

ADOPTED: 4 July 2019

doi: 10.2903/j.efsa.2019.5793

Efficacy of *Bacillus subtilis* DSM 28343 as a zootechnical additive (gut flora stabiliser) for calves for rearing

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Rosella Brozzi, Jaume Galobart, Lucilla Gregoretti, Gloria López-Gálvez, Matteo Lorenzo Innocenti, Maria-Vittoria Vettori and Konstantinos Sofianidis

Abstract

Bacillus subtilis DSM 28343 is a preparation of viable spores of a single strain of *B. subtilis* intended to be used as a zootechnical additive (functional group: gut flora stabilizer) in feed for calves for rearing to increase growth. In 2018, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) delivered a scientific opinion on the safety and efficacy of *Bacillus subtilis* DSM 28343 as a feed additive for calves for rearing. This species is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment. In that opinion, the FEEDAP Panel was unable to conclude on the efficacy of the additive, under the condition of use as proposed by the applicant, due to insufficient data provided. In the current opinion, additional data to demonstrate the efficacy of *Bacillus subtilis* DSM 28343 were assessed. Based on one study of this application and two studies submitted in the previous application, the Panel concluded that *Bacillus subtilis* DSM 28343 has the potential to be efficacious as gut flora stabiliser used in feed for calves for rearing at the proposed use level.

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: zootechnical additive, gut flora stabilisers, *Bacillus subtilis* DSM 28343, efficacy

Requestor: European Commission

Question number: EFSA-Q-2019-00261

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed, Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brozzi R, Galobart J, Gregoretto L, López-Gálvez G, Innocenti ML, Vettori M-V and Sofianidis K, 2019. Scientific Opinion on efficacy of *Bacillus subtilis* DSM 28343 as a zootechnical additive (gut flora stabiliser) for calves for rearing. EFSA Journal 2019;17(7):5793, 7 pp. <https://doi.org/10.2903/j.efsa.2019.5793>

ISSN: 1831-4732

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs License](https://creativecommons.org/licenses/by/4.0/), which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



Table of Contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Additional information.....	4
2. Data and methodologies	4
2.1. Data.....	4
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Efficacy for calves for rearing	5
3.1.1. Conclusions on efficacy.....	6
3.2. Post-market monitoring.....	6
4. Conclusions.....	6
5. Documentation as provided to EFSA/Chronology.....	6
References.....	6
Abbreviations.....	7

1. Introduction

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

Regulation (EC) No 1831/2003¹ established rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Lactosan GmbH & Co. KG, is seeking a Community authorisation of *Bacillus subtilis* DSM 28343 as a feed additive to be used as a gut flora stabiliser for calves for rearing (Table 1).

Table 1: Description of the substances

Category of additive	Zootechnical additive
Functional group of additive	Gut flora stabiliser
Description	<i>Bacillus subtilis</i> DSM 28343
Target animal category	Calves for rearing
Applicant	Lactosan GmbH & Co. KG
Type of request	New opinion

On 06 March 2018, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the efficacy of *Bacillus subtilis* DSM 28343 in calves for rearing under the condition of use proposed by the applicant.

The Commission (EC) gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of the Authority's opinion. The new data have been received on 28 September 2018.

In view of the above, the Commission asks the Authority to deliver a new opinion on *Bacillus subtilis* DSM 28343 as a feed additive for calves for rearing based on the additional data submitted by the applicant.

1.2. Additional information

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

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

In the opinion on calves for rearing (EFSA FEEDAP Panel, 2018a), the FEEDAP Panel was unable to conclude on the efficacy of the additive due to inadequacy of the data available.

The additive is currently authorised for use in feed for chickens for fattening² and in weaned piglets.³

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information⁴ following a previous application on the same product.⁵

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 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.

³ Commission Implementing Regulation (EU) 2018/1079 concerning the authorization of a preparation of *Bacillus subtilis* (DSM 28343) as a feed additive for weaned piglets (holder of authorisation Lactosan GmbH & Co. KG). OJ L 194, 31.7.2018, p. 131.

⁴ FEED dossier reference: FAD-2018-0069.

⁵ FEED dossier reference: FAD-2017-0006.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the efficacy of *Bacillus subtilis* DSM 28343 is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018c).

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 milk replacer for calves for rearing at a minimum dose of 1×10^9 CFU/kg.

The additive was fully characterised in the context of a previous opinion on the additive when used with chickens for fattening (EFSA FEEDAP Panel, 2016). In 2018, the FEEDAP Panel adopted another opinion on the additive when used with calves for rearing (EFSA FEEDAP Panel, 2018a). In that opinion, the Panel concluded that 'insufficient evidence was provided to conclude on the efficacy of *Bacillus subtilis* DSM 28343 in calves for rearing'. This conclusion was based on the lack of replication in two studies, which prevented the statistical analysis of the data.

The applicant has now provided one additional study to support the efficacy of *Bacillus subtilis* DSM 28343 as gut flora stabiliser in feed for calves for rearing, which is the subject of this assessment.

3.1. Efficacy for calves for rearing

In the previous opinion, the FEEDAP Panel assessed four studies in support of the efficacy of *Bacillus subtilis* DSM 28343 when used in calves for rearing (EFSA FEEDAP Panel, 2018a). Two studies could not be considered because of lack of replication. In the two remaining studies (Table 2, studies 1 and 2), significant effects of the additive on final weight, weight gain and energy intake to gain ratio were observed of the additive at the proposed dose.

In the new study submitted, a total of 179 male calves (Holstein, about 16 days-old) were allocated to two treatments (control and *Bacillus subtilis* DSM 28343), with four pens of 17–25 animals per treatment group (depending on pen size).^{7,8} *Bacillus subtilis* DSM 28343 was administered via the milk replacer 1×10^9 CFU/kg (concentration confirmed by analysis). 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 automatic feeding system. All animals had access to water and hay *ad libitum*. Additionally, calves received a total mix ration (TMR) *ad libitum* for the last week of the trial (week 8). The study lasted 56 days. Initial and final individual weights were measured and average daily weight gain was calculated. The 'milk replacer intake' was measured for each animal individually, while the concentrate intake, hay and TMR were recorded per pen. Mortality and morbidity of calves were monitored every day. The data were subject to analysis of covariance (ANCOVA), considering the treatment group as a fixed effect, the effect of the treatment within the pen and the initial body weight as the covariate in the model. The use of the covariate was considered due to the significant difference in the initial body weight between the groups (significantly higher in the control group compared to the treatment group). Significance was established at $p \leq 0.1$.

The results of this study are summarised in Table 2 (study 3), together with the main results of the two studies already assessed in the previous opinion (EFSA FEEDAP Panel, 2018a). No animals died. Final weight and daily weight gain of calves receiving *Bacillus subtilis* DSM 28343 were significantly higher than the control. The animals fed the additive consumed a significantly lower amount of milk replacer, compared to the control, while concentrate, hay and TMR intake were not different between the groups. The ratio of energy intake to weight gain was significantly lower for the treated animals when calculated as the sum of concentrate and milk replacer (Table 2), as in the two studies previously assessed. These results are confirmed when considering the total feed intake (sum of milk replacer, concentrate, hay and TMR) in the total energy intake to weight gain ratio (30.61 MJ/kg vs 27.68 MJ/kg, $p = 0.03$, for the control and the treatment group, respectively).⁹

⁶ 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.

⁷ Technical dossier/ KA 114ex-18_Report.

⁸ Technical dossier/ KA 114ex-18_TPDS.

⁹ Technical dossier/Supplementary information June 2019/Annex_EFSA_Q_2019_00261 and Annex_EFSA_Q_2019_00261_Part II.

Table 2: Overview of results of efficacy study with *Bacillus subtilis* DSM 28343 in calves for rearing in three efficacy studies

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/d)	Energy intake/weight gain (MJ/kg)*
1 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 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
3	0	51.3 ^a	86 ^b	754 ^a	457	625 ^b	28.2 ^a
	1 × 10 ⁹	50.7 ^b	91 ^a	742 ^b	487	710 ^a	25.4 ^b

CFU: colony forming unit.

^{a,b}: Means in a column within a given trial with different superscript letters are significantly different at $p \leq 0.1$.

*: Energy (milk replacer and concentrate, MJ) intake per kg of weight gain.

^{1,2}: Results assessed in the previous opinion (EFSA FEEDAP Panel, 2018a).

3.1.1. Conclusions on efficacy

Considering the results of the current study, along with the two efficacy studies already assessed in the previous opinion, in which significant effects of the additive on growth performance of calves were observed, the FEEDAP Panel concludes that *Bacillus subtilis* DSM 28343 at 1 × 10⁹ CFU/kg has the potential to be efficacious as a zootechnical additive in calves for rearing.

3.2. 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 Regulation¹⁰ and Good Manufacturing Practice.

4. Conclusions

Bacillus subtilis DSM 28343 has the potential to improve the performance in calves for rearing when used at 1 × 10⁹ CFU/kg complete feed.

5. Documentation as provided to EFSA/Chronology

Date	Event
28/9/2018	Dossier received by EFSA
16/4/2019	Reception mandate from the European Commission
29/5/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: Efficacy</i>
19/6/2019	Reception of supplementary information from the applicant – Scientific assessment re-started
4/7/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

- 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 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>

¹⁰ 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.

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2018c. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. <https://doi.org/10.2903/j.efsa.2018.5274>

Abbreviations

ANCOVA	analysis of covariance
CFU	colony forming unit
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
QPS	qualified presumption of safety
TMR	total mix ration