Assessment of the efficacy of two feed additives consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) strains ATCC 55058 and ATCC 55942 for all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the efficacy of two technological additives to improve ensiling of forages consisting of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) strains ATCC 55058 and ATCC 55942, respectively, for all animal species. Both additives are intended for use with all forages and for all animal species at a proposed minimum concentration of $5 \times 10^6$ colony forming units (CFU)/kg forage. In two previous opinions, the FEEDAP Panel could not conclude on their efficacy due to the absence of any significant evidence of nutrient preservation. Based on three new efficacy studies provided by the applicant as supplementary information, the FEEDAP Panel concluded that the addition of *L. plantarum* strains ATCC 55058 or ATCC 55942, have the potential to improve the ensiling process by reducing protein degradation in all type of forages as indicated by the reduction of ammonia production.

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**Keywords:** technological additive, silage additives, *Lactiplantibacillus plantarum* ATCC 55058, *Lactiplantibacillus plantarum* ATCC 55942, QPS, 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\(^1\) establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 thereof defines the terms of the authorisation by the Commission.

The applicant FEFANA asbl\(^2\) is seeking a Community authorisation of *Lactobacillus plantarum*\(^3\) – strains ATCC 55058 and ATCC 55942 as feed additives to be used as silage additives for all animal species (Table 1).

| Category of additive | Technological additives |
|----------------------|-------------------------|
| **Functional group of additives** | Silage additives |
| **Description** | *Lactobacillus plantarum* – strains ATCC 55058 and ATCC 55942 |
| **Target animal category** | All animal species |
| **Applicant** | FEFANA asbl |
| **Type of request** | New opinion |

On 10 October 2013, the Panel on Additives and Products or Substances used in Animal Feed 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 *Lactobacillus plantarum* – strains ATCC 55058 and ATCC 55942 as silage additives in all animal species.

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 3 September 2021 and the applicant has been requested to transmit them to EFSA as well.

In view of the above, the Commission asks EFSA to deliver a new opinion on *Lactobacillus plantarum* – strains ATCC 55058 and ATCC 55942 as feed additives for all animal 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

EFSA issued two opinions (EFSA FEEDAP Panel, 2012, 2013) on the safety and efficacy of the additives *L. plantarum* ATCC 55058 and *L. plantarum* ATCC 55942 when used in feed for all animal species.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information\(^4\) to previous application on the same products.\(^5\)

In accordance with Article 38 of the Regulation (EC) No 178/2002\(^6\) 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

\(^1\) 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.
\(^2\) FEFANA Asbl – SILAC ‘Silage Additives Authorisation Consortium’, Rue de Trèves 45, 1,040 Brussels, Belgium.
\(^3\) The request refers to the synonym *Lactobacillus plantarum*, corresponding to the current taxonomic unit of *Lactiplantibacillus plantarum*.
\(^4\) Dossier reference: EFSA-Q-2021-00594.
\(^5\) Dossier references: FAD-2010-0048 and FAD-2013-0001.
\(^6\) 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, a non-confidential version of the supplementary information has been published on Open.EFSA.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the efficacy of *L. plantarum* ATCC 55058 and ATCC 55942 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance document: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The two additives under assessment contain viable cells of either *L. plantarum* ATCC 55058 or *L. plantarum* ATCC 55942 and are intended to be added to forages to promote ensiling (category: technological additives, functional group: silage additives). Since the last opinions, the taxonomic unit of the species under assessment has been updated from the basionym *Lactobacillus plantarum* to *L. plantarum* (Zheng et al., 2020) and considered in the list of QPS-recommended biological agents (EFSA BIOHAZ Panel, 2020). The current taxonomic unit is used hereafter in the opinion. The additives composed by *L. plantarum* strains ATCC 55058 or ATCC 55942, when used individually, are intended to improve the ensiling process at proposed levels of $5 \times 10^6$ CFU colony forming units (CFUs)/kg forage.

In both previous opinions (EFSA FEEDAP Panel, 2012, 2013), the FEEDAP Panel could not conclude on the efficacy of the two additives owing to the lack of sufficient evidence for an improvement on the nutrient preservation during the ensiling process. The applicant has now provided new data to support the efficacy of the two products on the production of silage.

Three laboratory experiments were conducted with different forage samples representing the materials easy to ensile (study 1), moderately difficult to ensile (study 2) and difficult to ensile (study 3), as specified by Regulation (EC) No 429/2008 (Table 2). All the studies included a control (without inoculant), a group inoculated with *L. plantarum* ATCC 55058 and a group inoculated with *L. plantarum* ATCC 55942. The additives were certified at an intended concentration of $5 \times 10^6$ CFU/kg forage (confirmed by analysis of the applied suspension).

Forage for the control silos were without additive. Samples of forages, four replicates per treatment, were ensiled for 90 days in 2.75 L mini-silos which had the potential to vent gas. Experiments were conducted at room temperature.

Table 2: Characteristics of the forage samples used in the three ensiling experiments

| Study | Test material    | Dry matter content (%) | Water-soluble carbohydrates content (% fresh matter) |
|-------|------------------|------------------------|---------------------------------------------------|
| 1     | Maize            | 38.6                   | 5.2                                               |
| 2     | Alfalfa          | 38.8                   | 2.4                                               |
| 3     | High moisture maize | 67.2                 | 1.0                                               |

After 90 days, the mini-silos were opened, and the contents were analysed for dry matter (DM), pH, volatile fatty acids (VFAs), lactic acid, ethanol and ammonia concentration. The DM contents were not corrected for volatiles to calculate the DM loss.

Statistical analyses were performed using a non-parametric (Kruskal-Wallis) test and significance was declared at $p < 0.05$. Results are shown in Table 3.

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7 Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.
8 Available at: https://open.efsa.europa.eu/questions/EFSA-Q-2021-00594.
9 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.
10 Certificate of analysis – LP.
11 Annex 1 Efficacy Report 2_LP ATCC 55058 & 55942.
12 2022-06-21_SILAC L. Plantarum_SIn reply.
13 Annex 3 Statistical Summary 2_LP ATCC 55058 & 55942 and Annex 4 Statistical Results_LP.
At the end of the ensiling process, the forages treated with $5 \times 10^6$ CFU/kg forage of *L. plantarum* ATCC 55058 or ATCC 55942 showed a significantly lower ammonia-N content (expressed as percent of crude protein) compared to the control in all three studies, covering forages easy, moderately difficult and difficult to ensile. These results would indicate that the additives under assessment can contribute to reducing protein degradation of all type of forages during the ensiling process. As regards DM loss, a significant reduction was observed in the treated samples compared to control in all studies. However, considering that the DM contents were not corrected for volatiles, which may lead to an unreliable estimation of the DM loss, these results were not further considered in the assessment. Some significant and positive effects were observed also on pH (two studies), lactic acid (one study) and acetic acid content (two studies).

### 4. Conclusions

The addition of the additives *L. plantarum* ATCC 55058 and *L. plantarum* ATCC 55942 have the potential to improve the ensiling process by reducing protein degradation in all type of forages.

### 5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 07/12/2020 | Dossier received by EFSA, *L. plantarum* – strains ATCC 55058 and ATCC 55942 for all animal species. Submitted by FEFANA asbl |
| 12/10/2021 | Reception mandate from the European Commission                        |
| 25/10/2021 | Acceptance mandate from the European Commission by EFSA – Start of the scientific assessment |
| 17/12/2021 | Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. *Issues: efficacy* |
| 28/01/2022 | Reception of supplementary information from the applicant – Scientific assessment re-started |
| 30/03/2022 | Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. *Issues: efficacy* |
| 21/06/2022 | 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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**Abbreviations**

| Abbreviation | Description                            |
|--------------|----------------------------------------|
| CFU          | colony forming unit                    |
| DM           | dry matter                             |
| FEEDAP       | EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed |
| VFA          | volatile fatty acids                   |