Safety Assessment: Outcome of assessment of the safety and efficacy of *Bacillus velezensis* DSM 15544 (formerly *Bacillus subtilis* C-3102), (Calsporin®), as a feed additive for weaned piglets and all poultry and avian species

Reference number RP641

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

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Regulated Product Dossier Assessment

Safety Assessment finalised: 18/08/2023

Summary

An application was submitted to the Food Standards Agency (FSA) in March 2021 from Asahi Biocycle Co., Ltd ("the applicant") for the authorisation of an additive (Calsporin®) containing a minimum $1x10^{10}$ CFU/g of *Bacillus velezensis* DSM 15544, under the category of 'zootechnical' additives, functional group 'gut flora stabilisers'.

The application is for a request of extension of authorisation from chickens for fattening and laying hens to all other poultry and avian species for fattening, laying and breeding.

The additive Calsporin® was fully characterised and no major causes for concern were identified by the Joint Expert Group On Animal Feed And Feed Additives (AFFAJEG) in the identity, production, characterisation or conditions of use sections.

To support the FSA and Food Standards Scotland (FSS) in evaluating the dossier, the Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant. The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment, due to the Qualified Presumption of Safety (QPS) status of the microorganism *Bacillus velezensis* DSM 15544. The additive should be considered as a potential respiratory sensitiser and irritant.

The Group concluded that it is possible to extrapolate conclusions on efficacy from the original authorisation in chickens for fattening and laying hens and, therefore, the additive has the potential to be efficacious in other poultry, sporting and game birds, exotic and ornamental birds for rearing, fattening, egg production or breeding, when used at a minimum concentration of $3x10^{8}$ CFU/kg feed in complete feedstuff (with moisture content of 12%).

The views of AFFAJEG and 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 (Calsporin®, Asahi Biocycle Co., Ltd, Plaza Ausia March 1, 4th Floor, D01, Mirasol, ES-08195, Sant Cugat del Vallès, Spain, containing a minimum $1x10^{10}$ CFU/g of *Bacillus velezensis* DSM 15544, under Regulation (EC) No 1831/2003 the category of 'zootechnical' additives, functional group 'gut flora stabilisers'. To support the safety assessment by FSA and FSS, the AFFAJEG and the ACAF provided advice to the FSA and FSS outlined in this document. The application requested authorisation under two conditions of use:

- As a renewal for use in weaned piglets, chickens reared for laying, turkeys, minor avian species, and other ornamental and game birds.
- As an extension of use to all other poultry and avian species outside the categories covered under the renewal.

The dossier was evaluated on behalf of the FSA and FSS by the AFFAJEG. 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 AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Dr. 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 AFFAJEG at their October 2021 and February 2022 meetings. Further information was provided by the applicant in November 2021 responding to queries by FSA. The conclusions by the AFFAJEG were reviewed and approved by its successor body, the ACAF at their October 2022 meeting.

This document outlines the discussion and conclusions of the AFFAJEG's assessment on the safety and efficacy of Calsporin®, as a feed additive.

2. Assessment

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

The additive is a preparation of *Bacillus velezensis* DSM 15544, guaranteeing a minimum of $1x10^{10}$ CFU/g. The applicant provided data from seven batches supporting the specification values outlined below (Table 1).

Table 1: Specification table

Parameter	Specification
Bacillus velezensis DSM 15544	Minimum $1x10^{10}$ CFU/g
Heavy metals	< 20 ppm
Arsenic	< 2 ppm
Moisture	< 8% w/w
Dusting potential	3100 - 3215 mg/m^3
Particle size	<50 µm = 75-85% of total particles
Appearance	Pale granular powder
Odour	Characteristic fermented soy odour
Other	Complies with feed hygiene regulation

The Group evaluated the physico-chemical and technological properties of the additive, concluding that the manufacturing process, stability and homogeneity were well characterised. The microorganism *Bacillus velezensis* DSM 15544 was correctly characterised and equivalent to the organism listed in the QPS list.

In their first evaluation, members raised a query regarding the antimicrobial resistance potential of the microorganism. The FSA requested the applicant to provide further information. After careful consideration of the new data, the AFFAJEG noted antimicrobial resistance was not a cause for concern. Conditions of use of the additive are summarised in Table 2:

Table 2: Proposed mode of use of Calsporin® (*Bacillus velezensis* DSM 15544) as described in the application:

Proposed mode of use in animal nutrition			
Additive	Calsporin® (*Bacillus velezensis* DSM 15544)		
Registration Nº	4b1820		
Category of additive	Zootechnical feed additive		
Functional group(s) of additive	Gut flora stabiliser		
Description			
Composition, description	**Purity criteria**	**Method of analysis**	
A preparation of *Bacillus velezensis* DSM 15544	Containing a minimum of: $1x10^{10}$ viable spores (CFU) per gram	BS EN 15784:2009 Animal feedingstuffs: isolation & enumeration of presumptive *Bacillus* spp.	
Trade name (if appropriate)		Calsporin	
Name of the holder of authorisation (if appropriate)		Asahi Biocycle Co. Ltd.	
Conditions of use			
Species or category of animal	**Min. content**	**Max. content**	**Withdrawal period**
	CFU/kg of complete feedingstuffs with 12% moisture content		
Sows	$3x10^8$		Not applicable
Suckling piglets			
Weaned piglets			
Fattening pigs	$1.5x10^8$		
Chickens for fattening	$3x10^8$		
Breeding chickens			
Laying hens			
Chickens reared for laying & breeding			
All other avian species for rearing, fattening, laying & breeding			
Ornamental fish	$1x10^{10}$		
Dogs	$1x10^9$		

2.1.1. Conclusions on Section II

The AFFAJEG concluded that the 97% identity to a gene (clbA) belonging to the cfr-like antibiotic-resistance family of genes was not considered to be cause for concern, as it was found in the chromosome, as opposed to a bacterial plasmid.

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

2.2. Section III: Safety

As the organism *Bacillus velezensis* has QPS status, no studies are required to demonstrate the safety of the additive for the target species, the consumer or the environment.

2.2.1. Safety for the user/worker

The application presented two GLP-compliant dermal and ocular irritancy tests and one skin sensitisation test, all of which were negative. The AFFAJEG evaluated the studies and concluded that Calsporin® can be considered as non-irritant to the eyes and the skin and is not a potential skin sensitiser. Due to the proteinaceous nature of the additive, the Group concluded that it should be considered a potential respiratory sensitiser. Based on previous conclusions, and in the absence of new data, the AFFAJEG concluded that the additive should be considered a respiratory irritant.

2.2.2. Conclusions on safety

- The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment, due to the QPS status of the microorganism *Bacillus velezensis* DSM 15544.
- The additive should be considered as a potential respiratory sensitiser and irritant.

2.3. Section IV: Efficacy

The AFFAJEG evaluated Section IV of the dossier, for which no new efficacy studies were presented, for the possibility of extrapolating existing conclusions from previous authorisations of the additive to the new extensions of use proposed. The extrapolations proposed are:

- From chickens for fattening to other poultry for rearing or fattening, sporting and game birds for rearing or fattening, exotic and ornamental birds for rearing or fattening. (Minimum dose of $3x10^8$ CFU/kg feed).
- From laying hens to other poultry for egg production or breeding, sporting and game birds for egg production or breeding, exotic and ornamental birds for egg production or breeding. (Minimum dose of $3x10^8$ CFU/kg feed).

The Group determined that the existing conclusions on efficacy could be extrapolated to the newly proposed extensions of use. Therefore, the additive has the potential to be efficacious in other poultry, sporting and game birds, exotic and ornamental birds for rearing, fattening, egg production or breeding, when used at a minimum concentration of $3x10^8$ CFU/kg feed.

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 Calsporin® [2]:

"For the enumeration of *Bacillus subtilis* C-3102 in the feed additive, premixtures and feedingstuffs, the applicant proposes the CEN method – EN 15784:2009 – 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 CEN method– reported after logarithmic transformation of measured values (CFU) – are:

- For the premixtures: - a standard deviation for repeatability (s_r) of 0.09 $\log_{10}$ CFU/g and – a standard deviation for between-laboratory reproducibility (s_R) of 0.32 $\log_{10}$ CFU/g.
- For the feedingstuffs: - s_r = 0.07 $\log_{10}$ CFU/g - s_r = 0.35 $\log_{10}$ CFU/g and – a limit of quantification (LOQ) of $2x10^7$ CFU/kg of feedingstuffs, well below the minimum content proposed by the applicant ($3x10^8$ CFU/kg).

Molecular methods were used by the applicant for identification of the active agent. The EURL recommends for official control pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification. The CEN Technical Committee 327 is currently occupied with the harmonization of a European Standard for this identification method.

Further testing or validation is not considered necessary."

FSA/FSS accept the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The additive Calsporin® was fully characterised and no major causes for concern were identified by the AFFAJEG in the identity, production, characterisation or conditions of use sections.

The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment, due to the QPS status of the microorganism *Bacillus velezensis* DSM 15544. The additive should be considered as a potential respiratory sensitiser and irritant.

The Group concluded that the additive has the potential to be efficacious in other poultry, sporting and game birds, exotic and ornamental birds for rearing, fattening, egg production or breeding, when used at a minimum concentration of $3x10^8$ CFU/kg feed.

FSA/FSS accept 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 1831/2993 of the European Parliament and of the Council on additives for use in animal nutrition. Available at https://www.legislation.gov.uk/eur/2003/1831/contents
2. EURL-FA (European Reference Laboratory for Feed Additives), 2009. 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. Bacillus subtilis C-3102. Available at: https://joint-research-centre.ec.europa.eu/publications/fad-2009-0013_en

6. Abbreviations

FSA	Food Standards Agency
FSS	Food Standards Scotland
ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Joint Expert Group On Animal Feed And Feed Additives
WGS	Whole genome sequence
QPS	Qualified Presumption of Safety
FSA	Food Standard Agency
PFGE	Pulsed field gel electrophoresis
EURL	European Union Reference Laboratory
EURL-FA	European Union Reference Laboratory for Feed Additives
EC	European Commission

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