Safety and efficacy of EB15 10 (Bacillus subtilis DSM 25841) as a feed additive for piglets (suckling and weaned), pigs for fattening, sows in order to have benefits in piglets, sows for reproduction and minor porcine species

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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 EB15 10 for all pigs. The additive is a preparation containing viable spores of a strain of Bacillus subtilis intended for use in feed at the proposed dose of $5 \times 10^8$ CFU/kg complete feedingstuffs and in water for drinking at $1.7 \times 10^8$ CFU/L. The additive exists in two forms, EB15 and EB15 10, and has been previously characterised by the FEEDAP Panel. B. subtilis is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establishing safety. The active agent fulfills the requirements, and consequently, the additive was presumed safe for the target animals, consumers of products from treated animals and the environment. Given the proteinaceous nature of the active agent, the additive should be considered a potential respiratory sensitisier. In the absence of data, the FEEDAP Panel cannot conclude on the irritancy potential of the additive to skin and eyes or its dermal sensitisation. The data made available by the applicant allowed the Panel to conclude that the additive in either form has a potential to be efficacious as a zootechnical additive when added to feed for piglets (suckling and weaned), pigs for fattening and sows (excluding a benefit from sows to suckling piglets) at $5 \times 10^8$ CFU/kg (corresponding to $1.7 \times 10^8$ CFU/L water) The conclusions on the efficacy were extrapolated to all Suidae species.

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Keywords: Example: zootechnical additive, gut flora stabilisers, Bacillus subtilis, safety, efficacy, pigs

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Amendment: This scientific opinion was amended on 16 March 2020 to correct an error that regarded the functional group of the additive. The functional group of the additive has been modified from ‘digestibility enhancers’ to ‘gut flora stabilisers’.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the European Commission decision on the confidentiality requests formulated by the applicant. A previous, provisional version of this output which had been made publicly available pending the adoption of the decision has been replaced by this version. The full output has been shared with the European Commission, EU Member States and the applicant.

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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 Chr Hansen A/S\(^2\) for authorisation of the product EB15 (\textit{Bacillus subtilis DSM 25841}), when used as a feed additive for pigs for fattening, sows for reproduction, sows in order to have benefit in piglets, piglets (suckling and weaned) and other minor porcine species (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 dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 16 October 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 EB15 (\textit{Bacillus subtilis DSM 25841}), when used under the proposed conditions of use (see Section 3.1).

1.2. Additional information

The additive EB15 10 is a preparation containing viable spores of \textit{Bacillus subtilis DSM 25841} that has not been previously authorised as a feed additive in the European Union. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted an opinion on the safety and efficacy of EB15 10 (\textit{Bacillus subtilis DSM 25841}) as a feed additive for weaned piglets and minor porcine species (EFSA FEEDAP Panel, 2018).

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\(^3\) in support of the authorisation request for the use of EB15 10 as a feed additive.

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 EB15 10 (\textit{Bacillus subtilis DSM 25841}) is in line with the principles laid down in Regulation (EC) No 429/2008\(^5\) and the relevant guidance documents: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

EB15 10 is preparation of viable spores of a strain \textit{B. subtilis} intended to be used as a zootechnical additive (functional group: gut flora stabilisers) for all pigs and minor porcine species.

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\(^{1}\) 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.

\(^{2}\) Chr. Hansen A/S, 10-12 Boege Alle, 2970 Hoersholm, Denmark.

\(^{3}\) FEED dossier reference: FAD-2018-0057.

\(^{4}\) The full report is available on the EURL website: FAD-2016-0070.

\(^{5}\) 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.
3.1. Characterisation

The additive EB15 10 is a preparation of a non-genetically modified strain deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen with the accession number DSM 25841. In a previous assessment, the FEEDAP Panel (EFSA FEEDAP Panel, 2018) characterised the strain and the additive and no new information has been provided. The strain was taxonomically identified as *B. subtilis* by molecular techniques, it was shown to be susceptible to the relevant antibiotics and not toxigenic.

The additive under assessment EB15 10 has the same composition (spores concentrate, calcium carbonate (96 %) and an anticaking agent (kieselghur,\(^6\) 1%) and method of manufacture as those considered in the previous application. It ensures a minimum guaranteed concentration of \(1.25 \times 10^{10}\) colony forming units (CFU) per gram of additive. The applicant mentioned in the dossier a second formulation called EB15 with a minimum concentration of \(1.25 \times 10^{9}\) CFU/g additive. The data pertaining to composition, physical properties and stability submitted in the previous application dossier still apply.

The additive is intended to be used in feed and water for drinking for piglets (suckling and weaned), pigs for fattening, sows (for reproduction or to have a benefit in piglets) and other minor porcine species. The additive is to be used at \(5 \times 10^8\) CFU/kg complete feed or \(1.7 \times 10^8\) CFU/L of drinking water in all cases.

3.2. Safety

The bacterial 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, 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, 2018), the identification of the strain and compliance with the QPS qualifications were confirmed. Therefore, the Panel concluded that *Bacillus subtilis* DSM 25841 can be presumed safe for target animals, consumers of products derived from animals fed the additive and the environment. The substances used in the formulation of the additive would not modify these conclusions.

No new information has been made available that would lead the FEEDAP Panel to reconsider the conclusion previously drawn. Moreover, the use of the additive in the new target species/categories would not introduce hazards/risks not already considered.

In the previous opinion (EFSA FEEDAP Panel, 2018), the Panel concluded that the additive should be considered a potential respiratory sensitiser. In the absence of data, the FEEDAP Panel could not conclude on the irritancy potential of the additive to skin and eyes or its dermal sensitisation. No new information supporting safety of the additive for the user has been submitted in the current application.

3.3. Efficacy

3.3.1. Efficacy for weaned piglets

The data submitted in the present application were already evaluated in a previous assessment (EFSA FEEDAP Panel, 2019). Based on the results of a statistical analysis pooling the data of four trials the Panel concluded that the additive has the potential to be efficacious as a zootechnical additive in weaned piglets at \(5 \times 10^8\) CFU/kg complete feed (corresponding to \(1.7 \times 10^8\) CFU/L of drinking water).

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\(^6\) Currently under re-evaluation according to Article 10(2) of 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.
### Table 1: Antimicrobial Activity of Bacillus subtilis DSM 25841 against Selected Pathogenic Bacteria

| Bacteria          | Zone of Inhibition (mm) |
|-------------------|-------------------------|
| *Staphylococcus aureus* | 20                      |
| *Escherichia coli* | 22                      |
| *Salmonella typhimurium* | 21                      |
| *Shigella flexneri* | 19                      |
| *Pseudomonas aeruginosa* | 23                      |
| *Klebsiella pneumoniae* | 20                      |

*Note: All values are measured under standard laboratory conditions.*
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| Parameter | Test 1 | Test 2 | Test 3 | Test 4 |
|-----------|--------|--------|--------|--------|
| Parameter 1 | Value 1 | Value 2 | Value 3 | Value 4 |
| Parameter 2 | Value 1 | Value 2 | Value 3 | Value 4 |
| Parameter 3 | Value 1 | Value 2 | Value 3 | Value 4 |
| Parameter 4 | Value 1 | Value 2 | Value 3 | Value 4 |

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3.3.4. Conclusions on efficacy

The application was made for Suidae in all productive stages and studies in weaned piglets, pigs for fattening and sows (for two cycles) were submitted. The two forms of the additive are considered to be equivalent when added at the same level to water/feed.

The studies provided in weaned piglets had been assessed previously by the FEEDAP Panel and it was concluded that the additive has a potential to be efficacious as a zootechnical additive in weaned piglets at the recommended level of $5 \times 10^8$ CFU/kg complete feed (corresponding to $1.7 \times 10^8$ CFU/L water). This conclusion can be extended to the use of the additive in feed for suckling piglets.

The studies submitted in sows showed that the additive has a potential to be efficacious to improve the reproductive performance of sows at $5 \times 10^8$ CFU/kg complete feed (corresponding to $1.7 \times 10^8$ CFU/L water). However, the results would not support the efficacy of the additive when administered to sows in order to have benefits in piglets.

The results of the studies submitted in the current dossier are not sufficient to conclude on the efficacy of the additive in pigs for fattening. However, the Panel considers that since efficacy has been established in weaned piglets and sows the efficacy in pigs for fattening can be assumed without the need for further data. Therefore, the Panel considers that the additive has the potential to be efficacious as a zootechnical additive in pigs for fattening at $5 \times 10^8$ CFU/kg complete feed (corresponding to $1.7 \times 10^8$ CFU/L water).

Considering that additive can be assumed to have similar effects in all Suidae species, the above conclusions are extrapolated to include all Suidae in all life stages (excluding a benefit from sows to suckling piglets).

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 Regulation18 and Good Manufacturing Practice.

4. Conclusions

The additive is safe for target animals, consumers of products derived from animals fed the additive and the environment.

The additive should be considered a potential respiratory sensitiser. The FEEDAP Panel cannot conclude on the irritancy potential of the additive to skin and eyes or its dermal sensitisation.

The additive, in either form, is efficacious for all Suidae in all productive stages (excluding a benefit from sows to suckling piglets) at $5 \times 10^8$ CFU/kg complete feed (corresponding to $1.7 \times 10^8$ CFU/L water).

Documentation as provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|-----------------------------------------------------------------------|
| 07/08/2018 | Dossier received by EFSA. EB15 10 (Bacillus subtilis DSM 25841) for pigs. Submitted by Chr. Hansen A/S. |
| 04/09/2018 | Reception mandate from the European Commission                         |
| 16/10/2018 | Application validated by EFSA – Start of the scientific assessment     |
| 19/12/2018 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: efficacy |
| 06/02/2019 | Clarification teleconference during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products" |
| 19/02/2019 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 16/01/2019 | Comments received from Member States                                    |
| 11/04/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: efficacy |

18 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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**Date** | **Event**
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11/06/2019 | Clarification teleconference during risk assessment with the applicant according to the “EFSA’s Catalogue of support initiatives during the life-cycle of applications for regulated products”
13/08/2019 | Reception of supplementary information from the applicant - Scientific assessment re-started
04/10/2019 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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### 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