Assessment of the safety and efficacy of endo-1,4-beta-glucanase (Hostazym® C) as a feed additive for chickens for fattening, minor poultry species for fattening and weaned piglets and for new uses in turkeys for fattening and reared for breeding, chickens reared for laying, minor poultry species reared for laying or breeding, ornamental birds and suckling piglets

Reference number RP593

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
RDS_R	Standard deviation for reproducibility
R_{Rec}	Recovery rate

Summary

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

- A renewal of authorisation for chickens for fattening, minor poultry species for fattening, weaned piglets.
- A new authorisation for turkeys for fattening, turkeys reared for breeding, chickens reared for laying, minor poultry species reared for laying or breeding, ornamental birds and suckling piglets

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Advisory Committee on Animal Feedingstuffs (ACAF) was asked to review the dossier and the supplementary information from the applicant.

The safety and efficacy sections of the dossier were evaluated by the ACAF, and no new concerns were raised. Taking account of the literature review provided by the applicant, it was concluded that previous conclusions drawn by The European Food Safety Authority (EFSA) could be accepted and the additive could, therefore, be considered safe for the target species, the consumer and the environment. It was also concluded that the product should be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser. It was concluded the additive remains efficacious and that these conclusions can be extrapolated to the new uses proposed.

The views of 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-glucanase - Hostazym® C, 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 chickens for fattening, minor poultry species for fattening, weaned piglets.
- A new authorisation for turkeys for fattening, turkeys reared for breeding, chickens reared for laying, minor poultry species reared for laying or breeding, ornamental birds and suckling piglets

To support the safety assessment by FSA and FSS, 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 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 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 ACAF at their October 2022, April 2023 and July 2023 meetings. Further information was provided by the applicant in December 2022 and May 2023, responding to queries by the FSA.

This document outlines the discussion and conclusions of the AFFAJEG's assessment on the safety and efficacy of Hostazym® C 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-beta-glucanase, produced by the fermentation of the strain *Trichoderma citrinoviride* (IM SD142) with three formulations proposed:

- Hostazym® C 2000 MicroGranulate: with a minimum activity of 2000 CU/g
- Hostazym® C 7000 MicroGranulate: with a minimum activity of 7000 CU/g
- Hostazym® C 2000 Liquid: with a minimum activity of 2000 CU/g

The applicant provided data from several batches supporting the identity values outlined below (Table 1).

Table 1: Identity table of Hostazym® C 2000 MicroGranulate, 7000 MicroGranulate and 2000 Liquid

Hostazym® C 2000 MicroGranulate	
Composition	
Endo-1,4-beta-glucanase activity 32 000 CU/g	7.14%
Pregelatinised starch	0.68%
Wheat meal	Up to 100%
Appearance	
Granular form, beige	
Chemical-physical characteristics	
Dusting potential	< 00.00 mg/g^3 of air
Particle size distribution	1.289% of particles of diameter > 800µm; 17.655% of particles > 100 µm
Hostazym® C 7000 MicroGranulate	
Composition	
Endo-1,4-beta-glucanase activity 32 000 CU/g	24.98%
Pregelatinised starch	0.68%
Wheat meal	Up to 100%
Appearance	
Granular form, beige	
Chemical-physical characteristics	
Dusting potential	130 mg/g^3 of air
Particle size distribution	1.040% of particles of diameter > 800µm; 16.197% of particles > 100 µm

Hostazym® C 2000 and Hostazym® C 7000 MicroGranulate	
Microbiological profile	
Salmonella	Absent in 25 g of sample
Total coliforms	Not more than 30 CFU/g
E.coli	Absent in 25 g of sample
Heavy metals	
Arsenic	Not more than 4 mg/kg
Lead	Not more than 10 mg/kg
Cadmium	Not more than 0.5 mg/kg
Mercury	Not more than 0.2 mg/kg
Hostazym® C 2000 Liquid	
Composition	
Endo-1,4-beta-glucanase activity 32 000 CU/g	7.19%
Sorbitol, liquid	60.0%
Propylene, liquid	10.0%
Water, purified	Up to 100%
Appearance	
Viscous, slightly turbid liquid, light to dark brown	
Chemical-physical characteristics	
Relative density	1.10-1.60
Microbiological profile	
Salmonella	Absent in 25 ml of sample
Total coliforms	Not more than 30 CFU/g
E.coli	Absent in 25 ml of sample
Heavy metals	
Arsenic	Not more than 4 mg/kg
Lead	Not more than 10 mg/kg
Cadmium	Not more than 0.5 mg/kg
Mercury	Not more than 0.2 mg/kg

The ACAF requested clarification of the conditions of use in tabular form, new data to support the stability of the additive at 85^{0}C, a clarification of the homogeneity data, data to confirm the absence of the production strain and retention time data for the stability trials. These queries were satisfactorily answered by the applicant.

The applicant proposes that the additive is included in animal feed as shown in Table 2:

Table 2: Conditions of use

Proposed mode of use in animal nutrition	
Additive	3.2.1.4 endo-1,4-beta-glucanase
Registration number	4a1616
CAS number	9012-54-8

Category and functional group		Zootechnical additive, digestibility enhancer		
Description				
Composition, description	**Formula**		**Method of analysis**	
Preparation containing endo-1,4-beta-glucanase, produced by the fermentation of the strain *Trichoderma citrinoviride* Bisset (IM SD142)	Minimum activity Hostazym® C 2000, MicroGranulate Hostazym® C 2000 Liquid: 2000 CU/g MicroGranulate Hostazym® C 7000: 7000 CU/g		Colorimetric	
Trade name		Hostazym® C 2000 MicroGranulate Hostazym® C 7000 MicroGranulate Hostazym® C 2000 Liquid		
Name of the holder of authorisation		Huvepharma		
Conditions of use				
Species or category of animal	**Maximum age**	**Units of active substance per kg of complete feedingstuffs**		**Other provisions**
		Minimum content	**Maximum content**	
Chickens for fattening Chickens reared for laying Turkeys for fattening Turkeys reared for breeding Minor poultry species reared for laying/breeding Minor poultry species for fattening Ornamental birds	-	500 CU	-	1. In the direction for use of the additive and premixture, indicate the storage conditions and stability to pelleting. 2. For safety: breathing protection, glasses and gloves shall be used during handling. 3. Recommended dose range: growing poultry/poultry for fattening: 500 – 750 CU/kg feed Piglets: 350– 750 CU/kg feed 4. For use in weaned piglets until approximately 35 kg. 5. Use in suckling piglets limited to the period when solid feed is given
Piglets (suckling and weaned)	-	350 CU	-	

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 applicant presented evidence of safety for the target species through previous conclusions from EFSA and a comprehensive literature review. The ACAF agreed with the applicant's argument that the tolerance of Hostazym® C up to 75000 CU/kg in weaned piglets and chickens for fattening can be extrapolated to suckling piglets and all other poultry species for fattening i.e., turkeys for fattening, turkeys reared for breeding, chickens reared for laying, minor poultry species reared for laying, minor poultry species reared for breeding and ornamental birds. No new tolerance studies were carried out for the target species.

The applicant carried out a study to demonstrate that the active substance, endo-1,4-beta glucanase, is free from antibiotic activity.

The ACAF concluded that previous conclusions drawn by EFSA could be accepted and the additive could, therefore, be considered safe for the target species.

2.2.2. Safety for the consumer

The applicant presented previous conclusions by EFSA and a comprehensive literature review. The applicant provided new experimental data to support the safety of the fermentation product used to formulate the additive and the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the fermentation product is unlikely to present a genotoxic hazard to the consumers. The ACAF concluded that previous conclusions drawn by EFSA could be accepted and the additive could, therefore, be considered safe for the consumer.

2.2.3. Safety for the user

The applicant presented previous conclusions by EFSA and a comprehensive literature review. The ACAF concluded that previous conclusions drawn by EFSA could be accepted and that the additive should be considered a potential skin and eye irritant, and a potential skin and respiratory sensitiser.

None of the three forms of the additive were likely to form a respirable dust when handled by operators.

2.2.4. Safety for the environment

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

2.2.5. Conclusions on safety

The ACAF concluded that the additive can be considered safe for the target species, the 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 feed additives when the applicant does not propose amending the conditions of the original authorisation, which may have an impact on the efficacy of the additive.

It was concluded the additive remains efficacious and that these conclusions can be extrapolated to the new uses proposed.

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 Hostazym C®[2]:

For the determination of the activity of end-1,4-beta-lgucanase in the feed additive, premixtures and feedingstuffs, the Applicant proposed a single-laboratory validated and further verified colorimetric method based on the quantification of water soluble dyed fragments produced by the action of end-1,4-beta-glucanase on commercially available azurine crosslinked cellulose. The activity of the samples is calibrated against reference enzyme standards with known activity determined at the definition conditions of the activity unit. The following method performance characteristics for

feedingstuffs were recalculated by the EURL, based on the experimental data provided by the Applicant:

- A relative standard deviation for repeatability (RSD_r) ranging from 8.03 to 9.66 %;
- a relative standard deviation for intermediate precision (RSD_{ip}) ranging from 8.03 to 9.76 %;
- a recovery rate (R_{Rec}) ranging from 96 to 100 %, and
- a limit of quantification (LOQ) of 57.6 CU/kg, which is well below the minimum activity proposed by the Applicant.

Based on the satisfactory performance characteristics mentioned above, the EURL recommends for official control the single-laboratory validated and further verified method submitted by the Applicant for the determination of endo-1,4-beta-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 ACAF concluded that the additive was correctly identified and characterised.

The 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. The additive is a potential skin and eye irritant, and a potential skin and respiratory sensitiser.

It was concluded the additive remains efficacious and that these conclusions can be extrapolated to the new uses proposed.

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), 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-glucanase. Available at: FAD-2010-0062 (europa.eu)

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