SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 25 May 2020

doi: 10.2903/j.efsa.2020.6159

Safety and efficacy of *Lactobacillus buchneri* DSM 29026 as a silage additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Kos Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Lieve Herman, Boet Glandorf, Miguel Prieto Maradona, Maria Saarela, Jaume Galobart, Lucilla Gregoretti, Matteo Innocenti, Gloria López-Gálvez, Konstantinos Sofianidis, Maria Vittoria Vettori and Rosella Brozzi

Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on *Lactobacillus buchneri* DSM 29026 when used as a technological additive to improve ensiling of forage. The additive is intended for use with all forages and for all animal species at a proposed minimum concentration of 1×10^8 colony-forming units (CFU)/kg forage if used alone, or 5×10^7 CFU/kg forage if used in combination with other authorised microorganisms. The bacterial species *L. buchneri* is considered by the EFSA to be suitable for the qualified presumption of safety approach to safety assessment. As the identity of the strain has been clearly established and no acquired antimicrobial resistance determinants of concern were detected, the use of the strain as a silage additive is considered safe for livestock species, for consumers of products from animals fed the treated silage and for the environment. In the absence of data, the FEEDAP Panel cannot conclude on the potential of the additive to be a skin/eye irritant or skin sensitiser. Given the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser. The FEEDAP Panel concluded that *Lactobacillus buchneri* DSM 29026 at a minimum concentration of 5×10^7 CFU/kg may improve the production of silage from easy and moderately difficult to ensile forage material.

Keywords: *Lactobacillus buchneri*, technological additive, silage additive, safety, efficacy, QPS

Requestor: European Commission

Question number: EFSA-Q-2019-00180

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Kos Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Acknowledgements: The Panel wishes to acknowledge the contribution of Yolanda García Cazorla, Elisa Pettenati and Jordi Tarrés-Call to this opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Herman L, Glandorf B, Prieto Maradona M, Saarela M, Galobart J, Gregoretti L, Innocenti M, López-Gálvez G, Sofianidis K, Vettori MV and Brozzi R, 2020. Scientific Opinion on the safety and efficacy of *Lactobacillus buchneri* DSM 29026 as a silage additive for all animal species. EFSA Journal 2020;18(6):6159, 10 pp. https://doi.org/10.2903/j.efsa.2020.6159

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

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. 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 Microferm Ltd[2] for authorisation of the product *Lactobacillus buchneri* DSM 29026, 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 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 25 April 2019.

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 *Lactobacillus buchneri* DSM 29026, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive is a preparation containing viable cells of *Lactobacillus buchneri* DSM 29026 (current name *Lentilactobacillus buchneri*, Zheng et al., 2020). It has not been previously authorised as a feed additive in the European Union.

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 *Lactobacillus buchneri* DSM 29026 as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lactobacillus buchneri* DSM 29026 is in line with the principles laid down in Regulation (EC) No 429/2008 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 identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

[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] Microferm Ltd, Spring Lane North, WR141BU, Malvern, UK.
[3] FEED dossier reference: FAD-2018-0093.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0093_lactobacillus-buchneri-dsm29026.pdf

3. Assessment

The present additive is based on a preparation of viable cells of a single strain of *Lactobacillus buchneri* DSM 29026 (current name *Lentilactobacillus buchneri*, Zheng et al., 2020) and is intended to be added to forages to promote ensiling (technological additive, functional group: silage additive) with the eventual use of the silage in all animal species.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The strain of *L. buchneri* was originally isolated from cut grass and is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the accession number DSM 29026.[5] It has not been genetically modified.

The strain DSM 29026 was identified at species level as *L. buchneri* by whole genome sequence (WGS) analysis.[6] [redacted]

The susceptibility of the bacterial strain *L. buchneri* DSM 29026 was tested against the battery of antibiotics recommended by FEEDAP Panel (EFSA FEEDAP Panel, 2018a). All the minimum inhibitory concentration (MIC) values for the strain were equal to or fell below the cut-off values defined by the FEEDAP Panel for antimicrobials required for this species.[7]

The WGS was interrogated for the presence of antimicrobial resistance (AMR) genes [redacted] No relevant hits were identified.[8]

3.1.2. Characterisation of the product

[redacted] The product [redacted] has a minimum declared content of 2×10^{10} colony-forming units (CFU)/g. [redacted]

Analysis of five batches showed a mean value of 2.99×10^{10} CFU/g (range 2.35–4.40 $\times$ 10^{10} CFU/g).[9] [redacted] No microbial contaminants were detected in five batches (*Salmonella* spp. was absent in 25 g and < 10 CFU/g was obtained for the rest of microbial groups analysed).[10]

Five batches of the additive[11] and three batches of corn steep liquor[12] used in the growth medium were examined for the presence of heavy metals (Cd, Pb and Hg), arsenic and aflatoxins B1, B2, G1 and G2. In all cases, heavy metals and arsenic were found only in trace amounts (As < 0.05 mg/kg, Pb ≤ 0.22 mg/kg, Cd ≤ 0.07 mg/kg and Hg ≤ 0.02 mg/kg in the additive, and As ≤ 0.17 mg/kg, Pb ≤ 0.40 mg/kg, Cd: 0.09 mg/kg and Hg< 0.02 mg/kg in the corn steep liquor). No aflatoxins were detected in the steep liquor samples (Limit of detection (LOD): 0.1 μg/kg), whilst those in the batches of the additive were below the LODs (0.01 μg/kg for G1 and G2, ≤ 0.15 μg/kg for B1 and ≤ 0.03 μg/kg for B2).

No specific data were provided on the dusting potential of the additive under assessment.

[5] Technical dossier/Section II/Annex II.8.
[6] Technical dossier/Supplementary information January 2020/Annex Bioinformatic Analysis of DSM 29026.
[7] Technical dossier/Section II/Annex II.10.
[8] Technical dossier/Supplementary information January 2020/Annex Bioinformatic Analysis of DSM 29026 and Supplementary information April 2020/Annex Bioinformatic Analysis of DSM 29026 version 2.
[9] Technical dossier/Supplementary information January 2020/Annex Independent batch variation.
[10] Technical dossier/Section II/Annex II.3.
[11] Technical dossier/Section II/Annex II.2.
[12] Technical dossier/Section II/Annex II.1.

3.1.3. Stability

Three batches of the additive standardised to a count of 1×10^{11} CFU/g with maltodextrin and three batches standardised to a count of 2.5×10^{10} CFU/g with glucose were stored in aluminium foil bags at ambient temperature.[13] Losses after 18 months (the longest storage period) were < 0.5 Log units for both formulations.

L. buchneri DSM 29026 was standardised to a count of 1×10^{11} CFU/g using glucose as a carrier and including diammonium phosphate (5%) and dipotassium phosphate (2.5%) as buffers. Three samples (each of 5 g) were suspended in 1 L water giving a count of 5×10^{8} CFU/mL and stored for 7 days at room temperature. No loss of viability was detected after 4 days and even after 7 days losses were ≤ 0.2 Log units of the initial value.

3.1.4. Conditions of use

The additive is intended for use with all forages and for all animal species at a proposed minimum concentration of 1×10^{8} CFU/kg forage if used alone, or 5×10^{7} CFU/kg forage if used in combination with other authorised microorganisms. It is to be applied as such or as an aqueous suspension.

3.2. Safety

3.2.1. Safety for the target species, consumers and environment

The species L. buchneri is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show acquired resistance to antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the strain has been established as L. buchneri and the antibiotic resistance qualification met. Consequently, L. buchneri DSM 29026 is considered safe for the target species, consumers of products from animals fed treated silage and the environment.

3.2.2. Safety for the user

No specific data on skin/eye irritation or skin sensitisation were provided for the additive under application. Therefore, no conclusions can be drawn on the skin/eye irritancy or skin sensitisation of the additive. Given the proteinaceous nature of the active agent, the additive should be considered to be a respiratory sensitiser.

Once an active agent has been authorised as a silage additive, different formulations can be placed on the market with reference to that authorisation. The applicant listed several cryoprotectants and carriers which would allow multiple formulations of the additive to be produced, and consequently, not all forms can be directly tested for user safety. However, for assessing the safety for the user of the additive, the active agent is the principal concern provided that other components do not introduce safety issues. For this specific product, the excipients used in the preparation of the final formulation do not introduce additional risks.

3.3. Efficacy

Three laboratory experiments were made with different forage samples representing materials easy to ensile (study 1) and moderately difficult to ensile (studies 2 and 3), as specified by Regulation (EC) No 429/2008 (Table 1).[14] In the three studies, forage was ensiled in mini-silos with a capacity of 4.5 L. All of the silos were fitted with airlocks to vent gas. The additive was dissolved in water and sprayed on the forage at an intended concentration of 5×10^{7} CFU/kg fresh matter (not confirmed by analysis). Forage for the control silos were sprayed with an equal volume of water, but without the additive. Four replicate silos were prepared for each experimental treatment (with or without the additive). The ambient temperature during ensiling was controlled at 20 ± 1°C and the duration of the experiments was 90 days.

[13] Technical dossier/Section II.
[14] Technical dossier/Section IV/Annexes IV.1-3 and Supplementary information January 2020.

Table 1: Characteristics of the forage samples used in the three ensiling experiments

Study	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1	Grass/herbs (95:5)(1)	38.1	4.8
2	Grass/red clover/herbs (75:20:5)(2)	19.8	2.6
3	Grass/red clover/herbs (70:25:5)(3)	24.9	2.6

(1): Grass was predominantly timothy, perennial ryegrass and *L. multiflorum* × *F. arundinacea* hybrid.
(2): Grass was mainly timothy, perennial ryegrass and a minor proportion of orchard grass.
(3): Grass mainly timothy, meadow fescue and perennial ryegrass.

Silos were opened at the end of the experiment and the contents were analysed to determine silage dry matter (DM) and water-soluble carbohydrate (WSC) content, pH, lactic, formic, butyric, acetic and propionic acid as well as ethanol, and 1,2-butanediol concentrations. DM loss during ensiling was calculated and yeast, mould and lactobacilli counts were also measured. The method of Honig (1990) was used to determine aerobic stability of the silage. At the end of each experiment, samples were taken from each silo and exposed to air with continuous monitoring of temperature for at least 7 days. A rise of 3°C above room temperature was considered as indicator of silage deterioration, and the time at which that rise was observed was taken as a measure of the aerobic stability of treated and control silages.

Statistical evaluation of data was by a non-parametric test (Wilcoxon and Kruskal–Wallis test), comparing treated vs. control silos. Significance was declared at $p < 0.05$. Results are shown in Table 2.

Table 2: Summary of the analysis of ensiled material recovered at the end of the ensiling period with *L. buchneri* DSM 29026

Study	Application rate (CFU of each strain/kg forage)	Dry matter loss (%)	pH	Lactic acid (% dry matter)	Acetic acid (% dry matter)	Aerobic stability (days)
1	0	4.3	5.2	15.7	2.8	1.1
	5×10^7	5.5*	4.7*	10.3*	19.5*	> 7.2*
2	0	11.9	5.4	< 0.1	8.8	3.4
	5×10^7	10.7*	5.3*	0.1	7.4*	> 9.2*
3	0	8.4	5.7	1.2	7.7	1.5
	5×10^7	7.4*	5.3*	1.6	10.1*	> 8.1*

CFU: colony-forming unit.
*: Means in a column within a given trial are significantly different $p < 0.05$.

In the three studies, aerobic stability was significantly improved by the addition of the additive, extending the time with no deterioration of silage exposed to air for longer than 2 days and, therefore, complying with the minimum requirements specified in the guidance on the assessment of the efficacy of feed additives. However, the tested material was restricted to grass and with a range of dry matter content of 20–38%.

Dry matter losses were significantly reduced in both of the treated moderately difficult to ensile materials, but increased in the easy to ensile forage.

4. Conclusions

As the identity of the active agent has been established as *L. buchneri* and no antibiotic resistance of concern detected, following the QPS approach to safety assessment, the use of this strain as a silage additive is considered safe for target species, consumers of products from animals fed treated silage and for the environment.

In the absence of data, no conclusion can be drawn on the skin and eye irritancy or skin sensitisation of the additive. The additive should be considered a respiratory sensitiser.

The addition of *Lactobacillus buchneri* DSM 29026 at a minimum concentration of 5×10^7 CFU/kg has the potential to improve the aerobic stability of silage from easy and moderately difficult to ensile forage material. No conclusion can be drawn on the efficacy for difficult to ensile forage material due to the absence of data.

5. Documentation as provided to EFSA/Chronology

Date	Event
20/12/2018	Dossier received by EFSA. *Lactobacillus buchenri* DSM 29026 for all animal species. Submitted by Microferm Ltd.
11/03/2019	Reception mandate from the European Commission
25/04/2019	Application validated by EFSA – Start of the scientific assessment
26/06/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, user safety and efficacy*
22/07/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
03/01/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2020	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*
17/04/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
25/05/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. https://doi.org/10.2903/j.efsa.2007.587

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, Pei9e L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fern_andez Esc_amez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020. Scientific Opinion on the update of the list of QPS recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or 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

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2017a. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2017b. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018a. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

Honig H, 1990. Evaluation of aerobic stability. Proceedings of the EUROBAC Conference, Aug. 1986, Uppsala, Sweden. Swedish University of Agric. Sciences. Grass and Forage Reports 3, 76–82.

Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, Toole PWO, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG and Lebeer S, 2020. A taxonomic note on the genus Lactobacillus: description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. International Journal of Systematic and Evolutionary Microbiology, https://doi.org/10.1099/ijsem.0.004107

Abbreviations

CFU	colony-forming unit
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	Additives and Products or Substances used in Animal Feed
LOD	limit of detection
MIC	minimum inhibitory concentration
QPS	Qualified Presumption of Safety
WGS	whole genome sequence

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Lactobacillus buchneri* DSM 29026

In the current application authorisation is sought under Article 4(1) for a preparation of *Lactobacillus buchneri* DSM 29026 under the category/functional group 1(k) 'technological additives'/ 'silage additives', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* in *silage* for all animal species.

According to the Applicant, the *feed additive* contains as *active substance* viable spores of the non-genetically modified strain *Lactobacillus buchneri* DSM 29026. The *feed additive* is to be marketed as a powder preparation containing a minimum *Lactobacillus buchneri* DSM 29026 content of 2×10^{10} colony-forming unit (CFU)/g. The *feed additive* is intended to be added to *silage* at a minimum dose of 5×10^5 CFU/g of fresh *silage* if used alone, or at a minimum dose of 5×10^4 CFU/g of fresh *silage* if combined with other microorganisms.

For the identification of *Lactobacillus buchneri* DSM 29026, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Lactobacillus buchneri* DSM 29026 in the *feed additive*, the Applicant submitted the ring-trial validated spread plate method EN 15787. Based on the performance characteristics available, the EURL recommends this method for official control.

The Applicant did not provide any experimental method or data for the quantification of *Lactobacillus buchneri* DSM 29026 in *silage*. Since the unambiguous enumeration of *Lactobacillus buchneri* DSM 29026 initially added to *silage* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify the active substance in *silage*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.