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Safety and efficacy of Calsporin® (*Bacillus subtilis* DSM 15544) as a feed additive for pigs for fattening

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Abstract

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed was asked to deliver a scientific opinion on the safety and efficacy of Calsporin® when used in feed for pigs for fattening. The additive contains viable spores of a single strain of *Bacillus subtilis*. This species is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment. This approach requires the identity of the strain to be conclusively established, evidence that the strain is not toxigenic and that it does not show resistance to antibiotics of human and veterinary importance. The strain was found to meet the criteria for the QPS approach in the context of previous opinions and since concerns are not expected from other components of the additive, Calsporin® is presumed safe for all target species, including pigs, consumers of products derived from animals treated and for the environment. In a previous opinion, the Panel also concluded that the additive is not a dermal/eye irritant or a skin sensitiser but should be considered a potential respiratory sensitiser. The use of the additive in pigs for fattening is considered unlikely to introduce hazards for users of the product not already considered as part of the first assessment. Therefore, the conclusions reached apply to the current application. Based on the results of the pooled analysis of four studies, the Panel on additives and products or substances used in animal feed (FEEDAP) concludes that Calsporin® has the potential to improve performance of pigs for fattening at 1.5×10^8 CFU/kg feed.

Keywords: zootechnical additive, Calsporin®, *Bacillus subtilis* DSM 15544, pigs for fattening, QPS, safety, efficacy

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] 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 Asahi Calpis Wellness Co. Ltd.[2] for Calsporin® (*Bacillus subtilis* DSM 15544), when used as a feed additive for pigs for fattening (category: zootechnical additives; functional group: gut flora stabilisers).

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). The particulars and documents in support of the application were considered valid by EFSA as of 22 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 3.1.1).

1.2. Additional information

The additive Calsporin® is a preparation containing viable spores of a single strain of *Bacillus subtilis*. EFSA has issued several opinions on the safety and efficacy of this product when used with chickens for fattening (EFSA, 2006, 2007a), with weaned piglets (EFSA FEEDAP Panel, 2010a), with 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), with laying hens and minor avian species for laying (EFSA FEEDAP Panel, 2015a), with ornamental fish (EFSA FEEDAP Panel, 2015b), with dogs (EFSA FEEDAP Panel, 2017a) and with sows and suckling piglets (EFSA FEEDAP Panel, 2017b).

The additive is currently authorised for use with chickens for fattening,[3] weaned piglets,[4] chickens reared for laying, turkeys, minor avian species and other ornamental and game birds,[5] laying hens and ornamental fish,[6] dogs, sows and suckling piglets.[7]

[1] 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.

[2] Asahi Calpis Wellness Co., Ltd., represented in the EU by Asahi Calpis Wellness Co., Ltd. Europe Representative Office, 46 rue Paul Valery, 75116, Paris, France.

[3] 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.

[4] 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.

[5] 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.

[6] 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.

[7] 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[8] in support of the authorisation request for the use of Calsporin® as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[9] and the applicable EFSA guidance documents.

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.[10]

2.2. Methodologies

The approach followed by the panel on additives and products or substances used in animal feed (FEEDAP) to assess the safety and the efficacy of Calsporin® is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a) and Technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

3. Assessment

Calsporin® is a preparation of viable spores of a single strain of *B. subtilis* intended for use as a zootechnical additive (gut flora stabiliser) in feeds for pigs for fattening, in order to improve their performance.

3.1. Characterisation[11]

Calsporin® is a dry free-flowing powder with a minimum declared content of 1×10^{10} colony forming units (CFU) of *B. subtilis* DSM 15544[12] per gram of additive. It has the same formulation and method of manufacture as that considered in previous opinions (EFSA, 2006; EFSA FEEDAP Panel, 2010a,b, 2015a). Thus, the data pertaining to composition, impurities, physical properties and shelf-life still apply.

The stability and capacity of the additive to homogeneously mix with feed and premixtures for piglets and poultry was established in these previous opinions. Given the commonality of feed ingredients in diets for pigs for fattening, the FEEDAP Panel is of the opinion that the existing data are sufficient to establish the stability and capacity to homogeneously mix of the additive in premixtures and feeds for this category.

3.1.1. Conditions of use

Calsporin® is intended for use in feed for pigs for fattening at a minimum dose of 1.5×10^8 CFU/kg complete feedingstuffs.

3.2. Safety

The species *B. subtilis* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establishing safety for the target species, consumers and the environment (EFSA, 2007b; EFSA BIOHAZ Panel, 2017). This approach requires the identity of the strain to be conclusively established, evidence that the strain is not toxigenic and that it does not show resistance to antibiotics of human and veterinary importance. In a previous opinion (EFSA FEEDAP Panel, 2015a,b), the identification of the strain and compliance with the QPS qualifications were confirmed. Therefore, the Panel concluded that *B. subtilis* DSM 15544 can be presumed safe for target animals, consumers of

[8] FEED dossier reference: FAD-2016-0012.
[9] 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.
[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2009-0013.pdf
[11] This section has been amended following the confidentiality claims made by the applicant.
[12] The in-house identifier C-3102 has been used in previous assessments.

products derived from animals fed the additive and the environment. The Panel considers these conclusions to apply also in the current assessment. No concerns are expected from other excipients present in the product, so Calsporin® is also considered safe for target animals, consumers and the environment.

In a previous opinion on the use of Calsporin® in feed for chickens for fattening, the Panel concluded that the additive is not a dermal/eye irritant or a skin sensitiser but should be considered a potential respiratory sensitiser (EFSA, 2006). The use of the additive in pigs for fattening is considered unlikely to introduce hazards for users of the product not already considered as part of the first assessment. Therefore, the conclusions reached in the previous assessment apply to the current application.

3.3. Efficacy

Five studies were performed in four Member States to evaluate the effects of Calsporin® supplementation on the growth of pigs for fattening.

The designs of the studies are presented in Table 1 and the results in Table 2. Studies 1,[13] 2[14] and 5[15] involved single-sex pens whilst studies 3[16] and 4[17] had mixed-sex (50:50) pens. In study 3, two consecutive batches of animals were studied. In study 4, pigs were distributed in pens according to weight (in three light, three medium and three heavy pens per treatment). In all cases, pens were allocated to two experimental groups, one receiving the basal diets not supplemented and a second receiving the basal diets supplemented with the additive in order to provide 1.5×10^8 CFU/kg feed. Concentration in feed was confirmed by analysis. The animals were fed *ad libitum*. Pen feed intake and pen/individual body weight of animals were measured and the feed to gain ratio was calculated. Morbidity and mortality were also monitored. In studies 2 and 3, data were analysed as a randomized complete block design (in study 2 with 16 blocks corresponding to sex and location within the house and in study 3 with two consecutive batches of animals). In all cases, data were analysed using an analysis of variance (ANOVA), considering the pen as the experimental unit.

Table 1: Details on the study design for the studies performed in pigs for fattening

Study	Breed Sex	Total animals Replicates/ treatment × animals/replicate	Duration of the study (days)	Basal diets (main ingredients) form
1	Goland × Duroc ♀, castrated ♂	144 12 × 6	125	(wheat/soybean meal/barley/rye) mash
2	(Duroc × Landrace) × Piétrain ♀, ♂	128 16 × 4	111	(barley/wheat/soybean meal/maize) pelleted
3	Large White × Landrace ♀, ♂	3,715[(a)] 89 × 8–25	88	(maize/sunflower meal/soy oil) pelleted
4	Piétrain × hybrid ♀, castrated ♂	108 9 × 6	119	(wheat/maize/barley/soybean meal) pelleted
5	Goland × Bompieri ♀, castrated ♂	144 12 × 6	112	(wheat/soybean meal/barley/rye) mash

(a): 1,846 pigs in the control and 1,869 in the Calsporin group.

[13] Technical dossier/Section IV/Annex IV.3.1.
[14] Technical dossier/Section IV/Annex IV.3.2.
[15] Technical dossier/Supplementary information January 2017/Annex IV.3.6.
[16] Technical dossier/Section IV/Annex IV.3.3.
[17] Technical dossier/Section IV/Annex IV.3.4.

Table 2: Overview of results of efficacy studies with Calsporin® in pigs for fattening

Study	Calsporin® (CFU/kg feed)	Initial weight (kg)	Final weight (kg)	Average daily feed intake (kg/day)	Average daily gain (g/d)	Feed:gain	Mortality and removals (n/total)
1	0	27.6	107.9[b]	2.05	635[b]	3.24[a]	1/72
	1.5×10^8	27.7	113.3[a]	2.04	686[a]	2.98[b]	0/72
2	0	29.3	97.8	1.76	617	2.85	0/64
	1.5×10^8	29.4	98.1	1.76	618	2.84	2/64
3	0	49.0	102.4	1.83	608[a]	3.02	30/1,846
	1.5×10^8	49.2	103.0	1.87	625[b]	3.01	31/1,869
4	0	24.8	119.8	1.95	799	2.44	1/54
	1.5×10^8	24.8	121.5	1.99	815	2.45	2/54
5	0	26.5	116.8	2.90[a]	799	3.64	1/72
	1.5×10^8	26.8	120.2	2.89[b]	834	3.47	0/72

[a,b]Values within one column for the same study with different superscripts are different ($p < 0.05$).

Mortality in all studies was low and not influenced by treatment. Overall pigs in the Calsporin® group showed a significantly greater average daily gain (ADG) in two studies (1 and 3). In addition, final weight and feed to gain ratio were also improved in study (1).

The data on body weight at study start and study end, average daily feed intake (ADFI), ADG and feed to gain ratio of four of the five studies were tested for homogeneity and pooled.[18] The applicant excluded study 3 from this analysis because of its late start and shorter duration. The data were evaluated using the pen as the experimental unit, applying an ANOVA and using the Tukey test to separate means. The model included dietary treatment and study as main effects and their interaction. No interactions were detected except for the outcome for feed to gain ratio, where a significant interaction study treatment was found. Differences were considered significant at a level of $p < 0.05$. Results showed that supplementation with Calsporin® resulted in significant improvements in final body weight (control: 108.7 kg, Calsporin®: 111.5 kg) and in ADG (control: 698 g/d, Calsporin®: 724 g/d). No significant differences in ADFI were noted between groups (control: 2.14 kg, Calsporin®: 2.15 kg).

3.3.1. Conclusions on efficacy

Based on the results of the pooled analysis of four studies, the FEEDAP Panel concludes that Calsporin® has the potential to improve performance of pigs for fattening at 1.5×10^8 CFU/kg feed.

3.4. 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[19] and Good Manufacturing Practice.

4. Conclusions

The active agent fulfils the requirements of the QPS approach to the assessment of safety and no concerns are expected from other components of the additive. Consequently, Calsporin® can be presumed safe for the target animals, consumers of products from treated animals and the environment.

The additive is not a dermal/eye irritant or a skin sensitiser but should be considered a potential respiratory sensitiser.

Based on the results of the pooled analysis of four studies, the FEEDAP Panel concludes that Calsporin® has the potential to improve performance of pigs for fattening at 1.5×10^8 CFU/kg feed.

[18] Technical dossier/Supplementary information January 2017/Annex IV.3.7.

[19] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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2) CALSPORIN®. *Bacillus subtilis* C-3102, DSM 15544. Zootechnical feed additive for pigs for fattening. Supplementary information January 2017. Submitted by Asahi Calpis Wellness Co. Ltd.
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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ANOVA	analysis of variance
CFU	colony-forming unit
EURL	European Union Reference Laboratory
FEEDAP	the Panel on additives and products or substances used in animal feed
QPS	Qualified Presumption of Safety