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## Efficacy of the feed additive consisting of *Bacillus velezensis* NRRL B-67259 (Correlink™ ABS1781) as a feed additive for all growing poultry species (Elanco GmbH)

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### Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the efficacy of a product consisting of spores of *Bacillus velezensis* NRRL B-67257 as a zootechnical additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species. The additive has the tradename Correlink™ ABS1781 *Bacillus subtilis* and is not currently authorised in the EU. It is intended for use in complete feed for the target species at a minimum inclusion level of  $1.5 \times 10^8$  CFU/kg complete feed. In a previous opinion, the FEEDAP Panel could not conclude on the efficacy of the additive for the poultry species due to the potential cross-contamination of the control diets in two of the three studies provided. The applicant has provided supplementary information to exclude this possibility. The new data showed that the gene used as marker in the previous analyses is non-specific of the NRRL B-67259 strain, which precluded the adequate quantification of the active agent in the feeds used in the studies. Moreover, in a second analysis, the active agent could not be isolated from the field excreta samples collected from either the treated or the control group in the two formerly submitted efficacy studies. The Panel concluded that the methodology was not able to discriminate between the strain under assessment and the background. Additionally, two new efficacy trials with chickens for fattening were provided to support the efficacy of the additive. However, none could be further considered since the husbandry conditions in which the birds were kept were non-compliant with Directive 2007/43/EC. Therefore, the FEEDAP Panel was not in the position to conclude on the efficacy of Correlink™ ABS1781 for all growing poultry species.

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**Keywords:** zootechnical additives, gut flora stabilisers, Correlink™ ABS1781, *Bacillus velezensis* NRRL B-67257, poultry species, efficacy

**Requestor:** European Commission

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## 1. Introduction

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

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Elanco GmbH, is seeking a Community authorisation of *Bacillus subtilis* ABS1781 (*Bacillus velezensis* NRRL B-67259) as a feed additive to be used as a gut flora stabilisers for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species. (Table 1).

**Table 1:** Description of the substances

<b>Category of additive</b>	Zootechnical additives
<b>Functional group of additive</b>	Gut flora stabilisers
<b>Description</b>	<i>Bacillus subtilis</i> ABS1781 ( <i>Bacillus velezensis</i> NRRL B-67259)
<b>Target animal category</b>	Chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species
<b>Applicant</b>	Elanco GmbH
<b>Type of request</b>	New opinion

On 30 September 2020, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the product, could not conclude on the efficacy of *Bacillus subtilis* ABS1781 (*Bacillus velezensis* NRRL B-67259) for the target species and regarding the compatibility with monensin, lasalocid salinomycin, narasin, robenidine and maduramicin, due to lack of data.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 15 November 2021.

In view of the above, the Commission asks the Authority to deliver a new opinion on *Bacillus subtilis* ABS1781 (*Bacillus velezensis* NRRL B-67259) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species based on the additional data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

### 1.2. Additional information

The additive under assessment is a preparation containing viable spores of *B. velezensis* NRRL B-67259. It has not been previously authorised in the EU.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information<sup>1</sup> to a previous application on the same product.<sup>2</sup>

In accordance with Article 38 of the Regulation (EC) No 178/2002<sup>3</sup> and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39 e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical

<sup>1</sup> Dossier reference: EFSA-Q-2022-00264.

<sup>2</sup> Dossier reference: FAD-2019-0086.

<sup>3</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–48.

arrangements concerning transparency and confidentiality<sup>4</sup> a non-confidential version of the supplementary information has been published on Open.EFSA.<sup>5</sup>

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Bacillus velezensis* NRRL B-67259 (Correlink™ ABS1781 *Bacillus subtilis*) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>6</sup> and the relevant guidance documents: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).

## 3. Assessment

Correlink™ ABS1781 *Bacillus subtilis* (hereafter Correlink™ ABS1781) is a preparation of viable spores of *B. velezensis* NRRL B-67259 intended for use as a zootechnical additive (functional group: gut flora stabilisers) in feed for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor growing poultry species at the minimum concentration of  $1.5 \times 10^8$  CFU/kg complete feedingstuffs.

In its previous opinion (EFSA FEEDAP Panel, 2020), the FEEDAP Panel concluded that the additive is safe for the target species, consumers and the environment, that it is not irritant to skin and eyes or a skin sensitiser, but it is a respiratory sensitiser. However, the data provided were not sufficient to conclude on the efficacy of the additive for all poultry species. Moreover, the compatibility of *B. velezensis* NRRL B-67259 with diclazuril, decoquinatone and halofuginone was established, but not with monensin, salinomycin, narasin, robenidine, maduramicin and lasalocid.

The applicant has provided new information to address the gaps identified in the assessment of the efficacy of the additive which are the subject of this assessment.

### 3.1. Efficacy

In the former opinion, three efficacy studies conducted in chickens for fattening were submitted to support the efficacy of the additive. However, two of them could not be further considered due to the contamination of the control diets with the additive under assessment. In one study, the total bacilli counts in the diets of the control and treated groups were equivalent (i.e. control: 0.7/0.4/0.9  $\times 10^8$  CFU/kg feedingstuffs vs. Correlink™ ABS1781: 1.4/1.1/0.8  $\times 10^8$  CFU/kg feedingstuffs, in the starter, grower and finisher diets respectively). The presence of the active agent in all diets was confirmed by analysis using a polymerase chain reaction (PCR) targeting a functional gene of *B. velezensis* NRRL B-67259 (██████████) which the applicant declared to be strain specific. Therefore, it was inferred that the control diets were contaminated with the additive. Similarly, equivalent total bacilli counts in the control and treated diets were found in the second study (i.e. control: 0.3/0.3/0.4  $\times 10^8$  CFU/kg feedingstuffs vs. Correlink™ ABS1781: 1.7/3.2/4.0  $\times 10^8$  CFU/kg in the starter, grower and finisher diets, respectively), but the presence of *B. velezensis* NRRL B-67259 could not be confirmed due to the lack of samples. However, considering the similarities between the studies (i.e. they were both run in the same site and with the same design), and since *B. velezensis* NRRL B-67259 was identified in excreta samples collected from both control and treated groups using the same PCR method, the possibility that the control diets of this study were also contaminated with the additive could not be excluded. Therefore, the Panel considered that there were insufficient data to conclude on the efficacy of Correlink™ ABS1781 for chickens for fattening.

In the current application, two sets of data have been provided. The first aims to justify previous findings in the above-mentioned studies. The second set of data includes two new efficacy studies. Both are described below.

<sup>4</sup> Decision available at: <https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements>

<sup>5</sup> Available at: <https://open.efsa.europa.eu/questions/EFSA-Q-2022-00264>

<sup>6</sup> 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.

### 3.1.1. Data supporting previous findings

In the first set of data, the applicant declared that the [REDACTED] gene is not present only in the NRRL B-67259 strain but can also be found in other bacilli and provides some data to support it. To determine the prevalence of the [REDACTED] gene among bacilli,

[REDACTED]

Therefore, it can be concluded that the [REDACTED] gene is non-specific of the NRRL B-67259, and this precludes the adequate quantification of the strain in the samples.

Furthermore, two additional experiments were conducted with excreta samples from the above-cited trials to determine whether *B. velezensis* NRRL B-67259 could be detected after the gastrointestinal (GI) tract transit and to assess the genetic relatedness of strains recovered from the excreta samples, with the final aim of excluding the cross-contamination of control diets.<sup>8</sup>

[REDACTED]

[REDACTED]

The Panel has some doubts on the methodology applied

[REDACTED]

The Correlink™ ABS1781 active agent could not be substantially and consistently isolated from the field excreta samples collected from the treated (inoculated with the active agent) or control birds. These findings would suggest that either the Correlink™ ABS1781 strain was not included in the correct way in the treated feeds, or the strain was not able to survive the chickens' GI tract transit and/or the analysis was not able to discriminate between the strain under assessment and the background.

### 3.1.2. New efficacy studies

Two new efficacy studies with an experimental design similar to the ones previously submitted have been provided to support the efficacy of Correlink™ ABS1781. However, none was further considered as the total bacilli counts in most of the starter, grower and finisher diets fed to the birds in the control

<sup>7</sup> SIn\_Reply\_Oct\_178 and Annex\_IV\_3\_8\_NCBI Blast\_pksN2 and B velezensis genome list.

<sup>8</sup> Annex IV.3.2.2 and Annex IV.3.3.3/Supplementary information August 2022/Annex IV.3.2.2.Updated.CONF and IV.3.3.3.Updated.CONF

<sup>9</sup> Annex IV.3.2.2/Supplementary information August 2022/Annex IV.3.2.2.Updated.CONF

<sup>10</sup> Annex IV.3.3.3/Supplementary information August 2022/Annex IV.3.3.3.Updated.CONF

and treated groups were equivalent, suggesting a potential cross-contamination

<sup>11</sup>

<sup>12</sup>

<sup>13</sup>

<sup>14</sup>

Additionally, the husbandry conditions in which the birds were kept were not in line with Directive 2007/43/EC (e.g. used litter as bedding).

### 3.1.3. Conclusions on efficacy

With the data provided, it was not possible to draw any conclusion on the efficacy of Correlink™ ABS1781 for chickens for all growing poultry species.

## 4. Conclusions

The FEEDAP Panel is not in the position to conclude on the efficacy of Correlink™ ABS1781 for chickens for all growing poultry species.

## 5. Documentation provided to EFSA/Chronology

Date	Event
28/10/2021	Dossier received by EFSA. Correlink™ ( <i>Bacillus subtilis</i> ABS1781) for chickens for fattening, turkeys for fattening, chickens reared for laying, turkeys reared for breeding, minor poultry species. Submitted by Elanco GmbH
29/03/2022	Reception mandate from the European Commission
28/06/2022	Acceptance mandate from the European Commission by EFSA – Start of the scientific assessment
21/07/2022	Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 for 178 – Scientific assessment suspended. <i>Issues: efficacy</i>
08/08/2022	Reception of supplementary information from the applicant – Scientific assessment re-started
22/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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## Abbreviations

CFU	colony forming unit
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed

<sup>11</sup> Annex IV.3.4.

<sup>12</sup> Annex IV.3.5.

<sup>13</sup> Annex IV.3.6 and Annex IV.3.7.

<sup>14</sup> Pooling of two experiments.