REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment on the Safety and Efficacy of a Feed Additive That Contains Spores of *Bacillus paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903 (Bovacillus® 10 and Bovacillus® WS) for Use in Dairy Cows and Other Dairy Ruminants (RP2157)

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

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in December 2023 from Chr. Hansen A/S ('the applicant'), requesting the use of a new additive consisting of *Bacillus paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903 (Bovacillus® 10 and Bovacillus® WS), under the category 'zootechnical additives' and functional group 'gut flora stabiliser'. The additive is proposed to be used in feed and drinking water for dairy cows and other dairy ruminants at a minimum recommended dose of 3.8 x 10^8 CFU/kg complete feed, or minimum 7.4 x 10^7 CFU/l drinking water.

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. 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 respiratory sensitiser and dermal sensitiser for the user/worker.

ACAF concluded that the additive has the potential to be efficacious in dairy cows and other dairy ruminants.

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 paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903 (Bovacillus® 10 and Bovacillus® WS) (Chr. Hansen A/S, Boege Allé 10-12, DK-2970 Hoersholm, Denmark) under assimilated Regulation (EC) No 1831/2003 (EC, 2003) in the category 'zootechnical additives' and functional group 'gut flora stabiliser'. The application sought a new use authorisation for use in feed and drinking water for dairy cows and other dairy ruminants.

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 and concluded 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 February 2025 meeting, after which a request for further information (RFI) was communicated to the applicant. The applicant's response to this request and subsequent requests were evaluated by ACAF at its June 2025 and December 2025 meetings.

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 paralicheni*formis DSM 33902 and *Bacillus subtilis* DSM 33903	Feed additive	Zootechnical additive	Minimum content of 3.8×10^8 CFU/kg of complete feedingstuff with a moisture content of 12% and 7.4×10^7 CFU/l of drinking water

2. Assessment

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

The feed additive is produced in two formulations, Bovacillus™ 10, the solid-form, and Bovacillus™ WS, the solid water-soluble form. The additive is a microorganism product that contains spores of the active substances *Bacillus paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903 (concentrate of the *baciullus* spores at ~6* w/w% for both forms). The additive is produced by a fermentation-based manufacturing process, where the guaranteed minimum total concentration of viable spores of the active substances in both formulations is 3.2 x 10^{10} CFU/g.

The qualitative and quantitative composition and identity of the additive are outlined in Table 2 (Bovacillus™ 10) and Table 3 (Bovacillus™ WS).

Table 2. Composition and identity of the additive Bovacillus™ 10

Composition	
Concentration of the *bacillus* spores	~6* w/w%
Calcium carbonate	~94* w/w%
Appearance	
White powder	
Batch-to-batch variation of the active agent	
Concentrate of the *bacillus* spores	3.2 x 10^{10} CFU/g
Chemical-physical properties	
Particle size distribution	< 100 µm – 14.9 % < 50 µm – 9.9 % < 10 µm – 5.9 % < 4 µm – 0.4 % < 1 µm – 0.0 %
Bulk density	1400 kg/m^3
Dusting potential	5150 mg/m^3
Impurities	
Arsenic	< 2.0 mg/kg
Lead	< 5.0 mg/kg
Cadmium	< 0.5 mg/kg
Mercury	< 0.1 mg/kg
Coliforms	< 10 CFU/g
Enterobacteriaceae	< 10 CFU/g
Escherichia coli	< 10 CFU/g
Salmonella	Absent in 25 g
Yeast and moulds	< 1000 CFU/g
Bacillus cell count	4.6 x 10^{10} CFU/g

Aflatoxin B1	< 0.01 mg/kg

* Exact quantities depend on CFU/g of the bulk fermentation concentrate.

Table 3. Composition and identity of the additive Bovacillus™ WS

Composition	
Concentration of the *bacillus* spores	~6* w/w%
Maltodextrin	~94* w/w%
Appearance	
White powder	
Batch-to-batch variation of the active agent	
Concentrate of the *bacillus* spores	3.2 x 10^{10} CFU/g
Chemical-physical properties	
Particle size distribution	< 100 µm – 81.0 % < 50 µm – 54.8 % < 10 µm – 10.6 % < 4 µm – 3.8 % < 1 µm – 0.0 %
Bulk density	600 kg/m^3
Dusting potential	1130 mg/m^3
Impurities	
Arsenic	< 2.0 mg/kg
Lead	< 5.0 mg/kg
Cadmium	< 0.5 mg/kg
Mercury	< 0.1 mg/kg
Coliforms	< 10 CFU/g
Enterobacteriaceae	< 10 CFU/g
Escherichia coli	< 10 CFU/g
Salmonella	Absent in 25 g
Yeast and moulds	< 1000 CFU/g
Bacillus cell count	4.3 x 10^{10} CFU/g
Aflatoxin B1	< 0.01 mg/kg

* Exact quantities depend on CFU/g of the bulk fermentation concentrate

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 evidence demonstrating that the Peg 295 AMR gene detected in *Bacillus paralicheniformis* was not on or near a mobile element, provided an updated label stating that the additive, Bovacillus™ WS, is stable in water for 48 hours, demonstrated that the additive is homogenously distributed in water, clarified the location of the manufacturing plant the strain was produced in along with the HACCP plan, provided the quantitative composition of the fermentation media, provided an updated list of the raw materials used in the manufacturing process, provided MSDS's of all materials used in the manufacturing process within the last two years, provided an updated label to include

the temperature and time of exposure during the pelleting stability study, provided a justification for the dosage of the additive in drinking water based on the daily water consumption of the average cow, and clarified the specification used for testing coliforms.

The applicant proposed a shelf-life of the additive at 30°C for 2 years, however, the studies provided in the application were for a less concentrated version of the additive at 30°C and at 25°C. The applicant was asked if they wish to submit data at 30°C at the proposed concentration or accept the conclusion of the shelf-life at up to 25°C for 2 years. They were unable to provide further data to support the shelf life of the additive at 30°C for 2 years, so ACAF concluded that the additive is stable at up to 25°C for 2 years.

The applicant proposes that the additive is included in animal feed as shown in Table 4.

Table 4. Proposed conditions of use for *Bacillus paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903

Proposed mode of use in animal nutrition					
Additive			*Bacillus paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903		
Registration number/EC N°			Not yet available		
Category of additive			Zootechnical additive		
Functional group of additive			Gut flora stabiliser		
Description					
Composition, description	Chemical formula		Purity criteria	Methods of analysis	
A preparation Bovacillus™ 10 (carrier: calcium carbonate) and Bovacillus™ WS (carrier: Maltodextrin)	Minimum of 3.2×10^{10} CFU/g		Complies with EU law on undesirable substances	Validated microbiological methods	
Trade name			Bovacillus™ (other trade names will also be used)		
Name of the holder of authorisation			Chr. Hansen A/S		
Conditions of use					
Species or category of animal	Maximum age	Minimum content CFU/kg complete feedingstuff	Minimum content CFU/l drinking water	Minimum content CFU/ Head/ day	Withdrawal period
Dairy cows and other dairy ruminants (e.g., sheep, goat, buffalo)	Not applicable	3.8×10^8	7.4×10^7	9.6×10^9	Not applicable
Other provisions and additional requirements for the labelling					

Specific conditions or restrictions for use	Store at ambient temperatures (max. 25°C) in original, closed packaging in a dry area.
Specific conditions or restrictions for handling	For user safety: recommended to use a facemask, gloves and goggles to reduce contact with dust.
Post-market monitoring	Chr. Hansen A/S will conduct post-marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and traceability systems, and formal monitoring of customer feedback through product or service complaints.
Specific conditions for use in complementary feedingstuffs /drinking water	Content of *bacillus* spores in final feeds should be min. 3.8×10^8 CFU/kg, in drinking water 7.4×10^7 CFU/l, and 9.6×10^9 CFU/head/day, see above.

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
Not relevant	Not relevant	Not relevant	Not relevant

2.1.1. Conclusions on Section II

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. 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 paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903, which have the qualified presumption of safety (QPS) status. The other components of the additive, calcium carbonate in Bovacillus® 10 and maltodextrin in Bovacillus® WS, are widely used ingredients that are known to be safe. 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 paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903, which have the QPS status. The other components of the additive, calcium carbonate in Bovacillus® 10 and maltodextrin in Bovacillus® WS, are widely used ingredients that are known to be safe. Therefore, the additive is presumed to be safe for the consumer.

2.2.3. Section III: Safety for the user and worker

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

- Acute skin irritation study with Bovacillus™ 10 on rabbits, following OECD protocol 404;
- *In vitro* eye irritation test Bovacillus™ 10 on isolated chicken eyes, following OECD protocol 438.

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.

The additive contains the active substances, *Bacillus paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903, which are microorganism products. Such additives are presumed to be potential sensitisers by dermal or inhalation exposure. Both forms of the product are dusty and contain many small particles that could deposit in the respiratory systems of exposed workers. Therefore, measures should be taken to minimise such exposure.

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 paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903, which have the QPS status. The other components of the additive, calcium carbonate in Bovacillus® 10 and maltodextrin in Bovacillus® WS, are widely used ingredients that are known to be safe. 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 potential respiratory sensitiser and dermal sensitiser for the user/worker.

2.3. Section IV: Efficacy

2.3.1. Efficacy in lactating cows

The applicant presented the following three long-term efficacy studies in lactating dairy cows to assess the efficacy of this additive:

- Effects of Bovacillus™ supplementation on production performance of lactating dairy cows, USA, 2019-2020;
- Effects of Bovacillus ™ supplementation on production performance of lactating dairy cows, Sweden, 2021-2022;

- Effects of Bovacillus ^{TM} supplementation on production of lactating dairy cows, Spain, 2021-2022.

Members of ACAF discussed that the three trials presented were carried out to a high standard and demonstrate evidence of improvements in milk yield, milk components and milk production efficacies. ACAF concluded that the additive has the potential to be efficacious in dairy cows and other dairy ruminants.

2.3.2. Section IV: Conclusions on efficacy

ACAF concluded that the additive has the potential to be efficacious in dairy cows and other dairy ruminants.

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 paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903 (Bovacillus^{TM}) (EURL , 2023):

> "For the enumeration of total *Bacilli spp. (Bacillus paralicheniformis* DSM 33902 and *Bacillus subtilis* DSM 33903) in the feed additive, compound feed and water, the EURL recommends for official control the ring-trial validated EN 15784 method.
>
> Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary."

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.

ACAF concluded that the additive can be considered safe for the target species, the consumer and the environment. Both forms of the additive should be considered a potential respiratory sensitiser and dermal sensitiser for the user/worker.

ACAF concluded that the additive has the potential to be efficacious in dairy cows and other dairy ruminants.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
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
MSDS	Material safety data sheet
OECD	Organization for Economic Cooperation and Development
QPS	Qualified presumption of safety
RFI	Request for information

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