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Safety and efficacy of a feed additive consisting of *Lentilactobacillus buchneri* DSM 32650 as a feed additive for all animal species (BioCC OÜ)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of *Lentilactobacillus buchneri* DSM 32650 as a technological feed additive (functional group: silage additive) for all animal species. The additive is intended to improve the production of silage at a proposed application rate of 1×10^8 colony-forming units (CFU)/kg fresh material. The bacterial species *L. buchneri* is considered by the European Food Safety Authority to be suitable for the qualified presumption of safety (QPS) approach. As the identity of the strain has been established and no antimicrobial resistance determinants of concern were detected, the use of the strain as a silage additive is considered safe for the target species, consumers and 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 a skin sensitiser. Given the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser. The FEEDAP Panel concluded that *Lentilactobacillus buchneri* DSM 32650 at a minimum concentration of 1×10^8 CFU/kg fresh material may extend the aerobic stability of silage from easy and moderately difficult to ensile fresh material with a dry matter content ranging from 28% to 45%.

Keywords: technological additive, silage additive, *Lentilactobacillus buchneri* DSM 32650, safety, efficacy, QPS

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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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from BioCC OÜ[2] for the authorisation of the additive consisting of *Lentilactobacillus buchneri* DSM 32650, 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). The dossier was received on 19 May 2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00381. The particulars and documents in support of the application were considered valid by EFSA as of 2 August 2022.

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 *Lentilactobacillus buchneri* DSM 32650, when used under the proposed conditions of use (see **Section 3.1.4**).

1.2. Additional information

The product under assessment consists of *Lentilactobacillus buchneri* DSM 32650. It is not currently 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 *Lentilactobacillus buchneri* DSM 32650 as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 2 August 2022 to 2 November 2022 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[5] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 15 February 2023 to 8 March 2023 for which no comments were received.

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.[6]

[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. Kreutzwaldi 1, 51006 Tartu, Estonia.
[3] Dossier reference: FEED-2021-0246.
[4] 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, p. 1–48.
[5] Decision available online: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[6] Evaluation report received on 2 December 2022 and available on the EU Science Hub: https://joint-research-centre.ec.europa.eu/publications/feed-2021-0246_en

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lentilactobacillus buchneri* DSM 32650 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 assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) and EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021).

3. Assessment

The product under assessment consists of viable cells of a single strain of *Lentilactobacillus buchneri* DSM 32650 intended to be added to fresh material to promote ensiling (technological additive, functional group: silage additive) with the eventual use of the silage for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The strain of *Lentilactobacillus buchneri* DSM 32650 was originally isolated from silage. It is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the accession number DSM 32650.[8] It has not been genetically modified.

The taxonomical identification of the strain DSM 32650 was achieved with a digital DNA–DNA hybridisation (dDDH) based on the whole genome sequence (WGS). The results of this analysis showed a dDDH value of 99.1% with the type strain *L. buchneri* DSM 20057^T.[9] The strain was shown to harbour two plasmids (~ 48 and 20 kbp).[10]

The antimicrobial susceptibility of the strain DSM 32650 was tested against the battery of antibiotics recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018b).[11] All the minimum inhibitory concentration values were equal to or fell below the corresponding EFSA cut-off values for obligate heterofermentative lactobacilli. Therefore, the strain is considered susceptible to all the relevant antibiotics.

The whole genome sequence of the strain, including the plasmids, was interrogated for the presence of antimicrobial resistance (AMR) genes. The applicant briefly described an analysis from 2020 performed against the ResFinder database (thresholds applied: 70% identity; 40% length of coverage) which did not identify any AMR gene.[12] However, the FEEDAP Panel notes that the analysis was poorly reported and the results presented only in the form of a statement. The applicant conducted a second updated search for AMR genes in 2022, against ResFinder (using StarAMR, thresholds applied: 98% identity and 60% length coverage) and CARD (using RGI) databases.[13] No hits of concern were identified. The Panel notes that the thresholds used in the second search in ResFinder are higher than those recommended by EFSA (2021) and potential hits could have been missed. However, considering that the strain was susceptible to all the relevant antibiotics and none of the three WGS-based analyses submitted identified hits of concern, the data suggest that the strain does not carry any acquired AMR genes.

[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.
[8] Annex_II_7.
[9] Annex_II_11_WGS_part1.
[10] Annex_II_12.
[11] Annex_II_13.
[12] Annex_II_11_ part1 and Annex_II_11_ part4.
[13] Annex_II_11_ part3.

3.1.2. Characterisation of the additive

The active agent is produced by fermentation and concentrated by centrifugation. Cryoprotectants (e.g. [redacted]) are added, the mixture is freeze-dried and blended with carriers ([redacted]).[15] The final product consists of ~ [redacted] of carriers, [redacted] cell concentrate and should guarantee a minimum concentration of active agent of 1.0×10^{11} CFU/g of additive.

Analytical data of five independent batches confirmed compliance with the specifications (mean: 1.3×10^{11} CFU/g, range from 1.2–1.4 $\times 10^{11}$ CFU/g).[16]

Three independent batches of the additive were analysed for purity. Results of the analyses of arsenic, lead, cadmium and mercury showed values of 0.016 mg As/kg (range 0.015–0.017), 0.022 mg Pb/kg (range 0.021–0.023), 0.011 mg Cd/kg (range < 0.01–0.011) and < 0.01 mg Hg/kg.[17] The analysis of mycotoxins, including aflatoxins (B1, G1, B2 and G2), ochratoxin A, deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin and fumonisins (B1 and B2), showed values below the limit of quantification (LOQ) of the analytical methods.[18] As regards the microbiological quality, the results showed β-glucuronidase-positive *Escherichia coli* counts < 100 CFU/g, no detection of *Salmonella* spp. in 25 g samples, *Enterobacteriaceae* counts < 10 CFU/g and yeasts and filamentous fungi counts < 1,000 CFU/g.

The FEEDAP Panel considers that the microbial contamination and the detected amounts of the above-described impurities do not raise safety concerns.

The additive is a powder with a density of 584 kg/m^3.[19]

The dusting potential of the additive (anhydrous dextrose and silicon dioxide as carriers) was measured in three batches (Stauber–Heubach) and showed a mean value of 7,256 mg/m^3 (range 6,880–7,558 mg/m^3). The same batches were tested for particle size distribution by laser diffraction; results showed that ~ 40% of the additive consists of particles with diameters below 100 μm, 16% below 50 μm and 2.4% below 10 μm.[20]

3.1.3. Stability

The shelf-life of three batches of the additive (anhydrous dextrose and silicon dioxide as carriers) was studied when stored at 4°C and 20°C in sealed aluminium-polyethylene sachets for up to 15 months and 6 months, respectively. Negligible losses (< 0.5 log of the initial value) were observed under the above-mentioned conditions.[21]

The stability of the additive in water was studied in two batches of the bacterial concentrate (lyophilised powder). One gram of each batch was suspended in 1 L of tap water and the suspensions was stored for 48 h at 20°C.[22] No losses (< 0.5 log of the initial value) were observed at the end of the respective storage periods.

3.1.4. Conditions of use

The additive is intended for use with all fresh material and for all animal species at a proposed minimum concentration of 1×10^8 CFU/kg fresh material. It is to be applied as an aqueous suspension.[23]

3.2. Safety

3.2.1. Safety for the target species, consumers and the 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, 2023). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not harbour

[14] Currently under re-evaluation.
[15] Sect_II_Identity_2.3 and Annexes II_15–32.
[16] Annex_II_2 and Annex_II_2_ explanation.
[17] Annex_II_3. '<' means below the LOQ.
[18] LOQ: Aflatoxins B1, B2. G1 and G2 (0,4 μg/kg) ochratoxin A (0,8 μg/kg), deoxynivalenol (200 μg/kg), zearalenone (20 μg/kg), toxins T-2 and HT-2 (50 μg/kg), fumonisin B1 (80 μg/kg) and fumonisin B2 (24 μg/kg).
[19] Annex_II_5.
[20] Annex_II_4 and Annex_II_4_ part2.
[21] Annex_II_33.
[22] Annex_II_34 _part2.
[23] Sect_II_Identity_2.5_L. buchneri BioCC 203 DSM 32650.

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 32650 is considered safe for the target species, consumers and the environment.

3.2.2. Safety for the user

No specific studies investigating the effects of the additive on the respiratory system were submitted. The highest dusting potential reported (7,558 mg/m^3) suggests that exposure by inhalation is possible. Owing to the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser.

Despite the request, no data on skin and eye irritation were provided. Therefore, no conclusion can be drawn on the skin and eye irritation potential of the additive.

No data were provided on the skin sensitisation potential of the additive. The FEEDAP Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of microorganisms are available.[24] Therefore, no conclusion can be drawn on the skin sensitisation potential of the additive.

3.2.2.1. Conclusions on safety

The Panel concludes that *Lentilactobacillus buchneri* DSM 32650 is presumed safe for the target species, consumers and the environment. The additive should be considered a respiratory sensitiser. No conclusions can be drawn on its potential to be a skin/eye irritant or a skin sensitiser.

3.3. Efficacy

Six laboratory experiments were made with different feedingstuffs representing materials easy to ensile (studies 1, 2 and 3), moderately difficult to ensile (studies 4 and 5) and difficult to ensile (study 6), as specified by Regulation (EC) No 429/2008 (Table 1). All the studies included a non-inoculated control and a group inoculated with *L. buchneri* DSM 32650. The additive was dissolved in water and sprayed on the forage at intended concentrations ranging from 5.5×10^7 to 1.0×10^8 CFU/kg forage (confirmed by analysis of the water suspensions in studies 1, 2, 3 and 5). Forage material for the control silos were sprayed with an equal volume of water, but without the additive. Samples of forage/corn cob were ensiled for 90–103 days in 2.7 L (studies 1, 2, 3, 5) or 1.75 L (studies 4 and 6) minisilos (five replicates per treatment) at 20°C.

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

Study	Test material	Dry matter content (%)	Water-soluble carbohydrate content (% fresh matter)
1[25]	Grass–legume mixture(1)	45.0	4.9
2[26]	Grass mixture(2)	37.9	4.6
3[27]	Whole crop maize(3)	28.3	3.3
4[28]	Chopped whole crop maize (late dough stage)	33.9	2.9
5[29]	Whole crop maize(3)	30.2	2.5
6[30]	Corn–cob mix(3)	66.5	1.2

(1): Timothy (*Phleum pratense*), red clover (*Trifolium sativum*), meadow fescue (*Festuca pratensis*), 1st cut (wilted).
(2): Red fescue (*Festuca arundinacea*), Timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), festulolium (*Festutolium* sp.), cock's foot (*Dactylis glomerata*), meadow foxtail (*Alopecurus pratensis*), 1st cut (wilted).
(3): *Zea mays* L.

[24] https://www.efsa.europa.eu/sites/default/files/2022-07/feedap20220629-30_m.pdf
[25] Annex_IV_2, Annex_IV_7, Annex_IV_21, Annex_IV_22 and Annex_IV_24.
[26] Annex_IV_1, Annex_IV_10, Annex_IV_21, Annex_IV_22 and Annex_IV_27.
[27] Annex_IV_4, Annexes_IV_9, Annex_IV_21, Annex_IV_22 and Annex_IV_26.
[28] Annex_IV_5, Annex_IV_20, Annex_IV_22 and Annex_IV_28.
[29] Annex_IV_3, Annexes_IV_8, Annex_IV_21, Annex_IV_22 and Annex_IV_25.
[30] Annex_IV_6 CCM, Annex_IV_20, Annex_IV_22 and Annex_IV_29.

After the ensiling period (90–103 days), the silos were opened and the contents were analysed for dry matter (DM), crude protein, crude fibre, crude ash, metabolisable energy, pH, lactic and volatile fatty acid concentration, as well as ethanol, ammonia nitrogen in total nitrogen, 1,2-propanediol, 2,3-butanediol and numbers of *Clostridium* spp. spores, yeasts and filamentous fungi. The DM loss was calculated and corrected according to Weiβbach (1998). 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. 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 one-sided non-parametric test (Kruskal–Wallis test), and significance declared at $p < 0.05$. Results are shown in Table 2.

Table 2: Effects of *L. buchneri* DSM 32650 on the characteristics of ensiled material recovered at the end of the ensiling period (90–103 days)

Study	Application rate (CFU/kg forage)	Dry matter loss (%)(1)	pH	Lactic acid (g/kg)	Acetic acid (g/kg)	Ammonia-N (% of total N)	Aerobic stability (days)
1	0	3.69	5.5	6.5	4.8	1.8	3.6
	5.7×10^7	3.95	4.4*	49.8*	24.0*	3.0	9.0*
2	0	3.82	4.4	44.8	11.0	3.7	6.8
	8.9×10^7	4.09	4.5	38.0	36.8*	3.6	9.0
3	0	4.36	3.7	56.4	17.2	3.4	2.2
	5.5×10^7	4.80	3.8	49.5	27.6*	3.7	9.0*
4	0	4.08	3.7	79.1	12.3	7.7	7.9
	1.0×10^8	4.48	3.8	67.6	18.9*	7.7	10.6*
5	0	3.47	3.7	76.3	16.5	3.1	1.6
	6.5×10^7	3.86	3.7	51.6	36.7*	3.4	9.0*
6	0	3.24	4.1	19.4	3.5	0.6	17.7
	1.0×10^8	3.67	4.0*	22.3*	9.8*	2.2	18.0

*: Values in a column within a given trial are considered as significantly different ($p < 0.05$).
(1): Fermentation loss calculated according to Wei ▫ bach, 1998.

The addition of the additive showed a decrease of pH and an increase of lactic acid concentration in two studies (1 and 6), and a significant increase of acetic acid concentration in all studies. No significant results were obtained in other parameters or studies. The Panel has reservation on the procedures used to calculate DM loss corrected for volatiles, but this endpoint was not further considered as the assessment of efficacy was based on the effects on aerobic stability.

In four studies (1, 3, 4 and 5), the addition of the additive led to a significant extension of the time with no deterioration of the silage exposed to air for at least 2 days longer than the control. This was reached with forage material with a range of DM content between 28% and 45%.

3.3.1. Conclusions on efficacy

The use of *Lentilactobacillus buchneri* DSM 32650 at the proposed inclusion rate has the potential to improve the aerobic stability of silage from forage material with a DM ranging from 28% to 45%.

4. Conclusions

Lentilactobacillus buchneri DSM 32650 is safe for target species, consumers and 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 a skin sensitiser. Given the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser.

The addition of *Lentilactobacillus buchneri* DSM 32650 at a minimum concentration of 1×10^8 CFU/kg forage has the potential to improve the aerobic stability of silage from forage material with a DM content ranging from 28% to 45%.

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Abbreviations

AMR	antimicrobial resistance
CFU	colony forming unit

DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification
QPS	qualified presumption of safety
WGS	whole genome sequence