Assessment of the feed additive consisting of
*Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30236 for all animal species for the renewal of its authorisation (BioCC OÜ)

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**Abstract**

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of the application for renewal of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30236 as a technological additive for use in forage for all animal species. The additive aims at improving the production of silage and is currently authorised for all animal species. The applicant provided evidence that the additive currently on the market complies with the existing conditions of authorisation. There was no new evidence to lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concluded that the additive remains safe for all animal species, consumers and the environment under the authorised conditions of use. Regarding user safety, the additive should be considered a skin and respiratory sensitiser. No conclusions can be drawn on the eye and skin irritancy potential of the additive. There was no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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**Keywords:** technological additive, silage additive, *Lactiplantibacillus plantarum* NCIMB 30236, safety, efficacy, QPS, renewal

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Lactiplantibacillus plantarum NCIMB 30236 for all animal species

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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 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from (from BioCC OÜ)\(^2\) for the renewal of the authorisation of the additive consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30236, when used as a feed additive for all animal species (category: technological additives; functional group: silage additives).

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 14(1) (renewal of the authorisation). 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 19 March 2021.

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 feed additive consisting of an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30236, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive consists of viable cells of *L. plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30236. It is currently authorised for use in feed for all animal species in the European Union (1k2073).\(^3\)

EFSA has adopted one opinion on the safety and efficacy of this product for all animal species (EFSA FEEDAP Panel, 2011).

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 *L. plantarum* NCIMB 30236 as a feed additive.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the agent in animal feed are valid and applicable for the current application.\(^5\)

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *L. plantarum* NCIMB 30236 is in line with the principles laid down in Regulation (EC) No 429/2008\(^6\) and the relevant guidance documents: Guidance on the characterisation of microorganisms used as feed additives or as

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\(^1\) Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

\(^2\) BioCC OÜ, F.R.Kreuzwaldi 1, 51006, Tartu, Estonia.

\(^3\) Commission Implementing Regulation (EU) No 1111/2011 of 3 November 2011 concerning the authorisation of *Lactobacillus plantarum* (NCIMB 30236) as a feed additive for all animal species. OJ L 287, 4.11.2011, p. 30.

\(^4\) FEED dossier reference: FAD-2020-0112.

\(^5\) The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2011-0004?search&form-return

\(^6\) 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.
production organisms (EFSA FEEDAP Panel, 2018) and Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3. **Assessment**

The additive consisting of viable cells of *L. plantarum* NCIMB 30236 is currently authorised as a technological additive (functional group: silage additives) for use in forages for all animal species. This assessment regards the renewal of the authorisation of the additive.

3.1. **Characterisation**

3.1.1. **Characterisation of the active agent**

The active agent was isolated from ensiled grass material. It is deposited at the National Collection of Industrial, Food and Marine Bacteria (NCIMB) with the deposit number NCIMB 30236. It has not been genetically modified.

The taxonomic identification of the strain as *L. plantarum* was confirmed. The susceptibility of the strain to the antibiotics recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018) was tested by a microdilution method. All the minimum inhibitory concentration (MIC) values were equal or fell below the cut-off values established in the FEEDAP Guidance (EFSA FEEDAP Panel, 2018) for *L. plantarum*. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The whole genome sequence data of the strain, including the whole genome sequence part1 and Supplementary information November 2021/Annexes_II_2, were interrogated for the presence of antimicrobial resistance genes using the ResFinder and Plasmid profiling and genetic stability. No genes of concern were identified.

3.1.2. **Characterisation of the additive**

The product currently authorised consists of approximately 30–40% bacterial cells and 60–70% lactose as a carrier. The minimum concentration of the active agent (*L. plantarum* NCIMB 30236) is $1.2 \times 10^{11}$ colony forming units (CFU) per gram of additive.

The applicant declared that the manufacturing process of the additive has not been modified since the first authorisation. The only change introduced since then has been the change of the carrier from lactose to anhydrous dextrose.

Analysis of three recent batches showed that the intended minimum concentration of the active agent was exceeded in all cases (range: $1.69 \times 10^{11}$ to $1.75 \times 10^{11}$ CFU/g additive, average: $1.79 \times 10^{11}$ CFU/g additive).

Analysis of three recent batches of the additive showed that the levels of enterobacteria, beta-glucuronidase-positive *E. coli*, filamentous fungi and yeasts were all $< 10$ CFU/g. No *Salmonella* was detected in 25 g of the samples.

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7 Technical dossier/Supplementary information November 2021/Annex II_7 Certificate of deposition.
8 Technical dossier/Supplementary information November 2021/Annex II_11 WGS analysis.
9 Technical dossier/Section II/Annex II_11 Whole genome sequence analysis_part1 and Annex II_12 Plasmid profiling and genetic stability.
10 Technical dossier/Section II/Annex II_13 Antimicrobial susceptibility.
11 Technical dossier/Section II/Annex II_11 Whole genome sequence analysis_part1 and Supplementary information. November 2021/Annex II_11 WGS analysis_part1, Annex II_11 WGS analysis ResFinder_results_part4_Conf and Annex II_11 WGS analysis_rgi_results_part5_Conf.
12 Technical dossier/Section II/Annex II_16 and Supplementary information November 2021/Annex_SIn letter_FAD-2020-0112_EFSA-Q-2021-00076.
13 Technical dossier/Section II/Annex II_2 Batch to batch variations and Supplementary information November 2021/Annexes II_2 CoA part1-part5.
The levels of aflatoxins (B1, B2, G1, and G2), ochratoxin A, deoxynivalenol, zearalenone, Toxins T2 and HT-2, fumonisins, lead, mercury and cadmium in the same batches were below the respective limits of detection, while arsenic showed values in the range of 0.022–0.023 mg/kg. The particle size distribution of one of the same recent batches (carrier: anhydrous dextrose) was analysed by laser diffraction and the dusting potential by the Stauber–Heubach method. The results showed an average particle size of 211.78 µm with approximately 7% of particles with a diameter < 10 µm and 23% < 50 µm; and a dusting potential of 15.1 mg/m³.

3.1.3. Stability

The shelf-life of two recent batches of the additive was studied when stored at 4°C and 20°C in the original packaging for 23 and 30 months. A third batch was tested at 4°C in the original packaging for 8 months. In all tested conditions viability losses were below or equal to 0.5 log values. The stability of the additive (two batches) suspended in water was studied when stored at 4 and 20°C for 2 and 48 h. No losses were observed over 48 h (< 0.5 log).

3.1.4. Conditions of use

The additive is currently authorised for use in forages for all animal species.

Under other provisions of the authorisation, it is specified that:

- In the directions for use of the additive and premixture, indicate the storage temperature and storage life.
- Minimum dose of the additive when used without combination with other micro-organisms as silage additives: $2.4 \times 10^8$ CFU/kg fresh material.
- For safety: it is recommended to use breathing protection and gloves during handling.

The applicant wishes to maintain the same conditions of use.

3.2. Safety

In the previous opinion the Panel concluded that following the qualified presumption of safety (QPS) approach, the use of this strain in the production of silage was considered safe for target species, consumers and the environment (EFSA FEEDAP Panel, 2011). In the context of this application, the identity of the strain as L. plantarum NCIMB 30236 was confirmed and evidence that the strain does not show acquired antimicrobial determinants for antibiotics of human and veterinary importance was provided. Consequently, the conclusions already reached are still valid and L. plantarum NCIMB 30236 is considered to be safe for the target species, consumers and the environment.

The safety for the user was evaluated by the FEEDAP Panel in the previous assessment (EFSA FEEDAP Panel, 2011). The Panel concluded that: ‘Given its proteinaceous nature, the active agent has the potential to be a skin/respiratory sensitiser, although users at the farm level are exposed to the additive only for a short period of time when preparing the aqueous suspension’. In the current assessment, it has been shown that exposure by inhalation is likely.

The applicant performed a literature search in order to provide evidence that the additive remains safe under the approved conditions for target species, consumers, users and the environment. The literature search was conducted in June 2020 without time restrictions. The search term used was ‘Lactobacillus plantarum NCIMB 30236’ and the strategy followed was reported. The applicant searched in a total of seven relevant databases Agricola, Agris, Google scholar, Ingenta, PubMed, Science Direct and World Cat Library. The literature search retrieved seven publications. However, none was considered because they either regarded the assessment of the efficacy (two), referred to previous EFSA opinions (four) or did not regard the product under assessment (one).

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14 Technical dossier/Section II/Annex II_3 Purity.
15 Limit of determination: aflatoxins (B1, B2, G1, and G2): 0.4 µg/kg, deoxynivalenol 200 µg/kg, zearalenone 50 µg/kg, Pb 0.02 mg/kg, Hg 0.01 mg/kg, Cd 0.01 mg/kg and As 0.10 mg/kg.
16 Technical dossier/Section II/Annex II_4 Dusting potential and Supplementary information November 2021/Annex_SIn. letter_FAD-2020-0112_EFSA-Q-2021-00076 and Annex II_1.
17 Technical dossier/Section II/Annex II_31 Shelf life.
18 Technical dossier/Section II/Annex II_32 Stability in aqueous media.
19 Technical dossier/Section III/Annexes III_2-6.
Therefore, the FEEDAP Panel concludes that there is no new evidence that would lead to reconsider the previous conclusions that *L. plantarum* NCIMB 30236 is safe for the target species, consumers and the environment under the authorised conditions of use. Regarding user safety, *L. plantarum* NCIMB 30236 should be considered a skin and respiratory sensitiser but no conclusions can be drawn on its potential to be irritant to skin and eyes.

### 3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of use of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

### 4. Conclusions

The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation.

There is no new evidence to lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concludes that *L. plantarum* NCIMB 30236 remains safe for all animal species, consumers and the environment under the authorised conditions of use. Regarding user safety, the additive should be considered a skin and respiratory sensitiser, but no conclusions can be drawn on its potential to be irritant to skin and eyes.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

### 5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|------------------------------------------------------------------------|
| 04/12/2020 | Dossier received by EFSA. *Lactiplantibacillus plantarum* E-98 NCIMB 30236 for all animal species. Submitted by BioCC OU |
| 10/12/2021 | Reception mandate from the European Commission                           |
| 19/03/2021 | Application validated by EFSA – Start of the scientific assessment     |
| 21/06/2021 | Comments received from Member States                                   |
| 07/09/2021 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation* |
| 12/11/2021 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 26/01/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment  |

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Scientific Opinion on the safety and efficacy of Lactobacillus plantarum (NCIMB 30236) as a silage additive for all species. EFSA Journal 2011;9(6):2275, 65 pp. [https://doi.org/10.2903/j.efsa.2011.2275](https://doi.org/10.2903/j.efsa.2011.2275)

### Abbreviations

- **CFU**: colony forming unit
- **dDDH**: digital DNA–DNA hybridisation
- **EURL**: European Union Reference Laboratory
Lactiplantibacillus plantarum NCIMB 30236 for all animal species

| Acronym | Full Form |
|---------|-----------|
| FEEDAP  | EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed |
| LOD     | limit of detection |
| MIC     | minimum inhibitory concentration |
| NCIMB   | National Collection of industrial, food and marine bacteria |
| OECD    | Organisation for Economic Co-operation and Development |
| QPS     | qualified presumption of safety |
| TYGS    | type strain genome server |
| WGS     | whole genome sequence |