Assessment of the feed additive consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) DSM 8862 and *L. plantarum* DSM 8866 for all animal species for the renewal of its authorisation (Dr. Pieper Technologie- und Produktentwicklung GmbH)

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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 assessment of the application for renewal of *Lactiplantibacillus plantarum ssp. plantarum* (formerly *Lactobacillus plantarum ssp. plantarum*) DSM 8862 and *Lactiplantibacillus plantarum ssp. argentoratensis* (formerly *Lactobacillus plantarum ssp. argentoratensis*) DSM 8866 as a technological additive to improve ensiling of forage for all animal species. There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concludes that the additive remains safe for all animal species, consumer and the environment under the authorised conditions of use. Regarding user safety, the additive is not a skin irritant but no conclusions can be drawn on the eye irritancy potential of the additive nor to the skin sensitisation potential. The additive should be considered a respiratory sensitisser. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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**Keywords:** technological additives, silage additive, *Lactiplantibacillus plantarum ssp. plantarum* DSM 8862, *Lactiplantibacillus plantarum ssp. argentoratensis* DSM 8866, safety, efficacy, renewal

**Requestor:** European Commission

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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 1 year before the expiry date of the authorisation.

The European Commission received a request from Dr. Pieper Technologie- und Produktentwicklung GmbH\(^2\) for the renewal of the authorisation of the additive consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) strains DSM 8862 and DSM 8866, 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 7 June 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 containing the *L. plantarum* strains DSM 8862 and DSM 8866, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The additive is a preparation of viable cells of two lactobacilli strains (DSM 8862 and DSM 8866), intended for use as a technological additive (functional group: silage additive). It is currently authorised in the European Union (1 k20812)\(^3\) for use in feed for all animal species.\(^4\)

EFSA adopted one opinion on the safety and efficacy of this product for pigs, bovines, sheep, goats and horses (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\(^5\) in support of the authorisation request for the use of *L. plantarum* (formerly *Lactobacillus plantarum*) DSM 8862 and *L. plantarum* DSM 8866 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.\(^6\)

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *L. plantarum* DSM 8862 and *L. plantarum* DSM 8866 is in line with the principles laid down in Regulation (EC) No 429/2008\(^7\) and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the characterisation of

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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.

\(^2\) Dr. Pieper Technologie- und Produktentwicklung GmbH, Dorfstraße 34, 16,818 Wuthenow, Germany.

\(^3\) Commission Implementing Regulation (EU) No 93/2012 of 3 February 2012 concerning the authorisation of *Lactobacillus plantarum* (DSM 8862 and DSM 8866) as a feed additive for all animal species. OJ L 33, 4.2.2012, pp. 1–3.

\(^4\) Technical dossier/Supplementary Information June 2022/Supplementary Information BIO-SIL June 2022.

\(^5\) FEED dossier reference: FAD-2021-0012.

\(^6\) The full report is available on the EU Science Hub website: [https://joint-research-centre.ec.europa.eu/publications/20-fad-dossiers_en](https://joint-research-centre.ec.europa.eu/publications/20-fad-dossiers_en).

\(^7\) 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.
microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018) and Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2013).

3. **Assessment**

The additive under assessment is a preparation of viable cells of *L. plantarum* (formerly *Lactobacillus plantarum*) DSM 8862 and *L. plantarum* DSM 8866 and is currently authorised as a technological additive (functional group: silage additives) for all animal species. The herein assessment regards the renewal of the authorisation of the above-mentioned additive.

3.1. **Characterisation**

3.1.1. **Characterisation of the active agents**

The active agents are two non-genetically modified *L. plantarum*8 strains originally isolated from timothy-grass (DSM 8862) and chopped maize (DSM 8866) and deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ).9

The taxonomic identification of the strains under assessment was investigated by digital DNA–DNA hybridisation (dDDH) calculation based on the whole genome sequence (WGS).10 The highest dDDH values obtained were 93.1% between the strain DSM 8862 and *L. plantarum* ssp. *plantarum* DSM 20174T and 96.5% between strain DSM 8866 and *L. plantarum* ssp. *argentoratensis* DSM 16365T, confirming that the strains belonged to these subspecies respectively.11 The Panel notes that the identification of the strains in the former opinion was performed at the species level and not at the subspecies level (EFSA FEEDAP Panel, 2011).

The strains were subjected to antimicrobial susceptibility testing using broth microdilution method and included the list of antimicrobials recommended by EFSA (EFSA FEEDAP Panel, 2018). All the minimum inhibitory concentration values were equal or fell below the corresponding EFSA cut-off values for *L. plantarum/pentosus*.12 Therefore, the strains are considered susceptible to all the relevant antimicrobials.

The WGS of the strains DSM 8862 and DSM 8866 were interrogated for the presence of antimicrobial resistance genes in the CARD13 and ResFinder14 databases. The search in CARD was performed using strict, perfect and complete genes only criteria while the second database was queried with 90% identity and 60% of coverage as thresholds. No hits of concern were identified.

3.1.2. **Characterisation of the additive**

The product currently authorised consists of 40–60% bacterial cells (ratio 1:1 of *L. plantarum* DSM 8862 and *L. plantarum* ssp. *argentoratensis* DSM 8866) and 40–60% lactose as carrier.15 The additive is currently authorised with a minimum concentration of the active agents of $3.0 \times 10^{11}$ colony forming units (CFU)/g additive (ca. $1.5 \times 10^{11}$ CFU/g of each strain).

Analysis of five recent batches of the additive showed compliance with the specifications (mean $3.2 \times 10^{11}$ CFU/g additive, range $3.0 \cdot 3.7 \times 10^{11}$ CFU/g additive).16

Specifications for the final product are set for yeasts (< $10^3$ CFU/g), filamentous fungi (< $10^3$ CFU/g), Enterobacteriaceae (< $10^5$ CFU/g) and mesophilic aerobic spore-forming bacteria (< $10^3$ CFU/g). Additional specifications are set also for *Escherichia coli* (< $10$ CFU/g), presumptive *Bacillus cereus* (< $10^3$ CFU/g), coagulase-positive staphylococci (< $10^3$ CFU/g), sulfite-reducing clostridia (< $10^4$ CFU/g), *Salmonella* spp. (no detection in 25 g) and *Listeria monocytogenes* (no detection in 25 g). Three recent batches analysed were compliant with these specifications.17

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8 Technical dossier/Section II/Annex_II_29.
9 Technical dossier/Section II/Annex_II_30.
10 Technical dossier/Supplementary Information June 2022/ Annex II_126 and Annex II_128.
11 Technical dossier/Supplementary Information June 2022/Annex II_128.
12 Technical dossier/Section/Annex_II_41.
13 Technical dossier/Supplementary Information June 2022/Annex II_114 and Annex II_116.
14 Technical dossier/Supplementary Information June 2022/Annex II_115 and Annex II_117.
15 Technical dossier/Section II/Annex_II_5 and Annex II_6.
16 Technical dossier/Supplementary Information June 2022/Annex II_118, Annex II_119, Annex II_120, Annex II_121 and Annex II_122.
17 Technical dossier/Section II/Annex_II_12 and Annex_II_13.
Three batches of the additive were tested for the presence of mycotoxins (aflatoxins B1, B2, G1 and G2, deoxynivalenol and zearalenone), mercury, lead, cadmium and arsenic. Only lead (Pb) and cadmium (Cd) were detected with the mean values of 0.03 mg Pb/kg (range: 0.02–0.06 mg/kg) and 0.02 mg Cd/kg (range: 0.02–0.03 mg/kg). Results for mercury, arsenic and mycotoxins were below the limit of quantification (LOQ) in all batches.

Polychlorinated dibenzodioxins and dibenzofurans (PCDDs and PCDFs) and coplanar dioxin-like polychlorinated biphenyls (DL-PCBs) were analysed in three batches and were below the corresponding LOQs. In all the batches, the calculated (upper bound) levels of the sum of dioxins (88% dw) were ≤ 0.08 ng WHO-PCDD/F-TEQ/kg, of the sum of DL-PCBs were 0.012 ng WHO-PCB-TEQ/kg and of the sum of dioxins and DL-PCBs were ≤ 0.1 ng WHO-PCD/F-PCB-TEQ/kg. In all batches, the sum of non-DL PCBs was 0.6 μg/kg (88% d.w.).

The detected amounts of the above-described impurities do not raise safety concerns. The applicant has provided new data on dusting potential measured in three recent batches, and results showed a mean of 3,048 mg/m³ (range 2,395–3,825 mg/m³). Since no changes were introduced in the additive manufacturing process, the data on physico-chemical properties and stability of the additive described in the previous opinion still apply (EFSA FEEDAP Panel, 2011).

### 3.1.3. Conditions of use

The additive is currently authorised for use with 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 microorganism as silage additive: 3 × 10⁸ CFU/kg (ratio 1:1) fresh material.
- For Safety: it is recommended to use breathing protection and gloves during handling.

The applicant has requested to maintain the same conditions of use.

### 3.2. Safety

In the previous opinion the Panel considered that the active agents belong to a species suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007), and therefore the strains do not require any specific demonstration of safety other than confirming the absence of any determinants of resistance to antibiotics of human and veterinary clinical significance and the safety for the user (EFSA FEEDAP Panel, 2011). In the context of the current application, the identity of the strains DSM 8862 and DSM 8866 as L. plantarum ssp. plantarum and L. plantarum ssp. argentoratensis, respectively, was confirmed and evidence was provided that they do not show acquired antimicrobial determinants for antibiotics of human and veterinary importance, therefore meeting the QPS requirements (EFSA BIOHAZ Panel, 2020a,b). Both L. plantarum ssp. plantarum DSM 8862 and L. plantarum ssp. argentoratensis DSM 8866 are considered safe for the target species, consumers and the environment.

In the previous assessment (EFSA FEEDAP Panel, 2011), the Panel concluded regarding user safety: “Although users at farm level are exposed to the additive only for a short period of time when preparing the aqueous suspension, given the lack of specific information, its proteinaceous nature and the high dusting potential, the active agents should be considered to have the potential to be skin and respiratory sensitisers.”

The applicant submitted one study on skin irritation and one on eye irritation in order to address the safety for the user. The skin irritation potential of the additive was tested in a reconstructed human epidermis model in vitro under GLP principles and according to OECD TG 439 (2021). Results of the study showed no skin irritation potential under the test conditions used.

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18 Technical dossier/Supplementary Information June 2022/ Annex II_123, Annex II_124 and Annex II_125.
19 Technical dossier/Supplementary Information June 2022/ Supplementary Information BIO-SIL June2022: LOQ: aflatoxins B1, B2, G1 and G2: 0.5 μg/kg; deoxynivalenol and zearalenone: 10 μg/kg; mercury: 0.01 mg/kg; arsenic: 0.04 mg/kg.
20 Technical dossier/Supplementary Information June 2022/ Annex II_110.
21 Technical dossier/Supplementary Information June 2022/Annex III_20.
The eye irritation potential of the additive was tested using a Bovine Corneal Opacity Permeability Assay (BCOP) under GLP principles performed according to OECD TG 437 (2020). The results of the assay did not allow a conclusion concerning eye irritancy as some corneal opacity was observed (UN GHS “No stand-alone prediction can be made”), thus the FEEDAP Panel cannot conclude on the eye irritation potential of the additive.

In the absence of data, the FEEDAP Panel cannot draw conclusions on the skin sensitisation potential of the additive.

The applicant declares that no adverse effects on the health of workers have ever been reported.

3.2.1. Conclusions on safety

The FEEDAP Panel concludes that there is no new evidence that would lead it to reconsider the previous conclusions that \textit{L. plantarum} ssp. \textit{plantarum} DSM 8862 and \textit{L. plantarum} ssp. \textit{argentoratensis} DSM 8866 are safe for the target species, consumers and the environment under the authorised conditions of use. The additive is not a skin irritant, but it is considered a respiratory sensitisier. No conclusions can be drawn on the potential of the additive to be irritant for eyes or to cause skin sensitisation.

3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions 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.

Both strains under assessment based on the QPS approach to safety assessment, \textit{L. plantarum} ssp. \textit{plantarum} DSM 8862 and \textit{L. plantarum} ssp. \textit{argentoratensis} DSM 8866 are presumed safe for the target species, consumers and the environment.

There is no new evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concludes that the additive remains safe for all animal species, consumer and the environment under the authorised conditions of use. Regarding the user safety, the additive is not a skin irritant but should be considered a respiratory sensitisier. The FEEDAP Panel cannot draw conclusions on the eye irritation potential nor skin sensitisation potential of the additive.

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                                                                                                           |
|------------|-----------------------------------------------------------------------------------------------------------------|
| 17/02/2021 | Dossier received by EFSA. BIO-SIL (\textit{Lactobacillus plantarum} DSM 8862 and \textit{Lactobacillus plantarum} DSM 8866) for all pigs, all bovines, all sheep, all goats and horses. Submitted by Dr. Pieper Technologie- und Produktentwicklung GmbH |
| 25/02/2021 | Reception mandate from the European Commission                                                                  |
| 07/06/2021 | Application validated by EFSA – Start of the scientific assessment                                              |
| 10/09/2021 | Comments received from Member States                                                                           |
| 29/11/2021 | 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/safety for the user |
| 28/06/2022 | Reception of supplementary information from the applicant – Scientific assessment re-started                     |
| 27/09/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment                                          |

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22 Technical dossier/Supplementary Information June 2022/Annex III_21.

23 Technical dossier/Section III/Section III_safety.
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EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020b. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March 2020. EFSA Journal 2020;18(7):6174, 42 pp. https://doi.org/10.2903/j.efsa.2020.6174

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EFSA FEEDAP Panel (EFSA Panel on Additives and Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

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Abbreviations

CFU  colony forming unit

dDDH  digital DNA–DNA hybridisation

DSMZ  German Collection of Microorganisms and Cell Cultures

EURL  European Union Reference Laboratory

FEEDAP  EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed

LOQ  limit of quantification

MIC  minimum inhibitory concentration

OECD  Organisation for Economic Co-operation and Development

PCB  polychlorinated biphenyl

PCDD  polychlorinated dibenzodioxin

PCDF  polychlorinated dibenzofuran

QPS  qualified presumption of safety

TEQ  toxic equivalents

WGS  whole genome sequence

WHO  World Health Organization