REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive of *Bacillus Velezensis* as the Active Agent (Enviva® PRO 202 GT) Used as a Feed Additive for Turkeys for Fattening and Turkeys Reared for Breeding (RP1258)

Food Standards Agency[1], Food Standards Scotland[2]

[1] Regulated Products Risk Assessment, Food Standards Agency, UK, [2] Risk Assessment, Food Standards Scotland, UK

Keywords: Regulated Products, Safety assessment, Feed additives

https://doi.org/10.46756/001c.129717

FSA Research and Evidence

An application was submitted to the Food Standards Agency in September 2021 from Genencor International B.V, ('the applicant') for the new use application (extension of species) of an additive (Enviva® PRO 202 GT) containing *Bacillus amyloliquefaciens*, which has now been reclassified as *Bacillus velezensis*, under the category 'zootechnical additives" and functional group "gut flora stabiliser". The additive is proposed to be used at the minimum content of 7.5×10^7 CFU/kg complete feed for use in turkeys for fattening and turkeys reared for breeding.

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 FSA/FSS concluded, based on the ACAF's advice, 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 additive should be considered a skin sensitiser, respiratory sensitiser and a potential irritant to eyes for the user/worker.

The additive proved to be efficacious in chickens for fattening. The results can be extrapolated to turkeys for fattening and turkeys reared for breeding.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive containing *Bacillus velezensis*, formally *B. amyloliquefaciens* (Enviva® PRO 202 GT, Genencor International B.V, Willem Einthovenstraat 4, 2342 BH, Oegstgeest, The Netherlands) under assimilated Regulation (EC) No 1831/2003 (EC, 2003) in the category "zootechnical additives" and functional group "gut flora stabiliser", for use in turkeys for fattening and turkeys reared for breeding.

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 the ACAF at its September 2023 meeting, September 2024 meeting, October 2024 meeting, December 2024 meeting, and February 2025 meeting. The applicant was sent two Requests for Information (RFI) relating to queries by the ACAF, to which the applicant responded in July 2024 and November 2024. The response to the requests were reviewed by the ACAF at their September 2024 meeting and October 2024 meeting.

Further information was provided by the applicant during the suitability check stage; the applicant responded in July 2023.

The views of ACAF have been considered in this safety assessment, which represents the opinion of the FSA/FSS.

Table 1. Products included in this assessment

Title	Product type	Intended use/s	Intended species or categories of animals
Bacillus velezensis	Feed additive	Zootechnical additive	Turkeys for fattening and reared for breeding

2. Assessment

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

The additive is a microorganism product that contains the active substance *Bacillus amyloliquefaciens*, which has now been reclassified as *Bacillus velezensis.* The active substance is a preparation of three different strains of *Bacillus velezensis* (PTA-6507, NRRL B-50104 and NRRL B-50013); they are present in a 1:1:1 ratio. The additive is proposed for use at a minimum content of 30 mg/kg of complete feed, which corresponds to 7.5 x 10^7 colony forming units (CFU)/kg feed for use in turkeys for fattening and turkeys reared for breeding. The minimum guaranteed count of *Bacillus velezensis* in the additive is 2.5 x 10^9 CFU/g. The additive is available in a solid granular form.

The qualitative and quantitative composition and identity of the additive are outlined in Table 2.

The ACAF identified several pieces of information that the applicant would have to provide to inform the assessment of the product. In the RFI response, the applicant provided updated genetic stability analysis of the additive, an updated FAMI-QS certificate, updated material safety data sheets (MSDSs) for all ingredients, provided stability data in three feeds, and updated the conditions of use table and proposed label text to include the retention time and appropriate personal protective equipment (PPE).

Table 2. Composition and identity of the additive *Bacillus velezensis*

Composition	
Bacillus amyloliquefaciens (PTA-6507); *Bacillus amyloliquefaciens* (NRRL B-50104); *Bacillus amyloliquefaciens* (NRRL B-50013)	0.1 - 3 % (w/w)
Calcium carbonate (carrier)	95.8 - 98.9 %
TOS	0.80 - 1.32 %
Appearance	
Solid granular form	
Batch-to-batch variation of the active agent	
Bacillus cell count	3.48 x10^9 CFU/g
Chemical-physical properties	
Particle size distribution	< 100 µm – 1.5-3.2 % < 50 µm – 0.1-1.4 % < 10 µm – 0.0-0.2 %
Tapped density	2.51 g/L
Dusting potential	0.315 - 0.768 g/m^3
Impurities	
Mercury	<0.01 mg/kg
Cadmium	0.14 mg/kg
Coliforms	<10 CFU/g
Enterobacteriaceae	<10 CFU/g
Escherichia coli	<10 CFU/g
Bacillus cereus	<10 CFU/g
Staphylococcus aureus	<10 CFU/g
Salmonella	Absent in 25 g
Yeasts	<10 CFU/g
Moulds	<10 CFU/g
Aflatoxin	<5 µg/kg
Fumonisin	<30 µg/kg
HT-2 toxin	<10 µg/kg
Ochratoxins A	<5 µg/kg
T-2 Toxin	<1 µg/kg
Vomitoxin (Deoxynivalenol)	<10 µg/kg
Zearalenone	<5 µg/kg
Dioxins and PCBs	<1.5 ng WHO-PCDD/F-PCB-TEQ/kg

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

Table 3. Proposed conditions of use for *Bacillus velezensis*

Proposed mode of use in animal nutrition				
Additive	*Bacillus velezensis*			
Registration number/EC N°	4b1827i			
Category of additive	Zootechnical additive			
Functional group of additive	Gut floral stabiliser			
Description				
Composition, description	**Chemical formula**	**Purity criteria**	**Methods of analysis**	
Preparation of viable spores of three *B. amyloliquefaciens* strains in an excipient of calcium carbonate diluent	Enviva® PRO 202 GT: 2.5 x 10^9 CFU/g	Complies with EU feed hygiene law	BS EN 15784:2009	
Trade name	Enviva® PRO 202 GT			
Name of the holder of authorisation	Danisco (UK) Ltd, trading as Danisco Animal Nutrition and represented by Genencor International B.V.			
Conditions of use				
Species or category of animal	**Maximum age**	**Minimum content CFU/kg of complete feedingstuffs**	**Maximum content CFU/kg of complete feedingstuffs**	**Withdrawal period**
Turkeys for fattening and turkeys reared for breeding	Not applicable	7.5 x 10^7	Not applicable	Not applicable
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use	1. Conditioning and pelleting temperatures should not exceed 95°C for 30 seconds. Stable for 24 months when stored at 22°C. 2. The use is compatible in feed containing the following authorised coccidiostats: narasin/nicarbazin, maduramicin ammonium, lasalocid A sodium, salinomycin sodium, monensin sodium, robenidine hydrochloride, diclazuril, decoquinate, and semduramycin sodium.			
Specific conditions or restrictions for handling	May cause sensitisation by inhalation and irritation of respiratory tract. Potential skin sensitiser and irritant to eyes. Users are recommended to wear protective gloves, safety glasses with side shields, protective clothing and a respiratory mask.			
Post-market monitoring	In compliance with European Union (EU) feed hygiene legislation, traceability, Hazard Analysis and Critical Control Points (HACCP), formal product/service complaints procedure, and product recall capability.			
Specific conditions for use in complementary feedingstuffs	Not applicable			
Maximum Residue Limit (MRL)				
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**	
Not applicable	Not applicable	Not applicable	Not applicable	

2.1.1. Conclusions on Section II

The ACAF concluded that the additive was correctly identified and characterised and accepted the proposed conditions of use as detailed by the applicant.

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

2.2. Section III: Safety

2.2.1. Section III: Safety for the target species

No tolerance studies concerning the safety of use of the additive for the target species are required for microorganisms, such as *Bacillus velezensis*, which have the qualified presumption of safety (QPS) status. The Committee evaluated and agreed the use of the QPS approach to evaluate the safety of the microorganism strain. Therefore, the additive can be presumed to be safe for the target species. There were no concerns about the safety for the target species of the non-microbiological ingredients that make up the bulk of this additive.

2.2.2. Section III: Safety for the Consumer

No tolerance studies concerning the safety of use of the additive for the consumer are required for microorganisms, such as *Bacillus velezensis*, as it has the QPS status. Therefore, the additive is presumed to be safe for the consumer. There were no concerns about the safety for the consumer of the non-microbiological ingredients that make up the bulk of this additive.

2.2.3. Section III: Safety for the user and worker

The applicant presented the following tests to evaluate the safety of the additive for the users/workers:

- Acute dermal irritation in the rabbit
- Acute eye irritation in the rabbit
- Local lymph node assay in the mouse (skin sensitisation test)

The Committee was satisfied with the studies carried out on the effects on eyes and skin. It was concluded that the additive is an irritant to eyes, but not to skin. The Committee concluded that the additive, as a microorganism product, is a potential skin sensitiser and respiratory sensitiser. Although the dusting potential of the additive is quite low, it contains some particles that are small enough to deposit in the lungs. Therefore, the use of measures to limit exposure through inhalation when handling the additive is recommended.

2.2.4. Section III: Safety for the environment

No tolerance studies concerning the safety of use of the additive for the environment are required for microorganisms, such as *Bacillus velezensis*, as it has the QPS status. Therefore, the additive is presumed to be safe for the environment. There were no concerns about the safety for the environment of the non-microbiological ingredients that make up the bulk of this additive.

2.2.5. Section III: Conclusions on safety

The ACAF concluded that the additive is safe for the target species, consumer and the environment. The Committee concluded that the additive should be considered a skin sensitiser and respiratory sensitiser and a potential irritant to eyes but not to skin.

2.3. Section IV: Efficacy

2.3.1. Efficacy in chickens for fattening

The applicant presented the following long-term studies in chickens for fattening to assess the efficacy of the additive in turkeys for fattening and turkeys reared for breeding by favourably affecting animal performance:

- Efficacy of Enviva® PRO 202 GT on broiler performance, Spain, 2009;
- Efficacy of Enviva® PRO 202 GT on broiler performance, Spain, 2010;
- Efficacy of Enviva® PRO 202 GT in commercial growing broiler chickens, UK, 2010.

The three trials in chickens for fattening showed that the additive, *Bacillus velezensis*, at a dose of 7.5 x 10^7 CFU/kg feed, improved broiler performance. Members discussed the validity of extrapolation of results from older efficacy studies, based on the original study design and potential changes in current feed conversion ratios. The Committee concluded that the similarities between species would outweigh these concerns and, therefore, there is sufficient evidence to support the extrapolation to turkeys for fattening and turkeys reared for breeding.

2.3.2. Section IV: Conclusions on efficacy

The Committee concluded that the additive is efficacious. It was concluded by the ACAF that there is sufficient evidence to support an extrapolation from chickens for fattening to turkeys for fattening and turkeys reared for breeding. This demonstrates that *B. velezensis* favourably affects the stability of the gastro-intestinal flora of the target animals.

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 (EURL) for Feed Additives on the Method(s) of the Analysis for preparation of Enviva® PRO 202 GT (EURL 2015):

> "For the identification and characterization of *Bacillus amyloliquefaciens* (PTA-6507), *Bacillus amyloliquefaciens* (NRRL B-50104) and *Bacillus amyloliquefaciens* (NRRL B-50013), the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification. This standard methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.
>
> For the enumeration of all the *Bacillus amyloliquefaciens* strains (PTA-6507, NRRL B-50104, NRRL B-50013) in *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated spread plate method EN 15784 which was already evaluated by EURL in the frame of a previous *Bacillus amyloliquefaciens* dossier. Based on the performance characteristics available, the EURL recommends for official control this CEN method (EN 15784) for the enumeration of *Bacillus amyloliquefaciens* (PTA-6507, NRRL B-50104, NRRL B-50013) in the *feed additive*, *premixtures* and *feedingstuffs*."

FSA/FSS accepts the EURL 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 2015, at the time in which the UK was still an EU member.

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 Committee concluded that the additive should be considered a skin sensitiser and respiratory sensitiser and a potential irritant to the eyes for the user/worker.

The Committee concluded that the additive is efficacious, as the results from chickens for fattening and can be extrapolated to turkeys for fattening and turkeys reared for breeding.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
EC	European Commission
SERD	Science, Evidence and Research Directorate
FSA	Food Standards Agency
RFI	Request for information
CFU	Colony forming units
MSDS	Material safety data sheet
PPE	Personal protective equipment
EU	European Union
HACCP	Hazard Analysis and Critical Control Points
MRL	Maximum residue limit
QPS	Qualified presumption of safety

Acknowledgments

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, Susan MacDonald, Dr. Oonagh Markey, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Kristel Wake, Dr. Helen Warren and Professor Nick Wheelhouse.

Published: February 28, 2025 GMT.

References

EC (European Commission). (2003). *Regulation No 1831/2993 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2003/1831/contents

EURL-FA (European Reference Laboratory for Feed Additives). (2015). *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. Preparation of Enviva® PRO 202 GT*. https://joint-research-centre.ec.europa.eu/document/download/5bc23143-ab0f-447c-94d2-7b19325396b1_en?filename=finrep_fad_2015_0008_enviva_pro202gt.pdf