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Safety and efficacy of *Bacillus subtilis* DSM 28343 for pigs for fattening

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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 pigs for fattening. The additive is a preparation containing viable spores of a strain of *Bacillus 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 a previous opinion, the FEEDAP Panel concluded that *Bacillus subtilis* DSM 28343 is not an eye/skin irritant but should be considered as a potential respiratory sensitizer 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 2×10^8 CFU/kg complete feed has the potential to be efficacious in pigs for fattening.

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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 pigs 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 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 17 July 2018.

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 3.1).

1.2. Additional information

The additive *Bacillus subtilis* DSM 28343 is a preparation containing viable spores of a strain of *Bacillus subtilis*.

The additive is currently authorised for use in feeds for chickens for fattening and in weaned piglets.

EFSA issued an opinion on the safety and efficacy of this product when used with chickens for fattening (EFSA FEEDAP Panel, 2016), in calves (EFSA FEEDAP Panel, 2018a) and in weaned piglets (EFSA FEEDAP Panel, 2018b).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier³ in support of the authorisation request for the use of *Bacillus subtilis* DSM 28343 as a feed additive.

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

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.⁴

2.2. Methodologies

The approach followed by the FEEDAP Panel 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 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012),⁵ Guidance on

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 FEED dossier reference: FAD-2018-0044.

⁴ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2015-0006-bacillus_subtilis.pdf

⁵ 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>

the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA FEEDAP Panel, 2014)⁶ and Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008).⁷

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 (functional group: gut flora stabiliser) in feed for pigs for fattening.

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} colony forming unit (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). In that opinion, the strain taxonomically identified as *B. subtilis* and the lack of toxigenic potential and resistance to antibiotics of human and veterinary importance were demonstrated. No new data has been submitted and therefore, the data referred to the characterisation of the additive presented in previous opinions still apply (EFSA FEEDAP Panel, 2016, 2018b).

The product is intended for use in complete feed for pigs for fattening at a minimum inclusion level of 2×10^8 CFU/kg complete feed.

3.2. Safety

The bacterial species *B. subtilis* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment (EFSA BIOHAZ Panel, 2017). This approach requires the identity of the strain to be conclusively established and requires evidence that the strain lacks toxigenic potential and 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 *Bacillus 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 new target species (pigs for fattening), consumers and the environment.

In the same opinion (EFSA FEEDAP Panel, 2016), the Panel concluded that the additive is not an eye/skin irritant but should be considered a potential respiratory sensitizer and that no conclusion could be drawn on its skin sensitisation potential. 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

Three efficacy trials in pigs for fattening and sharing a common design were evaluated. The details on the study design are provided in Table 1 and the main results in Table 2. In all trials, fattening pigs with body weights ranging from 23 to 27 kg were penned in groups and allocated to two dietary treatments. In all trials, males and females were used, only in trial 1 animals were penned separately according to the gender. In all three studies, the animals were fed either a non-supplemented diet (control) or a diet containing the *Bacillus subtilis* DSM 28343 at 2×10^8 CFU/kg complete feed (confirmed by analysis). The diets were administered for 77 days (Trial 1) and 91 days (Trials 2 and 3). The health and mortality were monitored throughout the study and the body weight and feed intake were recorded. Feed to gain ratio was calculated. An analysis of variance (ANOVA) was done with the for body weight/body weight gain data and the model considered the treatment as a fixed

⁶ EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition. EFSA Journal 2014;12(5):3665, 10 pp. <https://doi.org/10.2903/j.efsa.2014.3665>

⁷ EFSA (European Food Safety Authority), 2008. Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition. EFSA Journal 2008;6(9):803, 5 pp. <https://doi.org/10.2903/j.efsa.2008.803>.

effect and the pen. Feed intake and feed to gain ratio (pen basis) were analysed with a t-test/U-test, respectively. Significance level was set at 0.05.

Table 1: Trial design and analysed levels of the additive in the efficacy trials performed in pigs for fattening

Trial	Total No of animals (animals × replicate) replicates × treatment	Breed sex (duration)	Composition feed (Form)	<i>Bacillus subtilis</i> DSM 28343 (CFU/kg feed)	
				Intended	Analysed
1 ^(a)	128 (64) 32	Dan × Pietrain Males/females (77 days)	Rye, barley, soybean meal (pellets)	2×10^8	2.4×10^8 (grower phase) 2.7×10^8 (finisher phase)
2 ^(b)	400 (25) 8	Duroc × DanAvl Males/females (91 days)	Rye, barley, soybean meal (mash)	2×10^8	2.0×10^8 (starter phase) 2.3×10^8 (grower phase) 2.2×10^8 (finisher phase)
3 ^(c)	350 (25) 7	Duroc × DanAvl Males/females (91 days)	Rye, barley, soybean meal (mash)	2×10^8	2.4×10^8 (starter phase) 2.1×10^8 (grower phase) 2.2×10^8 (finisher phase)

CFU: colony forming unit.

(a): Technical dossier/Section IV/Annex_1.

(b): Technical dossier/Section IV/Annex_4.

(c): Technical dossier/Section IV/Annex_6.

Table 2: Effects of *Bacillus subtilis* DSM 28343 on the performance of pigs for fattening

Trial	<i>Bacillus subtilis</i> DSM 28343 (CFU/kg feed)	Daily feed intake (kg)	Initial body weight (kg)	Final body weight (kg)	Daily weight gain (kg)	Feed to gain ratio	Mortality and culling (%)
1	0	2.52	27.1	105.9 ^b	1.02 ^b	2.47	0
	2×10^8	2.57	27.4	108.0 ^a	1.05 ^a	2.45	0
2	0	2.89	23.8	115.4 ^b	1.01 ^b	2.87	1
	2×10^8	2.88	24.0	117.4 ^a	1.03 ^a	2.81	1
3	0	2.81	27.3	118.7 ^b	1.01 ^b	2.82	4
	2×10^8	2.83	27.2	120.1 ^a	1.02 ^a	2.81	5

CFU: colony forming unit.

a,b: Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.

3.3.1. Conclusions on efficacy

Supplementation of *Bacillus subtilis* DSM 28343 at the recommended level of 2×10^8 CFU/kg feed has the potential to increase the body weight gain of pigs for fattening.

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 Regulation⁸ and Good Manufacturing Practice.

⁸ 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 additive *Bacillus subtilis* DSM 28343 is safe for pigs for fattening, consumers of products from treated animals and the environment. *Bacillus subtilis* DSM 28343 is not an eye/skin irritant, it should be considered a potential respiratory sensitizer and no conclusion can be drawn on its skin sensitisation potential. *Bacillus subtilis* DSM 28343 at 2×10^8 CFU/kg complete feed has the potential to be efficacious as a zootechnical additive in pigs for fattening.

Documentation provided to EFSA

- 1) *Bacillus subtilis* DSM 28343 for pigs for fattening. July 2018. Submitted by Lactosan GmbH & Co.KG
- 2) Comments from Member States.

Chronology

Date	Event
06/07/2018	Dossier received by EFSA
17/07/2018	Reception mandate from the European Commission
29/08/2018	Application validated by EFSA – Start of the scientific assessment
29/11/2018	Comments received from Member States
15/05/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

- EFSA (European Food Safety Authority), 2008. Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition. EFSA Journal 2008;6(9):803, 5 pp. <https://doi.org/10.2903/j.efsa.2008.803>
- 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, Cocconcetti PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gomez M, Barizzone 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), 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), 2014. Guidance on the assessment of the toxigenic potential of Bacillus species used in animal nutrition. EFSA Journal 2014;12(5):3665, 10 pp. <https://doi.org/10.2903/j.efsa.2014.3665>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. 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>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018a. Safety and efficacy of *Bacillus subtilis* DSM 28343 as a feed additive for calves for rearing. EFSA Journal 2018;16(3):5220, 7 pp. <https://doi.org/10.2903/j.efsa.2018.5220>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018b. Safety and efficacy of *Bacillus subtilis* DSM 28343 as a feed additive for piglets. EFSA Journal 2018;16(3):5221, 7 pp. <https://doi.org/10.2903/j.efsa.2018.5221>

Abbreviations

ANOVA	analysis of variance
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