SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Calsporin® (*Bacillus subtilis*) as a feed additive for piglets[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Calsporin® is a microbial feed additive based on a single strain of *Bacillus subtilis*. The applicant is seeking authorisation for the use of the product with piglets at a proposed dose of 3 x 10^8 CFU/kg complete feedingstuff. The additive is currently authorised for use with chickens for fattening. The identity of the active agent was established in the previous application and belongs to a bacterial species whose safety EFSA considers can be assessed using the QPS approach. The strain is susceptible to antibiotics and evidence was previously provided to demonstrate a lack of toxigenic potential. Thus the product strain meets the qualifications attached to this group of bacilli, and the additive can be presumed safe for the target species, consumers and the wider environment. Five feeding trials are reported, each made with piglets given the additive at the proposed dose compared to matched groups fed the same diet without the additive. A significant improvement in feed to gain ratio and increase in final body weight and average daily gain in animals given the additive compared to control animals was seen in three trials. Consequently, Calsporin® can be considered as effective at the proposed dose.

[1] On request from the European Commission, Question No EFSA-Q-2009-00533, adopted on 9 December 2009.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Micro-organisms including Guido Rychen for the preparation of this opinion.

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SUMMARY

Calsporin® is a microbial feed additive based on a single strain of *Bacillus subtilis.* The applicant is seeking authorisation for the use of the product as a zootechnical feed additive for piglets at a recommended dose of 3 x 10^8 CFU/kg complete feedingstuff. The additive has already been assessed for use as feed additive for chickens for fattening by the European Food Safety Authority (EFSA) and is currently authorised for this purpose in the European Union.

EFSA now has been requested by the European Commission to give an opinion on the efficacy of the additive and its safety for the target animals the consumer, the user of the product and the environment.

The identity of the active agent was conclusively established in the previous application and belongs to a bacterial species whose safety EFSA considers can be assessed using the QPS approach. The strain is susceptible to antibiotics when judged against current criteria and evidence was previously provided to demonstrate a lack of toxigenic potential. Thus the product strain meets the qualifications attached to this group of bacilli, and the additive can be presumed safe for the target species, consumers and the wider environment.

Five feeding trials are reported, each made with piglets given the additive at the proposed dose compared to matched groups fed the same diet without the additive. A significant improvement in feed to gain ratio and increase in final body weight and average daily gain in animals given the additive at the proposed dose compared to control animals was seen in three trials. Consequently Calsporin® can be considered as effective at the proposed dose of 3 x 10^8 CFU/kg complete feedingstuff.

KEY WORDS

Zootechnical additive, Calsporin®, *Bacillus subtilis*, piglets, efficacy, safety, QPS

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Calpis Co Ltd[5] for authorisation of the product Calsporin®, *Bacillus subtilis* C-3102 (DSM 15544), to be used as a feed additive for piglets (category: zootechnical additive; functional group: gut flora stabiliser) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 29 September 2009.

The additive Calsporin® is a preparation of *Bacillus subtilis* C-3102 (DSM 15544). This product is authorised for use in chickens for fattening until October 2016 (4b1820).[7]

EFSA issued two opinions on the safety and efficacy of Calsporin® for chickens for fattening (EFSA, 2006 and 2007).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the efficacy and the safety for the target animal, consumer, user and the environment of the product Calsporin®, *Bacillus subtilis* C-3102 (DSM 15544), when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Micro-organisms, including Guido Rychen for the preparation of this opinion.

[4] OJ L 268, 18.10.2003, p.29
[5] Calpis Co Ltd, Europe Representative Office, 153 rue de Corcelles, 75017 Paris Cedex 17, France
[6] EFSA dossier reference: FAD-2009-0013
[7] OJ L 50, 23.2.2008, p. 6

Table 1. Description and conditions of use of the additive as proposed by the applicant

Additive	Calsporin®
Registration number/EC No/No	4b1820
Category of additive	Zootechnical feed additive
Functional group of additive	Gut flora stabiliser

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
A preparation of *Bacillus subtilis* C-3102 (DSM 15544)	Minimum of 1.0 x 10^{10} viable spores per gram	Complies with EU feed hygiene law	Validated microbial enumeration technique with heat treatment of samples

Trade name	Calsporin®
Name of the holder of authorisation	Calpis Co. Ltd., Japan, represented in the EU by the Calpis Co. Ltd. Europe Representative Office, Paris, France

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
Weaned piglets	120 days	3 x 10^8	3 x 10^8	Not applicable

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	None
Specific conditions or restrictions for handling	R42, potential respiratory sensitiser Breathing protection during handling and safety glasses
Post-market monitoring	No specific requirements
Specific conditions for use in complementary feedingstuffs	Dosage used should supply 3 x10^8 CFU/kg final complete feedingstuff

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

Calsporin® is the trade name for a feed additive based on viable spores of *Bacillus subtilis* C-3102. The applicant has requested an authorisation for use with piglets for a period of ten years under the category of a zootechnical additive (functional group: gut flora stabiliser). This product is currently authorised for use in chickens for fattening (see Background).

B. subtilis is now considered by the European Food Safety Authority (EFSA) to be a species suitable for QPS status (EFSA, 2008a). However, Calsporin® was first considered before the introduction of QPS as an assessment tool. In the course of this first assessment the identity of bacterial strain was conclusively established and the absence of a toxigenic potential demonstrated. As no resistance was found to the battery of antibiotics recommended by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) at that time, the strain satisfies the qualifications specified for the species and so can be treated as meeting the requirements of the QPS system. Consequently no further assessment of safety for the target species, the consumer and the environment is required. In addition, the use of the additive with piglets is considered unlikely to introduce hazards for users of the product not already considered as part of the first assessment. Consequently, in the present Opinion, the FEEDAP Panel has reviewed some additional information updating that previously available and assessed the data specific to the use of the additive with piglets.

2. Characterisation

The active agent in the additive consists of viable endospores of single strain of *B. subtilis* originally isolated from soil in Japan and deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 15544.[8] It has not been genetically engineered and does not harbour plasmids. After fermentation the medium and cells are dried and pasteurised to kill vegetative cells and sufficient amounts of calcium carbonate are added to produce a final product meeting the specification of 1.0 x 10^{10} CFU/g of *B. subtilis*.[9] Typically the final product contains 25–30% of the dried ferment and calcium carbonate to 100%. It is a dry free-flowing powder with an average particle size of about 23 μm.[10]

2.1. Additional characterisation of the product/active agent

The original assessment considered antibiotic resistance of the Calsporin® strain against the battery of antibiotics and using the breakpoints recommended by SCAN (EC, 2001/2003).[11] The FEEDAP Panel subsequently revised both the list of recommended antibiotics and the breakpoints used to establish susceptibility (EFSA, 2008b). The strain remains susceptible to all of the antibiotics when data were judged against the revised criteria. The FEEDAP Panel also introduced clindamycin to the battery of antibiotics recommended for testing. In a separate analysis the MIC value of *B. subtilis* C-3102 for clindamycin was found to be well below the breakpoint defined by FEEDAP for *Bacillus* spp.[12]

The EFSA Opinion (2006) recommended that the final product should be routinely monitored for the specific presence of *B. cereus* as part of the quality control process. In the current application an internationally recognised method for the enumeration of *B. cereus* is specified, accompanied by a statement that the concentrated fermentation product is routinely tested prior to dilution to specification.

[8] Technical dossier/Section II/Annex II_2_1_2a
[9] Technical dossier/Section II/Annex II_1_3
[10] Technical dossier/Section II/Annex II_1_5
[11] Technical dossier/Section II/Annex II_2_2_2d
[12] Technical dossier/Section II/Annex II_2_2_2e

2.2 Stability and homogeneity

The stability of the additive was established under two storage conditions (25°C/60% RH and 40°C/75% RH) and its shelf-life shown to be at least one year and six months respectively. However, at the time of the first assessment the stability data were incomplete. Observations at the lower temperature have now been extended to 37 months without any apparent loss of viability.[13]

The study protocol designed to measure stability in typical vitamin-mineral premixtures and complete feeds gives equal weight to piglet and broiler feedstuffs. However, only the data from the broiler premixture and feed are fully analysed. Results for piglet premixture and feed are provided only as raw data and do not include time zero values against which to measure change. Taken in isolation, the data relating to piglets is inadequate.

No reduction in microbial counts were seen in the broiler premixture after six months storage at 25°C/60% RH and only a limited reduction was seen over the same period at 40°C/75% RH. The similarity of counts in the piglet premixture between two and six months storage at the lower temperature suggests a comparable stability. Results at 40°C/75% RH were more variable but suggested that significant losses occurred.[14]

The additive survived pelleting at temperatures up to 90°C and the strain was fully recovered from pelleted broiler feed samples after one month at 25ºC/60% RH. However, counts were reduced by approximately one-third after three months storage. The product was not stable in pelleted broiler feed held at 40ºC. As the data made available for pelleted piglet feed consisted only of observations made after two and three months storage no conclusions can be drawn.

The capacity of the product to homogeneously distribute in feed has been previously assessed for poultry feed (EFSA, 2006).

Overall, given the similarity of broiler and piglet premixtures and feeds, it is unlikely that the behaviour of the additive in feed for piglets will differ from that seen in the broiler feed. However the data do suggest a susceptibility of the additive when mixed into feed to storage at the high ambient temperatures which may be encountered in southern Europe.

2.3 Conditions of use

The product is intended for use in feed for weaned piglets at a dose of 3 x 10^8 CFU/kg complete feedingstuff.

2.4 Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the CRL report can be found in Appendix A.

3. Safety

The species *B. subtilis* is considered by EFSA to have QPS status and not to require any specific demonstration of safety for the target species. However, the applicant included details of an early tolerance study made with piglets.[15] None of the results from the study would conflict with the QPS approach.

The safety of Calsporin® for consumers, users of the additive and the wider environment has been established as part of the previous authorisation (see Background). The FEEDAP Panel is not aware of

[13] Technical dossier/Section II/Annexes II_4_1a and II_4_1b
[14] Technical dossier/Section II/Annexes II_4_1c and II_4_1d
[15] Technical dossier/Section IV/Annex IV 6b

any additional data which would require a reconsideration of these safety assessments. Accordingly, no further assessment of safety is required for the product.

4. Efficacy

Five trials are described, made in three different European countries. Each trial consisted of a control group and a treatment group in which animals were fed the control diet supplemented with the proposed dose of the additive (in each case confirmed by analysis) or, in one case, the proposed dose and a higher dose. The numbers of animals and replicates per treatment varied between trials but even the study with the lowest number of animals (trial 1) still had 12 replicates per treatment. The duration of the trials was 42 or 43 days. Four of the trials used equal numbers of males and female Large white x Landrace piglets. Trial 1, however, differed and was made with male Duroc piglets only.

In each trial animals were monitored for zootechnical performance (intake, daily weight gain, body weight and efficiency of feed conversion), general health status and mortality. Measurements of intake were made on a pen basis.

Although occasional animals needed veterinary intervention, in four of the trials, the numbers of animals treated were small and were not test group related. The exception was trial 1 in which a more general occurrence of meningitis and respiratory disease mid-way through the study required all animals to be treated with antibiotics in the drinking water. This trial is not considered further. In the remaining four studies, the incidence of diarrhoea was low and the percentage mortality fell well within the range considered normal for the experimental facilities.

No significant differences were found in any measured parameter in trial 2 (Table 2). In contrast, the remaining three (trials 3, 4 and 5) showed highly significant increases in final body weight and average daily gain compared to control animals and an improvement in feed to gain ratio in the treated group. There was a numerical reduction in feed intake in the treated group compared to controls. Although this did not reach significance it was probably a contributory factor in the improved feed to gain ratio.

Table 2. Summary of performance data of piglets receiving Calsporin®

	Animals (replicates per treatment × animals/pen)	Calsporin® (CFU/kg feed)	Final body weight (kg)	Average daily gain (kg/day)	Feed/gain (kg/kg)
Trial 2[16]	336 (16 x 7)	0	21.4	0.31	1.57
		3×10^8	21.1	0.30	1.59
		1×10^9	21.1	0.30	1.58
Trial 3[17]	280 (14 x 10)	0	28.6	0.54	1.53
		3×10^8	29.8[2]	0.58[1]	1.41[1]
Trial 4[18]	426 (24 x 8–9)	0	25.6	0.43	1.93
		3×10^8	27.5[3]	0.48[3]	1.72[2]
Trial 5[19]	421 (24 x 8–11)	0	25.3	0.41	1.88
		3×10^8	26.3[3]	0.44[3]	1.73[3]

Treatment means differ significantly from controls [1]P<0.05, [2]P<0.001, [3]P<0.0001

CONCLUSIONS

The identity of the active agent was established previously and is one whose safety can be assessed using QPS approach. The strain remains susceptible to a battery of antibiotics when judged against current criteria and evidence was previously provided to demonstrate a lack of toxigenic potential. Thus the strain meets the qualifications attached to this group of bacilli, and the additive can be presumed safe for the target species, consumers and the environment.

As a significant improvement in feed to gain ratio and increase in final body weight and average daily gain in animals given the additive compared to control animals was seen in three trials, Calsporin® can be considered as effective in piglets at the proposed dose of 3×10^8 CFU/kg complete feedingstuffs.

DOCUMENTATION PROVIDED TO EFSA

1. Calsporin® (EU No. 4b1820) (*Bacillus subtilis* C-3102) Zootechnical feed additive for weaned piglets. March 2009. Submitted by EU Pen & Tec Consulting.
2. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for *Bacillus subtilis*.
3. Comments from Member States received through the ScienceNet.

REFERENCES

EC (European Commission), 2001, updated 2003. Opinion of the Scientific Committee on Animal Nutrition on the criteria for assessing the safety of micro-organisms resistant to antibiotics of human and veterinary importance. http://ec.europa.eu/food/fs/sc/scan/out108_en.pdf

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the microbiological product "Calsporin", a

[16] Technical dossier/Section IV/Annex IV 3 2
[17] Technical dossier/Section IV/Annex IV 3 3
[18] Technical dossier/Section IV/Annex IV 3 4
[19] Technical dossier/Section IV/Annex IV 3 6

preparation of *Bacillus subtilis* as a feed additive for chickens for fattening in accordance with Regulation (EC) 1831/2003. EFSA Journal (2006) 336, 1-15
http://www.efsa.europa.eu/en/scdocs/scdoc/336.htm

EFSA (European Food Safety Authority), 2007. Safety and efficacy of Calsporin®, a preparation of *Bacillus subtilis* as a feed additive for chickens for fattening in accordance with Regulation (EC) 1831/2003. EFSA Journal (2007) 543, 1-8
http://www.efsa.europa.eu/en/scdocs/scdoc/543.htm

EFSA (European Food Safety Authority), 2008a. The maintenance of the list of QPS microorganisms added to food or feed. Scientific Opinion of the Panel on Biological Hazards. EFSA Journal (2008) 923,1-48
http://www.efsa.europa.eu/en/scdocs/scdoc/923.htm

EFSA (European Food Safety Authority), 2008b. Technical guidance prepared by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. EFSA Journal (2008) 732, 1-15
http://www.efsa.europa.eu/en/scdocs/scdoc/732.htm

APPENDIX A

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis of Calsporin®

In the current application authorisation is sought for the microbial feed additive *Bacillus subtilis* C-3102, DSM 15544 under the category 'zootechnical additive', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) 1831/2003. Specifically, authorisation is sought for the use of *Bacillus subtilis* C-3102 for piglets (weaned). The feed additive consists of a minimum of $1x10^{10}$ colony forming units (CFU) per gram of viable spores of *Bacillus subtilis* C-3102. The feed additive is a pale granular powder intended to be mixed into complete feedingstuffs at a final concentration of $3x10^{8}$ CFU/kg.

For the enumeration of *Bacillus subtilis* C-3102 in the feed additive, premixturetures 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 premixturetures 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 premixturetures: - a standard deviation for repeatability (s_r) of 0.09 log10 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 bellow 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 CRL 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.