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

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Abstract

Following a request from the European Commission, the 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 dogs. 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 dogs, 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 dogs 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. Four studies were supplied aiming at investigating the effects of the supplementation of Calsporin® to the faecal consistency of dogs. Overall, there is a small but significant increase in faecal dry matter content and benefits seen in a subjective assessment of faecal consistency. However, the biological relevance of changes of this magnitude for the animal is questionable, as are the practical benefits for the owner.

Keywords: zootechnical additive, Calsporin, *Bacillus subtilis*, dogs, safety, efficacy, faeces

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

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 Calpis Co. Ltd.[2] for authorisation of the product Calsporin®, *Bacillus subtilis* DSM 15544, when used as a feed additive for dogs (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). EFSA received directly from the applicant the technical dossiers in support of these applications. The particulars and documents in support of the application were considered valid by EFSA as of 16 February 2015.

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 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 3.2.1)).

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 in 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 2010, 2011b), with laying hens and minor avian species for laying (EFSA FEEDAP Panel, 2015a) and with ornamental fish (EFSA FEEDAP Panel, 2015b).

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] and laying hens and ornamental fish.[5]

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, 2013). This approach requires the identity of the active agent to be established and the absence of toxigenic potential and susceptibility to a selected range of antibiotics to be demonstrated.

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[6] in support of the authorisation request for the use of Calsporin® (*Bacillus subtilis* DSM 15544)

[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] On 26 January 2016, the applicant communicated the change from Calpis Co. Ltd. to Asahi Calpis Wellness Ltd., represented in the European Union 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] FEED dossier reference: FAD-2014-0041.

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[7] 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.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Calsporin® is in line with the principles laid down in Regulation (EC) No 429/2008, the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012), Technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a), and Guidance on the assessment of additives intended to be used in pets and other non food-producing animals (EFSA FEEDAP Panel, 2011b).

3. Assessment

Calsporin® is a preparation of viable spores of *Bacillus subtilis* DSM 15544 intended for use as a zootechnical additive (other zootechnical additive) in feed for dogs to exert beneficial effects in their gastrointestinal tract, leading to an increase in faecal consistency/harder stools.

3.1. Characterisation of the additive[9]

Calsporin® is a dry free-flowing powder with a minimum declared content of 1×10^{10} colony-forming units (CFU) of *Bacillus subtilis* DSM 15544[10] 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; EFSA FEEDAP Panel, 2015a). Thus, the data pertaining to impurities, physical properties and shelf-life assessed in these opinions still apply.

3.2. Stability in pet food

A study was performed to investigate the stability of Calsporin® incorporated in a premixture (not containing choline chloride) in order to supply the recommended dose in the final feed and in two types of feeds for dogs.[11] One of the feeds is described as extruded kibbles (moisture 8%, proteins 32%, fat 19%, minerals 7.5%) mixed with soybean oil containing Calsporin®. The second feed is described as pelleted kibbles manufactured by mixing the additive with fat in the mixer, then pelleted at temperatures up to 74°C (moisture 9%, proteins 24%, fat 12%, minerals 7%). The samples were stored in plastic bags at 18°C in a dark room for 3 (premixture) and 25 months (extruded and pelleted feed), respectively. No losses (< 0.5 log-values) were observed at the end of the experimental period for any of the test items.

Another study, intended to mimic domestic use, was performed mixing Calsporin® with moistened feed (at 2.4×10^8 CFU/kg dry feed, in triplicate), prepared by wetting a dry pelleted feed and then incubating at 30°C for up to 6 h.[12] Half of the samples were subjected to heat treatment to eliminate vegetative cells. The recovery rates of the four samples showed no losses (< 0.5 log-counts).

Evidence was also provided to show that the viability of the additive was not affected by the form of compression used in the manufacture of solid treats.[13]

3.2.1. Conditions of use

The product is intended for use in feed for dogs at a minimum dose of 1×10^9 CFU/kg complete feedingstuffs.

[7] 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.
[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2009-0013.pdf
[9] This section has been amended following the confidentiality claims made by the applicant.
[10] The in-house identifier C-3102 has been used in previous assessments.
[11] Technical dossier/Supplementary information October 2016/Annexes IV.4.1.8 and IV.4.1.9.
[12] Technical dossier/Supplementary information October 2016/Annex IV.4.1.7.
[13] Technical dossier/Supplementary information October 2016/Annex IV.4.1.10.

It may be administered via dry feed, in the form of top-dressing to be mixed with wet feed, or in complementary feeds or solid treats.

3.3. 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. In a previous opinion (EFSA FEEDAP Panel, 2015a), the identification of the strain and compliance with the QPS qualifications were confirmed. Therefore, the FEEDAP Panel concluded that *Bacillus subtilis* DSM 15544 can be presumed safe for target animals 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 and the environment.

In a previous opinion on the use of Calsporin® in feed for chickens for fattening, the FEEDAP 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 dogs 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.

3.4. Efficacy for dogs

The additive is intended to exert beneficial effects in the gastrointestinal of dogs tract leading to an increase in faecal consistency/harder stools.

The original dossier included two studies aimed at investigating the effects of the supplementation of Calsporin® to the faecal consistency in dogs and a literature search including studies performed with other animal species (e.g. piglets, turkeys for fattening, laying hens). These later studies focused mainly on the evaluation of the effects of the Calsporin® supplementation on the zootechnical performance of the animals. Considering the different end-points/effects observed in the major species and those in dogs, efficacy demonstration in the major species cannot be used to support that in dogs. The applicant supplied two additional studies in dogs. The four studies are described below. They all investigate the effect of Calsporin® administration on the characteristics of stools.

The first trial is a publication of a study performed in a non-European country (Portella Félix et al., 2010).[14] The study involved 12 dogs (7–8 months old, both sexes, Beagle breed) distributed between two dietary treatments, with six replicates per treatment: control or Calsporin® added at the minimum recommended dose, 1×10^9 CFU/kg feed. The additive was mixed with soybean oil and added to a basal commercial extruded feed for the treatment group. Compliance of the additive with specifications and concentration of the additive in feed were confirmed by analysis. Controls received the same basal feed mixed with the same amount of soybean oil, but without the additive. The treatment diets were offered once a day to the dogs for 30 days. The dogs were individually housed for the first 20 days and then transferred to metabolic cages (5 days cage adaptation, followed by 5 days of total faeces collection). Faeces were collected, weighed and subjected to analyses of dry matter, pH, ammonia content, and score[15] and digestibility of nutrients (crude protein, crude fibre, mineral matter and ether extract). For faecal characteristics, the study design was completely randomised and a split-plot in time was applied (main plot: treatments; subplot: days), with 5 days of data collection and six dogs per treatment. Also, for diet digestibility, the study design was completely randomised with two treatments of six replicates per treatment. Data were subjected to an analysis of variance (ANOVA) and means were compared using the Tukey–Kramer (faecal characteristics) or Tukey's test (digestibility parameters). Significant differences were declared at $p < 0.05$.

Faeces of dogs supplemented with Calsporin® showed significantly greater dry matter contents (Calsporin®: 39.1% vs control: 36.5%, $p < 0.05$), better faecal scores (Calsporin®: 3.4 vs control: 3.0, $p < 0.05$, although the author recognises both as optimal) and lower ammonia content than that from dogs fed the control diet (Calsporin®: 0.45% vs control: 0.56%, $p < 0.05$). Diet digestibility, faecal pH and faecal output were not different between treatments.

The second trial was a double-blind study with 14 puppies from three litters (approximately 8 weeks old, both sexes, medium-large (German Shepherd) and small breeds (Fox and Hungarian Jagd Terriers)) housed in individual kens.[16] Puppies were distributed by sex and initial body weight in two homogeneous

[14] Technical dossier/Section IV/Annex IV.3.1.
[15] Using a five point-scale with 1 denoting very soft faeces and 5 shaped, dry and hard faeces.
[16] Technical dossier/Section IV/Annex IV.3.2.

treatment groups. Animals were fed a daily diet composed of approximately two-thirds of wet feed and one-third of dry feed. Calsporin® or a placebo (based on soybean meal) were added as a top-dressing to a small portion of the wet feed to guarantee full consumption (100 g Calsporin®/10 kg soybean meal, to produce a top-dressing of 1×10^8 CFU/g) delivering 1×10^9 CFU/kg complete diet (equivalent to $3–7 \times 10^8$ CFU/dog per day, depending on body weight). Compliance of the additive with specifications and concentration of the additive in the top-dressing were confirmed by analysis. Faeces were collected daily to monitor faecal scores,[17] ammonia content, moisture and immunoglobulin A (IgA) concentration. Blood samples were taken from all animals at study start (day 1, to establish baseline values), after 15 days and at the end of the study at day 43 to monitor immune parameters (serum vaccinal titres, IgG and IgA) and a stress parameter (serum C-reactive protein). Feed intake and body weight were measured on a weekly basis and general health monitored during the whole experimental period. Data were analysed with ANOVA considering the treatment as main factor and each dog as the experimental unit. The model included treatment and gender as main factors. Significant differences were declared at $p \leq 0.10$.

Health of puppies was good throughout the study and there was no mortality. No significant differences in growth and food intake were observed between treatments. As regards serum antibody titres, the only significant differences observed were related to lower C-reactive protein values at day 15 in blood samples from the treated animals compared to control animals (Calsporin®: 0.06 vs control: 0.11 mg/dL, $p < 0.0075$). As regards faecal parameters, the only significant effect was observed in faecal IgA counts at the end of the trial which were greater in samples from treated animals compared to samples from control animals (Calsporin®: 6.76 vs control: 3.48 mg/g wet matter, $p < 0.0087$).

The third study involved 16 Beagle dogs (4–8 years old, both sexes), individually housed and evenly distributed (based on sex and weight) between two dietary treatments: control or Calsporin® supplemented at the minimum recommended dose of 1×10^9 CFU/kg feed.[18] Dogs were fed a dry extruded feed during a 2-week acclimatisation period before being given the experimental diets. Compliance of the additive with specifications and concentration of the additive in feed were confirmed by analysis. Dogs were monitored for weight, feed intake and health status during the entire experimental period of 28 days. Faecal scores were monitored approximately every 2 days.[19] Faecal samples were collected at several time points (0, 7, 14, 21 and 28 days), and subjected to analyses to monitor dry matter, pH, and ammonia content (at start, day 7 and end of the study). Data were analysed using ANOVA. The effect of diet on the studied parameters was analysed considering diet as fixed effect and with the single dog as experimental unit and as random effect. The factor time (day or week) was included in the model as a repeated measure. The inclusion of basal values (day or week zero) as covariate in the model was also considered for faecal parameters in which the effect of the covariate was significant. Significant differences were established at $p \leq 0.10$.

A small but significant decrease in faecal scores (Calsporin®: 2.85 vs control: 3.30 after 4 weeks; $p = 0.01$) was detected in Calsporin® treated-animals. Although faecal dry matter content was increased in the early part of the study (Calsporin: 30.8% vs control: 27.3% at day 7 and Calsporin: 30.9% vs control: 26.4% at day 14; $p = 0.021$), no differences were observed after 4 weeks (control: 29.1% vs Calsporin: 29.7%). Faecal pH remained normal throughout the study (6.0–6.7) and no significant changes to faecal ammonia content were seen.

The fourth study involved 16 Beagle dogs (4–8 years old, both sexes), individually housed and evenly distributed (based on sex and weight) between two dietary treatments: control and Calsporin®.[20] Dogs were fed a dry extruded feed without or with Calsporin® at the minimum recommended dose of 1×10^9 CFU/kg feed) during the 33 days of the study. Compliance of the additive with specifications and concentration of the additive in feed were confirmed by analysis. The dogs were monitored for weight, feed intake and health status overall the experimental period. Faecal scores were monitored daily.[21] Faecal samples were collected at the end of the trial and subject to analysis of dry matter and ammonia content. Data were analysed according to a completely randomised design with the diet as effect and the single dog as experimental unit. Student's t-test was

[17] Using a five point-scale with 1 denoting very soft faeces, 5 denoting dry and hard faeces and 4 as ideal score.
[18] Technical dossier/Supplementary information October 2016/Annex IV.3.3.
[19] Using a seven point-scale with 1 denoting dry and hard faeces and 7 watery faeces.
[20] Technical dossier/Supplementary information October 2016/Annex IV.3.4.
[21] Using a five point-scale with 1 denoting soft stools and formless and 5, well-formed stools, hard and dry.

used to compare means of body weight, condition score and feed intake and Kruskal–Wallis test to compare faecal parameters. Significant differences were declared at $p < 0.10$.

Dry matter of faeces of the Calsporin® group was significantly greater (denoting harder faeces) than that of the control group at the end of the experimental period (Calsporin: 33.9% vs control: 30.3%; $p = 0.021$). Overall, the Calsporin® group showed a significantly greater distribution of faecal score 4 compared to the control (Calsporin: 55.9% vs control: 32%; $p < 0.01$). However, baseline values for faecal scores were not recorded. Faecal ammonia content was not influenced by treatment.

3.4.1. Conclusion on the efficacy for dogs

Overall, there is a small but significant increase in faecal dry matter content and benefits seen in a subjective assessment of faecal consistency. However, the biological relevance of changes of this magnitude for the animal is questionable, as are the practical benefits for the owner.

3.5. 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[22] 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 dogs and for the environment.

The additive is not a dermal/eye irritant or a skin sensitiser but should be considered a potential respiratory sensitiser. Use of the additive in dogs is considered unlikely to introduce hazards for users of the product not already considered as part of the first assessment.

Overall, there is a small but significant increase in faecal dry matter content and benefits seen in a subjective assessment of faecal consistency. However, the biological relevance of changes of this magnitude for the animal is questionable, as are the practical benefits for the owner.

Documentation provided to EFSA

1) CALSPORIN®. *Bacillus subtilis* C-3102, DSM 15544. Zootechnical feed additive for dogs. December 2014. Submitted by Calpis Co. Ltd.
2) CALSPORIN®. *Bacillus subtilis* C-3102, DSM 15544. Zootechnical feed additive for dogs Supplementary information October 2016. Submitted by Asahi Calpis Wellness Co. Ltd.
3) Comments from Member States.

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[22] 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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Abbreviations

ANOVA	analysis of variance
CFU	colony-forming units
EURL	European Union Reference Laboratory
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
Ig	immunoglobulin
QPS	qualified presumption of safety