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

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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 calves for rearing. 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. 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 the target species, consumers of products derived from animals fed the additive 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 a potential respiratory sensitiser and that no conclusion could be drawn on its skin sensitisation potential. These conclusions apply also to the current application. Insufficient evidence was provided to conclude on the efficacy of the additive in calves for rearing.

Keywords: Zootechnical additive, gut flora stabilisers, *Bacillus subtilis* DSM 28343, safety, efficacy, QPS, calves for rearing

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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 Lactosan GmbH & Co. KG.[2] for authorisation of the product *Bacillus subtilis* DSM 28343 (*Bacillus subtilis* DSM 28343), when used as a feed additive for calves for rearing (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 20 April 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 (*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 dossier[4] 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 429/2008[5] 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 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, 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-2017-0006.
[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 calves for rearing to increase growth.

3.1. Characterisation

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

The stability of one batch of *Bacillus subtilis* DSM 28343 was tested when mixed at the proposed conditions of use with four milk replacers, and stored at 20°C for 1, 2 and 3 months.[7] No losses (< 0.5 log) were observed in the bacterial counts over these periods. In a second study, short-term stability of one batch of the additive was tested in order to mimic practical conditions (additive incorporated at the dose proposed by the applicant in a liquid milk replacer and stored at 37°C and 55°C).[8] Bacilli counts made up to 4 h showed negligible losses (< 0.5 log) at both temperature conditions.

The ability of the additive to be uniformly distributed was evaluated using a single batch of the additive mixed into a milk replacer (at 1%).[9] Analysis of bacterial counts of 10 subsamples resulted in a coefficient of variation of 8%.

3.2. Conditions of use

The product is intended for use in milk replacer for calves for rearing at a minimum dose of 1×10^9 CFU/kg.

3.3. 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, 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. Moreover, since no concerns are expected from other excipients present in the product, the Panel concluded that the additive *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.

In the same opinion on the use of *Bacillus subtilis* DSM 28343 in feed for chickens for fattening (EFSA FEEDAP Panel, 2016), 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 calves for rearing 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

Four studies were performed in two different locations of one Member State to investigate the effects of *Bacillus subtilis* DSM 28343 on the performance of calves. Two studies could not be considered because of lack of replication.[10,11] The applicant pooled the data from these two studies in order to increase the number of experimental units (replicates) per treatment group (i.e. three in total).[12] The Panel considers that pooling of data from studies that do not meet the minimum quality standards is not appropriate. Therefore, the pooled analysis was not further considered for the assessment.

[7] Technical dossier/Section II/Annexes II.4-3.
[8] Technical dossier/Section II/Annex II.4-4.
[9] Technical dossier/Section II/Annex II.4-5.
[10] Technical dossier/Section IV/Annexes IV_5-6/Supplementary information August 2017/Annex 3.
[11] Technical dossier/Section IV/Annexes IV_5-6/Supplementary information August 2017/Annexes 4 and 5.
[12] Technical dossier/Section IV/Annexes IV_5-6/Supplementary information August 2017/Annex 6.

In the two remaining studies, the experimental design was the same.[13,14] In each study, a total of 80 male calves (Holstein, 12–25 days old) were allocated to two treatments (control and *Bacillus subtilis* DSM 28343), with four pens of 10 animals per treatment group. *Bacillus subtilis* DSM 28343 was administered via the milk replacer at 1×10^9 CFU/kg (concentration confirmed by analysis). Milk replacer was mixed at 140 g/L water, and animals were fed 2–7 kg of milk replacer solution/calf per day (depending on the week of the experiment). Animals received also concentrate (0.5–2 kg/calf per day) through an automated feeding system. All animals had free access to water and hay *ad libitum*. Both studies lasted 56 days. Initial and final individual weights were measured and average daily weight gain was calculated. Individual milk replacer and concentrate intake were measured daily. Mortality and morbidity of calves were monitored daily. The data were subjected to analysis of variance, considering the fixed effect of the group and the random effect of the pen. The pen was the statistical unit. Significance was established at $p < 0.1$.

Table 1: Overview of results of efficacy studies with *Bacillus subtilis* DSM 28343 in calves for rearing

Study	Additive (CFU/kg milk replacer)	Initial weight (kg)	Final weight (kg)	Daily milk replacer intake (g)	Daily concentrate intake (g)	Daily weight gain (g/day)	Energy intake/ weight gain (MJ/kg)*
1	0	56.1	99[b]	808	312	766[b]	24.0[a]
	1×10^9	56.2	102[a]	814	291	812[a]	22.3[b]
2	0	56.0	95[b]	767	363	694[b]	27.3[a]
	1×10^9	55.7	103[a]	755	379	843[a]	21.6[b]

CFU: colony-forming unit.
a,b: Means in a column within a given trial with different superscript letters are significantly different at $p < 0.1$.
*: Energy (milk replacer and concentrate, MJ) intake per kg of weight gain.

Results of both studies are summarised in Table 1. Mortality was low (only two animals died in the control group of study 1), and not influenced by treatment. Final weight and weight gain of calves were significantly increased in the *Bacillus subtilis* DSM 28343 group in both studies. The daily energy intake to gain ratio was also significantly improved in calves receiving the additive.

3.4.1. Conclusions on efficacy

Significant effects of the additive on growth performance of calves were observed only in two studies. Therefore, there is insufficient evidence to conclude on the efficacy of *Bacillus subtilis* DSM 28343 in calves for rearing.

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[15] 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, *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.

Insufficient evidence was provided to conclude on the efficacy of *Bacillus subtilis* DSM 28343 in calves for rearing.

[13] Technical dossier/Section IV/Annexes IV_1-2/Supplementary information August 2017/Annex 1.
[14] Technical dossier/Section IV/Annexes IV_3-4/Supplementary information August 2017/Annex 2.
[15] 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.

Documentation provided to EFSA

1) *Bacillus subtilis* DSM 28343. Calves for rearing. January 2017. Submitted by Lactosan GmbH & Co. KG.
2) *Bacillus subtilis* DSM 28343. Calves for rearing. Supplementary information. August 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, 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), 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 usedin 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