REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of an Additive Consisting of *Bacillus Velezensis* ATCC PTA-6737 (PB6) Used as a Feed Additive for Use in Turkeys for Fattening, Turkeys Reared for Breeding and All Growing Poultry (RP1696)

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in September 2022 from Kemin Europa N.V. ('the applicant'), for the renewal and modification of an additive consisting of *Bacillus velezensis* ATCC PTA-6737 (PB6), under the category 'zootechnical additives' and functional group 'gut flora stabiliser'. The additive is proposed to be used in turkeys for fattening, turkeys reared for breeding and all growing poultry, to modify the recommended dose for chickens for fattening from 1 x 10^7 CFU/kg to 1 x 10^8 CFU/kg and to authorise the use of the additive with halofuginone.

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 ACAF's advice, that the additive was correctly identified and characterised, the additive is compatible with halofuginone, and the recommended minimum dose for chickens for fattening can be modified to 1 x 10^8 CFU/kg.

ACAF concluded that the additive can be considered safe for the target species, the consumer and the environment. The additive should be considered a skin sensitiser and a respiratory sensitiser for the user/ worker.

ACAF concluded that the additive has the potential to be efficacious in turkeys for fattening turkeys and reared for breeding and can be extrapolated to all growing poultry.

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.

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive containing *Bacillus velezensis* ATCC PTA-6737 (previously identified as *Bacillus subtilis*) (PB6, Kemin Europa N.V., Toekomstlaan 42, 2200 Herentals, Belgium) under assimilated Regulation (EC) No 1831/2003 (EC, 2003) in the category 'zootechnical additives' and functional group 'gut flora stabiliser'. The application sought a renewal authorisation for use in turkeys for fattening and turkeys reared for breeding; to extrapolate the use of the additive to the category 'all growing poultry'; a modification of authorisation of the recommended dose for chickens for fattening from 1×10^7 CFU (colony forming units)/kg to 1×10^8 CFU/kg and a change of authorisation, to authorise the use of *Bacillus velezensis* in feed containing the permitted coccidiostat (halofuginone).

To support the safety assessment by the FSA and FSS, ACAF provided advice to the FSA and FSS 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.

The dossier was evaluated by ACAF at its September 2024 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 by ACAF at its October 2024, December 2024 and February 2025 meetings.

Further information was provided by the applicant during the suitability check stage; the applicant responded in April 2024.

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 dose/intake
Bacillus velezensis ATCC PTA-6737	Feed additive	Zootechnical additive	Minimum content of 1 x 10^8 CFU/kg of complete feedingstuff

2. Assessment

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

The additive is a microorganism product that contains spores of the active substance *Bacillus velezensis* ATCC PTA-6737. The additive is produced by a fermentation-based manufacturing process, where there is a minimum concentration of 8.0 x 10^{10} CFU/g of *Bacillus velezensis* ATCC PTA-6737 spores. The additive is available as a tan, free flowing, dry powder.

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

ACAF identified several pieces of information that the applicant would have to provide to inform the assessment of the product. In the request for information (RFI) response, the applicant provided confirmation that the manufacturing process has not been changed since the original submission, clarity in which plant manufactures the active ingredient with the relevant assurance and HACCP certification, data to confirming the absence of AMR genes and of plasmids, updated material safety data sheets (MSDSs) for all ingredients, an updated product label to include the compatibility of the additive with the administration of halofuginone, and an updated conditions of use label to include the exposure time and temperature during the pelleting and storage trial.

Table 2. Composition and identity of the additive *Bacillus velezensis* ATCC PTA-6737

Composition	
Dried ferment	5 – 25%
Sodium bicarbonate	75 – 95%
Appearance	
Tan colour, free flowing, dry powder	
Batch-to-batch variation of the active agent	
Bacillus cell count	8.0×10^{10} CFU/g
Chemical-physical properties	
Particle size distribution	< 100 µm – 97.47-99.85% < 50 µm – 89.43-97.30% < 10 µm – 19.63-32.28%
Bulk density	500 to 600 g/L
Dusting potential	1,045- 1,540 mg/m^3
Impurities	
Arsenic	≤ 2 ppm
Lead	≤ 10 ppm
Cadmium	≤ 1 ppm
Mercury	≤ 0.1 ppm
Coliforms	< 10 CFU/g
Enterobacteriaceae	< 10 CFU/g
Escherichia coli	< 10 CFU/g
Bacillus cereus	< 100 CFU/g
Staphylococcus aureus	< 10 CFU/g
Salmonella	Absent in 25 g
Yeasts and moulds	< 10 CFU/g
Dioxin and dioxin-like PCBs	< 1.25 ppt TEQ
Deoxynivalenol	< 900 ppb
Fumonisin	< 5,000 ppm
Aflatoxin B1	< 20 ppb
Ochratoxin A	< 50 ppb
Zearalenone	< 100 ppb

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* ATCC PTA-6737

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, turkeys reared for breeding and all growing poultry	Not applicable	1.0×10^8	Not applicable	None
Other provisions and additional requirements for the labelling				
Directions for use, and any safety recommendations regarding the use and, where applicable, the specific requirements mentioned in the authorisation	May be used in feed containing the permitted coccidiostats: halofuginone, diclazuril, decoquinate, salinomycin sodium, narasin/ nicarbazin, lasalocid A sodium, maduramycin ammonium, monensin sodium, narasin or robenidine hydrochloride. The additive can be used during the complete life cycle without any withdrawal period.			
Specific conditions or restrictions for use	Store in a dry place in closed containers at room temperature. Stable to pelleting at maximum 90°C for 45 seconds.			
Specific conditions or restrictions for handling	Wear personal protective equipment (PPE)/face protection. Ensure adequate ventilation. Avoid contact with skin, eyes, or clothing. Avoid ingestion and inhalation. Avoid dust formation. Observe good industrial hygiene practices. Wash hands after handling. Store away from incompatible materials. Dispose of waste and residues in accordance with local authority requirements.			

2.1.1. Conclusions on Section II

ACAF concluded that the additive was correctly identified and characterised, the additive is compatible with halofuginone, and the recommended minimum dose for chickens for fattening can be modified to 1×10^8 CFU/kg.

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* ATCC PTA-6737, which have the qualified presumption of safety (QPS) status. Therefore, the additive is presumed to be safe for the target species.

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* ATCC PTA-6737, as it has the QPS status. Therefore, the additive is presumed to be safe for the consumer.

2.2.3. Section III: Safety for the user and worker

In the applicant's RFI response, the applicant provided clarification that the additive is considered a potential respiratory sensitiser, not an irritant, and provided the studies on the effects of the additive on eyes and skin.

The applicant presented the following tests to evaluate the safety of the additive for the user/worker:

- Acute inhalation toxicity study on rats, following OECD protocol 403;
- Acute dermal irritation/corrosion study on white rabbit, following OECD protocol 404;
- Acute eye irritation/corrosion study on white rabbit, following OECD protocol 405.

ACAF was satisfied with the studies provided relating to skin and eye irritation and concluded that the additive is non-irritant to the eyes and skin.

It was concluded that the additive is considered a skin sensitiser and respiratory sensitiser. The additive is considered a dusty material that may deposit in the lungs of workers exposed to its dust. Therefore, measures are needed to minimise inhalation exposure of workers.

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* ATCC PTA-6737, as it has the QPS status. Therefore, the additive is presumed to be safe for the environment.

2.2.5. Section III: Conclusions on safety

ACAF concluded that the additive can be considered safe for the target species, the consumer and the environment. The additive should be considered a skin sensitiser and respiratory sensitiser for the user/worker.

2.3. Section IV: Efficacy

2.3.1. Efficacy in chickens for fattening

The applicant presented three *in vivo* studies in chickens for fattening to assess the efficacy of this application.

Members of ACAF discussed the three trials presented; they found discrepancies in the data submitted. In the RFI response, the applicant uploaded the unedited original reports. ACAF was satisfied with the documents that were provided.

The three trials were evaluated by the members; they agreed that the studies show potential for efficacy in chickens for fattening at 1 x 10^8 CFU/kg. Therefore, the additive has the potential to be efficacious in turkeys for fattening and reared for breeding, can be extrapolated to all growing poultry and the recommended minimum dose can be modified to 1 x 10^8 CFU/kg.

2.3.2. Section IV: Conclusions on efficacy

ACAF concluded that the additive has the potential to be efficacious in turkeys for fattening, turkeys reared for breeding, can be extrapolated to all growing poultry, and the recommended minimum dose can be modified to 1 x 10^8 CFU/kg.

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 *Bacillus subtilis* ATCC PTA-6737 (EURL, 2009):

> "For the enumeration of *Bacillus subtilis* ATCC PTA-6737 in the feed additive, premixtures and feedingstuffs, the applicant proposes the draft CEN method – prEN 15784:2008 E – an internationally recognised spread plate method. This method was ring-trial validated using the premixtures and feedingstuffs samples containing *Bacillus subtilis* spores. The performance characteristics of the draft CEN method reported after logarithmic transformation of measured values (CFU) are:
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> - For the premixtures: (1) a standard deviation for repeatability (s_r) of 0.09 $\log_{10}$ and (2) a standard deviation for between-laboratory reproducibility (s_R) of 0.32 $\log_{10}$.

> - For the feedingstuffs: (1) a s_r of 0.07 $\log_{10}$ and (2) a s_R of 0.35 $\log_{10}$.
>
> The applicant used the above mentioned spread plate method to analyse the various matrices containing *Bacillus subtilis* ATCC PTA-6767 spores and reported the following results: (a) 1 x 10^9 to 1.5 x 10^{11} CFU/g of feed additive; (b) 1 x 10^7 to 1.5 x 10^9 CFU/kg for premixtures and (c) 1 x 10^7 to 1.5 x 10^8 for feedingstuffs. The results obtained for feed additives and premixtures are considered acceptable; this method is therefore recommended for official controls for the feed additives and premixtures in the frame of the authorisation.
>
> As regards feedingstuffs, the CRL notes that the limit of quantification reported by the applicant upon request (LOQ = 1 x 10^7 CFU/kg feedingstuffs) is identical to the minimum dose proposed and is below the LOQ reported in the draft CEN method (2 x 10^7 CFU/kg). On the basis of the available information, the draft CEN method is recommended for official control of the feedingstuffs containing *Bacillus subtilis* PB6 at the dosages above the LOQ reported by CEN. Below 2 x 10^7 CFU/kg the CRL is not able to conclude on the suitability of this method for official control purposes.
>
> Molecular methods were used by the applicant for identification of the active agent. For official controls pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended."

ACAF recognised that PFGE is a valid method for microbial identification when using a validated reference pattern for authorised probiotic strains of *Lactobacillus, Pediococcus, Enterococcus* and *Bacillus* species. It was also recognised that whole genome sequencing would be the preferred method.

The FSA/FSS accepts the EURL analytical method evaluation reports and ACAF's assessment of microbial identification methodology. The FSA/FSS determined the analytical methods proposed as appropriate for official controls for this feed additive.

4. Conclusions

ACAF concluded that the additive was correctly identified and characterised, the additive is compatible with halofuginone, and the recommended minimum dose for chickens for fattening can be modified to 1 x 10^8 CFU/kg.

ACAF concluded that the additive can be considered safe for the target species, the consumer and the environment. The additive should be considered a skin sensitiser and a respiratory sensitiser for the user/ worker.

The additive has the potential to be efficacious in turkeys for fattening, turkeys reared for breeding and can be extrapolated to all growing poultry with a minimum dose of 1 x 10^8 CFU/kg.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
CEN	Comité Européen de Normalisation
CFU	Colony forming units
EC	European Commission
EU	European Union
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
HACCP	Hazard Analysis and Critical Control Points
LOQ	Limit of quantification
MSDS	Material safety data sheet
OECD	Organization for Economic Cooperation and Development
PPE	Personal protective equipment
QPS	Qualified presumption of safety
RFI	Request for information
S_r	Standard deviation for repeatability
s_R	Standard deviation for between-laboratory reproducibility

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