

ADOPTED: 13 June 2018

doi: 10.2903/j.efsa.2018.5340

## Assessment of the application for renewal of authorisation of Calsporin<sup>®</sup> (*Bacillus subtilis* DSM 15544) for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP),  
Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis,  
Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli,  
Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso,  
Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos,  
Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Jaime Aguilera, Rosella Brozzi and  
Maria Saarela

### Abstract

Calsporin<sup>®</sup> is the trade name for a feed additive based on *Bacillus subtilis* DSM 15544. It is currently authorised for use in chickens for fattening, weaned piglets, chickens reared for laying, turkeys, minor avian species and other ornamental and game birds, and ornamental fish. This opinion concerns the renewal of the authorisation of Calsporin<sup>®</sup> as a zootechnical additive for chickens for fattening. In 2006 and 2007, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted two opinions on the safety and efficacy of Calsporin<sup>®</sup> when used in chickens for fattening, and the active agent was further characterised in an opinion from 2015. The applicant has provided data demonstrating that the additive currently in the market complies with the conditions of authorisation. Furthermore, according to the information provided in the technical dossier, no new evidence has been identified that would make the FEEDAP Panel reconsider the previous conclusions on the safety of the additive. The FEEDAP Panel confirms its previous conclusion that Calsporin<sup>®</sup> is safe for the target species, consumers of products from animals fed the additive, users and the environment.

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**Keywords:** zootechnical additive, Calsporin<sup>®</sup>, *Bacillus subtilis*, safety, efficacy, QPS, Chickens

**Requestor:** European Commission

**Question number:** EFSA-Q-2016-00089

**Correspondence:** feedap@efsa.europa.eu

**Panel members:** Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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**Acknowledgements:** The FEEDAP Panel wishes to thank the following for the support provided to this scientific output: Jaume Galobart, Matteo L. Innocenti and Paola Manini.

**Suggested citation:** EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Villa RE, Wallace RJ, Wester P, Aguilera J, Brozzi R and Saarela M, 2018. Scientific Opinion on the assessment of the application for renewal of authorisation of Calsporin® (*Bacillus subtilis* DSM 15544) for chickens for fattening. EFSA Journal 2018;16(7):5340, 9 pp. <https://doi.org/10.2903/j.efsa.2018.5340>

**ISSN:** 1831-4732

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# 1. Introduction

## 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from Asahi Calpis Wellness Co. Ltd.<sup>2</sup> for renewal of the authorisation of the Calsporin® (*Bacillus subtilis* DSM 15544), when used as a feed additive for chickens for fattening (category: zootechnical additive; functional group: gut flora stabiliser).

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 14(1) (renewal of the authorisation). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 11 April 2016.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product Calsporin® (*Bacillus subtilis* DSM 15544), when used under the proposed conditions of use (see Section 3.1.2).

## 1.2. Interpretation of the Terms of Reference

The application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive; therefore efficacy is not assessed. The present opinion will focus only on the safety aspects.

## 1.3. Additional information

EFSA has issued several opinions on the safety and efficacy of Calsporin® when used in chickens for fattening (EFSA, 2006, 2007a), weaned piglets (EFSA FEEDAP Panel, 2010a), turkeys for fattening, ducks, geese, pigeons and other game birds for meat production, ducks, geese, pigeons, game birds, ornamental and sporting birds for rearing to point of lay, turkeys reared for breeding and chickens reared for laying (EFSA FEEDAP Panel, 2010b), laying hens and avian species for laying (EFSA FEEDAP Panel, 2015a), ornamental fish (EFSA FEEDAP Panel, 2015b), sows and suckling piglets (EFSA FEEDAP Panel, 2017a), dogs (EFSA FEEDAP Panel, 2017b) and pigs for fattening (EFSA FEEDAP Panel, 2018).

The additive is authorised as a zootechnical additive (functional group: gut flora stabiliser) for use in chickens for fattening,<sup>3</sup> weaned piglets,<sup>4</sup> chickens reared for laying, turkeys, minor avian species and other ornamental and game birds,<sup>5</sup> laying hens and ornamental fish,<sup>6</sup> dogs, sows and suckling piglets.<sup>7</sup>

<sup>1</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>2</sup> Asahi Calpis Wellness Co., Ltd., represented in the EU by Asahi Calpis Wellness Co., Ltd. Europe Representative Office, 46 rue Paul Valéry, 75116, Paris, France.

<sup>3</sup> Commission Regulation (EC) No 1444/2006 of 29 September 2006 concerning the authorisation of *Bacillus subtilis* C-3102 (Calsporin) as a feed additive. OJ L 271, 30.9.2006, p. 19 plus amendments.

<sup>4</sup> Commission Regulation (EU) No 333/2010 of 22 April 2010 concerning the authorisation of a new use of *Bacillus subtilis* C-3102 (DSM 15544) as a feed additive for weaned piglets (holder of authorisation Calpis Co. Ltd. Japan, represented in the European Union by Calpis Co. Ltd. Europe Representative Office). OJ L 102, 23.4.2010, p. 19 plus amendments.

<sup>5</sup> Commission Regulation (EU) No 184/2011 of 25 February 2011 concerning the authorisation of *Bacillus subtilis* C-3102 (DSM 15544) as a feed additive for chickens reared for laying, turkeys, minor avian species and other ornamental and game birds (holder of authorisation Calpis Co. Ltd Japan, represented by Calpis Co. Ltd Europe Representative Office). OJ L 53, 26.2.2011, p. 33 plus amendments.

<sup>6</sup> Commission Implementing Regulation (EU) 2016/897 of 8 June 2016 concerning the authorisation of a preparation of *Bacillus subtilis* (C-3102) (DSM 15544) as a feed additive for laying hens and ornamental fish (holder of authorisation Asahi Calpis Wellness Co. Ltd) and amending Regulations (EC) No 1444/2006, (EU) No 333/2010 and (EU) No 184/2011 as regards the holder of the authorisation. OJ L 152, 9.6.2016, p. 7

<sup>7</sup> Commission Implementing Regulation (EU) 2017/2312 of 13 December 2017 concerning the authorisation of a new use of the preparation of *Bacillus subtilis* C-3102 (DSM 15544) as a feed additive for sows, suckling piglets and dogs (holder of the authorisation Asahi Calpis Wellness Co. Ltd, represented by Asahi Calpis Wellness Co. Ltd Europe Representative Office), OJ L 331, 14.12.2017, p. 41.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier<sup>8</sup> in support of the authorisation request for the use of Calsporin® (*Bacillus subtilis* DSM 15544) as a feed additive. The technical dossier was prepared following the provisions of Article 14 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008<sup>9</sup> and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.<sup>10</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of Calsporin® (*Bacillus subtilis* DSM 15544) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013).

## 3. Assessment

### 3.1. Characterisation

The additive is a preparation consisting of dried cells of *Bacillus subtilis* DSM 15544 intended for use as a zootechnical additive (gut flora stabiliser) in feed for chickens for fattening at a minimum content of  $5 \times 10^8$  colony forming units (CFU)/kg.

The additive is authorised with a minimum declared content of  $1.1 \times 10^{10}$  CFU/g [REDACTED]. The applicant declared that additive has not been modified since the last authorisation and provided data from recent batches on the composition and purity of the additive. Compliance with specifications was confirmed by analysis of three batches produced in 2014 (mean value of  $1.2 \times 10^{10}$  CFU/g additive, coefficient of variation (CV) = 3%).<sup>11</sup>

Three batches produced in 2014 and 2015 were analysed for microbial and chemical contaminants.<sup>12</sup> Results confirm compliance with limit levels (*Escherichia coli* < 10 CFU/g, *Salmonella* absence in 25 g, Enterobacteriaceae < 10 CFU/g, *Staphylococcus aureus* ≤ 30 CFU/g, *Bacillus cereus* < 100 CFU/g, yeasts and filamentous fungi < 100 CFU/g, aflatoxins B1, B2, G1 and G2 < 0.005 mg/kg, deoxynivalenol < 100 µg/kg, fumonisin B1 and B2 < 5 µg/kg, HT-2-toxin < 10 µg/kg, ochratoxin A < 1 µg/kg, T-2 toxin < 50 µg/kg, zearalenone < 5 µg/kg, arsenic ≤ 1 mg/kg, cadmium 0.6 mg/kg, mercury < 0.01 mg/kg, lead ≤ 0.8 mg/kg and sum of dioxins and dioxin-like polychlorinated biphenyls < 0.137 ng/kg).

#### 3.1.1. Characterisation of the active agent

The active agent is a *B. subtilis* strain deposited in the German Collection of Microorganisms and Cell Cultures with accession number DSM 15544 and in the National Institute of Advanced Industrial Science and Technology (Japan) with accession number FERM BP-1096.<sup>13</sup> The identity of the strain was evaluated using molecular methods (full 16S rRNA gene sequencing and pulsed field gel electrophoresis (PFGE)).<sup>14</sup> The PFGE patterns of the strain isolated from one batch of the product from 2015 were identical to those from the strain deposited (DSM 15544), confirming that the strain has not

<sup>8</sup> FEED dossier reference: FAD-2015-0038.

<sup>9</sup> Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

<sup>10</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2009-0013.pdf>

<sup>11</sup> Technical dossier/Section II/Annex II.1.4.1.3.

<sup>12</sup> Technical dossier/Section II/Annexes II.1.4.1.1 and II.1.4.1.2.

<sup>13</sup> Technical dossier/Supplementary information October 2016/Annex II.2.1.2.1.

<sup>14</sup> Technical dossier/Supplementary information October 2016/Annexes II.2.1.2.1-II.2.1.2.3.

been modified overtime.<sup>15</sup> Susceptibility to relevant antibiotics and lack of toxigenic potential were demonstrated in a recent opinion (EFSA FEEDAP Panel, 2015a) and are considered still valid.

### 3.1.2. Conditions of use

Calsporin® is currently authorised for chickens for fattening at the minimum of  $5 \times 10^8$  CFU/kg feed with no maximum level. It is allowed to be used simultaneously with coccidiostats: monensin sodium, salinomycin sodium, semduramycin sodium, lasalocid sodium, maduramycin sodium, narasin-nicarbazin and diclazuril.

The applicant proposes to maintain the same conditions of use.

## 3.2. Safety

The species *B. subtilis* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establish safety for the target species, consumers and the environment (EFSA, 2007b; EFSA BIOHAZ Panel, 2017). This approach requires the identity of the active agent to be established and the absence of a toxigenic potential and susceptibility to antibiotics of human clinical and veterinary importance to be demonstrated. The identity of the strain Calsporin® (*Bacillus subtilis* DSM 15544) was established and the lack of resistance to relevant antibiotics and of toxigenic potential was demonstrated in a recent assessment (EFSA FEEDAP Panel, 2015a). Accordingly, this strain is considered by EFSA to be suitable for the QPS approach to safety and is presumed safe for the target species, consumers of products from animals fed the additive and the environment.

In a previous opinion (EFSA FEEDAP Panel, 2006), the FEEDAP Panel concluded that Calsporin® is not irritant to skin and eye and is not a skin sensitiser. Given the small particle size and propensity to dust formation, sensitisation via respiratory route cannot be excluded.

The applicant maintains records of quality control parameters, non-conformities and recalls. No adverse effects have been reported.<sup>16</sup> The applicant performed two literature searches on the safety of Calsporin® using three databases: CAB Abstracts, Medline and Veterinary Science Database.<sup>17</sup> The first search covered the period 2000–2016 and the search terms included Calsporin®, *Bacillus subtilis* C-3102,<sup>18</sup> poultry, broiler, fowls and game birds. The second covered the period 2005–2016 and the search terms included Calsporin®, *Bacillus subtilis* C-3102, poultry, broiler, fowls, game birds, adverse event, toxicity, poison, danger, risk and safety. The searches did not reveal publications reporting safety issues with the additive under assessment. The only studies reported (Appendix A) are related to efficacy, and concluded that mortality was not affected by treatment with the additive.

Therefore, the FEEDAP Panel concludes that there is no new evidence that would lead the Panel to reconsider its previous conclusions on the safety of the product for target species, consumers, users and the environment under the authorised conditions of use.

## 3.3. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation<sup>19</sup> and Good Manufacturing Practice.

## 4. Conclusions

The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation.

The FEEDAP Panel confirms its previous conclusion that Calsporin® is safe for the target species, consumers of products from animals fed the additive, users and the environment.

<sup>15</sup> Technical dossier/Supplementary information October 2016/Annex II.2.1.2.4.

<sup>16</sup> Technical dossier/Supplementary information October 2016/Adverse Events, 2016.

<sup>17</sup> Technical dossier/Supplementary information October 2016.

<sup>18</sup> C-3102 is an in-house identifier; the active agent is *Bacillus subtilis* DSM 15544.

<sup>19</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.



## Documentation provided to EFSA

- 1) Calsporin®. *Bacillus subtilis* C-3102, DSM 15544. Zootechnical additive for chickens for fattening. October 2015. Submitted by Asahi Calpis Wellness Co. Ltd.
- 2) Calsporin®. *Bacillus subtilis* C-3102, DSM 15544. Zootechnical additive for chickens for fattening. Supplementary information. October 2016. Submitted by Asahi Calpis Wellness Co. Ltd.
- 3) Comments from Member States.

## Chronology

Date	Event
16/10/2016	Dossier received by EFSA
16/11/2015	Reception mandate from the European Commission
11/7/2016	Comments received from Member States
30/5/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species, consumer, user and environment</i>
13/6/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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- EFSA (European Food Safety Authority), 2007a. Scientific Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the product Calsporin®, a preparation of *Bacillus subtilis*, as a feed additive for chickens for fattening in accordance with Regulation (EC) No 1831/2003. EFSA Journal 2007;5(9):543, 8 pp. <https://doi.org/10.2903/j.efsa.2007.543>
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- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcini PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlcek JM, Aguilera-Gomez M, Barizzzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C and Fernández Escámez PS, 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal 2017;15(3):4664, 177 pp. <https://doi.org/10.2903/j.efsa.2017.4664>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010a. Scientific Opinion on the safety and efficacy of Calsporin® (*Bacillus subtilis*) as a feed additive for piglets. EFSA Journal 2010;8(1):1426; 11 pp. <https://doi.org/10.2903/j.efsa.2010.1426>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010b. Scientific Opinion on the safety and efficacy of Calsporin® (*Bacillus subtilis*) for turkeys for fattening, ducks, geese, pigeons and other game birds for meat production, ducks, geese, pigeons, game birds, ornamental and sporting birds for rearing to point of lay, turkeys reared for breeding and chickens reared for laying. EFSA Journal 2010;8(10):1867, 13 pp. <https://doi.org/10.2903/j.efsa.2010.1867>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Guidance on the renewal of the authorisation of feed additives. EFSA Journal 2013;11(10):3431, 8 pp. <https://doi.org/10.2903/j.efsa.2013.3431>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015a. Safety and efficacy of Calsporin® (*Bacillus subtilis* DSM 15544) as a feed additive for laying hens and avian species for laying. EFSA Journal 2015;13(9):4231, 10 pp. <https://doi.org/10.2903/j.efsa.2015.4231>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015b. Scientific Opinion on the safety and efficacy of Calsporin® (*Bacillus subtilis* DSM 15544) as a feed additive for ornamental fish. EFSA Journal 2015;13(11):4274, 10 pp. <https://doi.org/10.2903/j.efsa.2015.4274>

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## Abbreviations

CFU	colony forming unit
CV	coefficient of variation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
PFGE	pulsed field gel electrophoresis
QPS	qualified presumption of safety



## Appendix A – List of references retrieved from the literature search provided by the applicant to support safety of the additive

- Jeong JS and KIM IH, 2014. Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. *Poultry Science*, 93, 3097–3103.
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