-chemical properties and impurities' values outlined below (**Table 1**).

Table 1: Identity table AGal-Pro BL and AGal-Pro BL L

AGal-Pro BL (solid)	
Alphagalactosidase activity	1000 - 1300 U/g
Glucanase activity	5700 - 7410 U/g
Enzyme	5-10 %
Water	8-12 %
Wheat flour	75-80 %
Physico-chemical properties	
Dusting potential	479 - 679 mg/m^3
Particle size distribution	> 215 µm (15 %) 45 - 215 µm (85 %) < 45 µm (5 %)
Purity	
Coliform bacteria	< 10 CFU/g
Yeast and moulds	10 - 65 CFU/g
Salmonella spp.	Absent in 25g
E. coli	Absent in 25g
B. cereus	Not tested
Lead	< 0.05 mg/kg
Aflatoxin total	< 1.5 µg/kg
Deoxynivalenol	< 100 µg/kg
Fumonisin B1	< 5.0 µg/kg
Fumonisin B2	< 5.0 µg/kg
Ochratoxin A	< 1.0 µg/kg
Zearalenone	< 1.0 µg/kg
AGal-Pro BL L (liquid)	
Alphagalactosidase activity	500 - 650 U/g

Glucanase activity	2850 - 3705 U/g
Enzyme	5 – 10 %
Water	40 – 55 %
Glycerol	40-55 %
Sodium benzoate	0.2 %
Potassium sorbate	0.1 %
Physico-chemical specifications	
Specific gravity	1.02
Purity specifications	
Coliform bacteria	< 10 CFU/g
Yeast and moulds	10 – 30 CFU/g
Salmonella spp.	Absent in 25g
E.coli	Absent in 25g
B. cereus	Not tested
Lead	< 0.05 mg/kg
Aflatoxin total	< 1.5 µg/kg
Deoxynivalenol	< 100 µg/kg
Fumonisin B1	< 5.0 µg/kg
Fumonisin B2	< 5.0 µg/kg
Ochratoxin A	< 1.0 µg/kg
Zearalenone	< 1.0 µg/kg

The enzymes are produced from a strain of *Aspergillus niger* (beta-glucanase) and a genetically modified strain of *Saccharomyces cerevisiae* (alpha-galactosidase). After request by the FSA, data from several batches were presented showing no DNA of the production organism in the final formulations of the product.

The additive showed shelf-life stability for up to 12 months and stability in mash and pelleted feed for 6 months. Homogeneity was also demonstrated. Queries were successfully answered by the applicant providing further data on mycotoxin testing, critical control points of the production system and dusting potential.

The conditions of use of the additive are summarised in **Table 2**.

Table 2: Conditions of use of AGal-Pro BL and AGal-Pro BL L

Proposed mode of use in animal nutrition		
Additive	Alpha-galactosidase, beta-glucanase	
Category(-ies) of additive	Zootechnical	
Functional group(s) of additive	Digestibility enhancer	
Description		
Composition description	**Purity criteria**	**Method of analysis**

Preparation of Alpha-galactosidase, beta-glucanase (solid)	Min 1000 U/g alpha-galactosidase Min 5700 U/g glucanase	In-house validated method (SAM 0202 – alpha-galactosidase) In-house validated method (SAM 0109 – beta-glucanase)		
Preparation of Alpha-galactosidase, beta-glucanase (liquid)	Min 500 U/g alpha-galactosidase Min 2850 U/g glucanase	In-house validated method (SAM 0202 – alpha-galactosidase) In-house validated method (SAM 0109 – beta-glucanase)		
Trade name (if appropriate)		AGal-Pro BL, AGal-Pro BL L		
Name of holder authorisation (if appropriate)		-		
Conditions of use				
Species or category of animal	**Product**	**Min. content**	**Max. content**	**Withdrawal period**
		mg/kg of feed		
Chickens for fattening, minor poultry species for fattening and chickens reared for laying	**AGal-Pro BL**	50	100	-
Chickens for fattening, minor poultry species for fattening and chickens reared for laying	**AGal-Pro BL L**	100	200	-

2.2.1. 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

The applicant presented a comprehensive review of recent scientific literature to support the safety for the target species, consumer and the environment, where no papers were identified showing adverse effects for any of these safety areas. The original safety studies were carried out on the product “Biogalactosidase BL”, therefore, the Committee requested access to these studies and evidence that the product under renewal was the same as that of the original application. The ACAF also questioned whether the updated EFSA Guidelines on Mutagenicity would affect the validity of the original conclusion with regards to clastogenicity and aneugenicity.

The applicant clarified the difference between AGal-Pro BL and Agal-Pro, as the two products are the same, the Committee concluded that conclusions were still

relevant, and that no further tests would be required from the applicant. Therefore, the additive can be considered safe for the target species, consumers and the environment.

No new studies were provided to evaluate the safety for users, so the Committee reiterated the previous conclusions that the product should be considered a potential skin and eye irritant, as well as a respiratory and skin sensitiser. The solid formulation of the product (Agal-Pro BL) has a high proportion of small particles and is moderately dusty, so precautions should be taken to minimise inhalation exposure.

2.2.1. Conclusions on safety

The ACAF concluded that the additive can be considered safe for the target animals, consumers and the environment.

The additive should be considered a respiratory and skin sensitiser, as well as a skin and eye irritant. Dust formed from the solid formulation of the additive could contain a high amount of particles that are small enough to deposit in the respiratory system of users.

It was concluded that further information would not be required on clastogenicity and aneugenicity as the in vivo test originally provided by the applicant was sufficiently conclusive, therefore, no further mutagenicity tests would be required.

2.3. Section IV: Efficacy

Efficacy evaluation is not required for renewals of authorisation.

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 of Biogalactosidase BL[5]:

"For the determination of α-galactosidase the applicant proposes two single laboratory validated and further verified colorimetric methods for the analysis: - in feed additives; and in premixtures and feedingstuffs. These methods are based on the production of p-nitrophenol (pNP) and D-galactose from p-nitrophenyl-α-galactopyranoside (pNPG) at pH = 5.0 at 37°C. The following performance characteristics were reported: (a) relative standard deviations for repeatability (RSDr) ranging from 2.7 to 10 %; (b) relative standard deviations for reproducibility (RSDR) ranging from 3 to 6 %; (c) recovery rates (RRec) ranging from 81 to 103 %; and a limit of quantification (LOQ) of 10 U/kg feedingstuffs.

For the determination of the activity of endo-1,4-β-glucanase in the feed additive the applicant proposes a single laboratory validated and further verified colorimetric method, based on the hydrolyses of a barley beta-D-glucan at 50°C and pH = 5.0 to release reducing sugars reacting with 3.5-dinitro salicylic acid (DNS). As for the determination of the enzyme activity of endo-1,4-β-glucanase in the premixtures and feedingstuffs the applicant proposes another single laboratory validated colorimetric method using azurine cross-linked barley glucan substrate.

The following performance characteristics were reported: (a) RSDr ranging from 3.3 to 10 %; (b) RSDR ranging from 1.3 to 15 %; (c) RRec around 100 %; and a limit of detection (LOD) and a limit of quantification (LOQ) of 66 and 132 U/kg feedingstuffs, respectively.

Based on the above mentioned performance characteristics, the CRL recommends for official control the single laboratory validated and further verified colorimetric methods submitted by the applicant for the determination of α-galactosidase and endo-1,4-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 FSA/FSS have considered and agree with the conclusions reached by the ACAF on the safety and efficacy of the feed additive.

The ACAF concluded that the additive was correctly characterised and no causes for concern were identified.

The additive can be considered safe to the consumer, the target animal and the environment. The additive should be considered a respiratory and skin sensitiser, as well as a skin and eye irritant.

5. References

1. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Scientific Opinion on the safety and efficacy of Biogalactosidase BL (alpha-galactosidase and beta-glucanase) as feed additive for chickens for fattening. EFSA Journal 2011. Available at Biogalactosidase BL for chickens for fattening | EFSA (europa.eu)

2. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of AGal-Pro (alpha-galactosidase and endo-1,4-beta-glucanase) as a feed additive for chickens reared for laying and minor poultry species for fattening. EFSA Journal 2013. Available at AGal-Pro for poultry | EFSA (europa.eu)

3. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of AGal-Pro BL-L (alpha-galactosidase and endo-1,4-beta-glucanase) as a feed additive for chickens for fattening. EFSA Journal 2014. Available at AGal-Pro BL for chickens for fattening | EFSA (europa.eu)

4. EC (European Commission), 2003. Regulation No 1831/2993 of the European Parliament and of the Council on additives for use in animal nutrition. Available at Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition (Text with EEA relevance) (legislation.gov.uk)

5. 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. Biogalactosidase BL-AlphaGal BL. Available at: FAD-2009-0014 - European Commission (europa.eu)

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