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Safety and efficacy of *Bacillus subtilis* DSM 28343 as a feed additive for piglets

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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 (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of *Bacillus subtilis* DSM 28343 when used in feed for weaned piglets. The additive is a preparation containing viable spores of a strain of *B. subtilis*. This species is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment which 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 a previous opinion and since concerns are not expected from other components of the additive, the additive is presumed safe for all target species, consumers and the environment. In the same opinion, the FEEDAP Panel concluded that *Bacillus subtilis* DSM 28343 is not an eye/skin irritant but should be considered as a potential respiratory sensitiser and that no conclusion could be drawn on its skin sensitisation potential. These conclusions apply also to the current application. *Bacillus subtilis* DSM 28343 at 1×10^9 CFU/kg feedingstuffs has the potential to be efficacious in weaned piglets.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 Lactosan GmbH & Co. KG.² for authorisation of the product *Bacillus subtilis* DSM 28343, when used as a feed additive for piglets (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 12 June 2017.

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 *Bacillus subtilis* DSM 28343, when used under the proposed conditions of use (see Section 3.2).

1.2. Additional information

The additive *Bacillus subtilis* DSM 28343 is a preparation containing viable spores of a strain of *Bacillus subtilis*. EFSA issued an opinion on the safety and efficacy of this product when used with chickens for fattening (EFSA FEEDAP Panel, 2016).

The additive is currently authorised for use in feeds for chickens for fattening.³

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁴ in support of the authorisation request for the use of *Bacillus subtilis* DSM 28343 as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁵ 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.⁶

2.2. Methodologies

The approach followed by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) to assess the safety and the efficacy of *Bacillus subtilis* DSM 28343 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) and Technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

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 Lactosan GmbH & Co. KG, Industriestrasse West 5, 8605, Kapfenberg, AT.

³ Commission Implementing Regulation (EU) 2017/187 of 2 February 2017 concerning the authorisation of a preparation of *Bacillus subtilis* (DSM 28343) as a feed additive for chickens for fattening (holder of authorisation Lactosan GmbH & Co. KG). OJ L 29, 3.2.2017, p. 35.

⁴ FEED dossier reference: FAD-2017-0018.

⁵ 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.

⁶ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2015-0006-bacillus_subtilis.pdf

3. Assessment

Bacillus subtilis DSM 28343 is a preparation of viable spores of a single strain of *B. subtilis* intended for use as a zootechnical additive (gut flora stabiliser) in feed for weaned piglets.

3.1. Characterisation

The additive is a preparation of viable spores of *Bacillus subtilis* DSM 28343 with a minimum declared concentration of 1×10^{10} CFU/g additive. It has the same formulation (bacterial spores (2–4%), calcium carbonate (93–95%), maltodextrins (2%) and silicon dioxide (1%)) and method of manufacture as that considered in a previous application (EFSA FEEDAP Panel, 2016). Thus, the data pertaining to composition, impurities, physical properties and shelf life still apply.

Stability of three batches of *Bacillus subtilis* DSM 28343 was tested when mixed (at the proposed concentration) with a mineral premixture and a minerals/vitamins premixture and with mash and pelleted feed for pigs and piglets, and stored at 20°C for 6 months.⁷ Differences in the bacterial counts were negligible in all cases (< 0.5 log).

The stability to pelleting (at 70°C) of the additive (three batches) was tested when mixed (at the proposed concentration) in piglets feed.⁸ The enumeration of bacilli spores showed a recovery close to 90% or higher after the thermal treatment.

The ability of the additive to be uniformly distributed in feed was examined using a single batch of the additive incorporated at the proposed use level into a pig feed.⁹ The enumeration of bacilli spores of 10 subsamples showed a coefficient of variation of 6%.

3.2. Conditions of use

The product is intended for use in feed for weaned piglets at a minimum inclusion level of 1×10^9 CFU/kg feedingstuffs.

3.3. Safety

The bacterial species *B. subtilis* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; 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, 2016), the identification of the strain and compliance with the QPS qualifications were confirmed. Therefore, the Panel concluded that *B. subtilis* DSM 28343 can be presumed safe for target animals, consumers of products derived from animals fed the additive and the environment. The Panel considers these conclusions to apply also in the current assessment. Consequently, the additive *Bacillus subtilis* DSM 28343 is considered safe for the target animals, consumers and the environment.

In the same opinion on the use of *Bacillus subtilis* DSM 28343 in feed for chickens for fattening, the Panel concluded that the additive is not an eye/skin irritant but should be considered a potential respiratory sensitiser and that no conclusion could be drawn on its skin sensitisation potential. The use of the additive in weaned piglets 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

Three studies were performed in two different locations of the same Member State to investigate the effects of *Bacillus subtilis* DSM 28343 on the performance of weaned piglets.

The detailed design of the studies is presented in Table 1 and the results in Table 2. Piglets were divided based on weight and gender (where relevant) in two homogeneous experimental groups and

⁷ Technical dossier/Section II/Annex II.4-3.

⁸ Technical dossier/Section II/Annex II.4-4.

⁹ Technical dossier/Section II/Annex II.4-5.

within each group, animals were randomly assigned to seven (study 1¹⁰) or four pens (studies 2¹¹ and 3¹²). The groups received either the basal diets (not supplemented) or the basal diets supplemented with the additive in order to provide 1×10^9 CFU/kg feed. Intended cell counts were confirmed by analysis. The diets were offered to the animals *ad libitum*. Health status was monitored throughout the experimental period of 42 days. Body weight on a pen (study 1) or animal (studies 2 and 3) basis and pen feed intake were measured and the feed to gain ratio (per pen) was calculated. In the three studies, an analysis of variance was performed considering the fixed effect of the treatment and the random effect of the pen (in trials 2 and 3). The pen was the experimental unit for all parameters.

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

Study	Additive (CFU/kg feed)	Breed (sex)	Total animals replicates/treatment × animals/replicate	Basal diets (main ingredients) form
1	0 1×10^9	Large White × Landrace ♀, castrated ♂	28 7 × 2	(maize/wheat/soybean meal) Mash
2	0 1×10^9	Duroc × DanAvl ♀, ♂	200 4 × 25	(wheat/barley/maize/soybean meal) Mash
3	0 1×10^9	Duroc × DanAvl ♂	200 4 × 25	(wheat/barley/maize/rye/soybean meal) Mash

CFU: colony forming unit.

Table 2: Overview of results of efficacy studies with *Bacillus subtilis* DSM 28343 in piglets

Study	Additive (CFU/kg feed)	Initial weight (kg)	Final weight (kg)	Total feed intake (kg)*	Weight gain (kg)	Feed to gain	Mortality (n)
1	0	7.3	26.9	29	19.6	1.49 ^a	0
	1×10^9	7.3	28.0	28	20.7	1.36 ^b	0
2	0	7.8	31.6 ^b	990	23.8 ^b	1.66	0
	1×10^9	7.9	32.7 ^a	1,016	24.9 ^a	1.63	0
3	0	7.0	30.7 ^b	944	23.7 ^b	1.61 ^a	1
	1×10^9	7.0	32.0 ^a	960	25.0 ^a	1.56 ^b	1

CFU: colony forming unit.

a,b: Means in a column within a given trial with different superscript letters are significantly different at $p < 0.05$.

*: Per piglet in study 1 and per pen in studies 2 and 3.

Supplementation of the additive led to a significantly greater final weight and weight gain in two trials (2 and 3) and a significantly improved feed to gain ratio in one of these (3) and in the remaining study (1).

3.4.1. Conclusions on efficacy

Supplementation of *Bacillus subtilis* DSM 28343 at the recommended dose has the potential to increase the growth rate or feed to gain of weaned piglets.

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 Regulation¹³ and Good Manufacturing Practice.

¹⁰ Technical dossier/Section IV/Annexes IV.1-2.

¹¹ Technical dossier/Section IV/Annexes IV.3-4.

¹² Technical dossier/Section IV/Annexes IV.5-6.

¹³ 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.

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, the additive *Bacillus subtilis* DSM 28343 can be presumed safe for the target animals, consumers of products from treated animals and the environment.

Bacillus subtilis DSM 28343 is not an eye/skin irritant, should be considered a potential respiratory sensitiser and no conclusion can be drawn on its skin sensitisation potential.

Bacillus subtilis DSM 28343 at 1×10^9 CFU/kg feedingstuffs has the potential to be efficacious in weaned piglets.

Documentation provided to EFSA

- 1) *Bacillus subtilis* DSM 28343. Weaned piglets. March 2017. Submitted by Lactosan GmbH & Co. KG.
- 2) *Bacillus subtilis* DSM 28343. Weaned piglets. Supplementary information October 2017. Submitted by Lactosan GmbH & Co. KG.
- 3) Comments from Member States.

References

- EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. <https://doi.org/10.2903/j.efsa.2007.587>
- 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, Cocconcelli PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak 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), 2011. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. <https://doi.org/10.2903/j.efsa.2011.2175>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. <https://doi.org/10.2903/j.efsa.2012.2536>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on safety and efficacy of *Bacillus subtilis* DSM 28343 as a feed additive for chickens for fattening. EFSA Journal 2016;14(6):4507, 11 pp. <https://doi.org/10.2903/j.efsa.2016.4507>

Abbreviations

CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
QPS	qualified presumption of safety