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SCIENTIFIC OPINION

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Assessment of the feed additive consisting of *Lentilactobacillus buchneri* (formerly *Lactobacillus* buchneri) DSM 22501 for all animal species for the renewal of its authorisation (Chr. Hansen A/S)

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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 the authorisation of the additive consisting of *Lentilactobacillus buchneri* DSM 22501 as a technological feed additive to improve ensiling of fresh material for all animal species. The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation. 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 irritant to skin and eye, but owing to its proteinaceous nature it should be considered a respiratory sensitiser. No conclusions could be drawn on the 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.

KEYWORDS

Lentilactobacillus buchneri DSM 22501, QPS, renewal, safety, silage, technological additives

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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 Chr. Hansen A/S[2] for the renewal of the authorisation of the additive consisting of *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 22501, when used as a feed additive for all animal species (category: technological; functional group: silage).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) under Article 14(1) (renewal of the authorisation). The dossier was received on 09/11/2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00789. The particulars and documents in support of the application were considered valid by EFSA as of 23 January 2023.

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* (formerly *Lactobacillus buchneri*) DSM 22501, when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

The additive is a preparation containing *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 22501. The additive is currently authorised for use in feed for all animal species (1k20738).[3]

EFSA issued one opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2013).

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 *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 22501 as a feed additive.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] 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,[6] 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,[7] EFSA carried out a public consultation on the non-confidential version of the technical dossier from 1 September to 22 September 2023 for which no comments were received.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 27 January 2023 to 27 April 2023 for which the received comments were considered for the assessment.

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 active agent in animal feed are valid and applicable for the current application.[8]

[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]Chr. Hansen A/S, Boege Allé 10–12, DK-2970, Hoersholm, Denmark.

[3]Commission Implementing Regulation (EU) No 1113/2013 of 7 November 2013 concerning the authorisation of preparations of *Lactobacillus plantarum* NCIMB 40027, *Lactobacillus buchneri* DSM 22501, *Lactobacillus buchneri* NCIMB 40788/CNCM I-4323, *Lactobacillus buchneri* LN 40177/ATCC PTA-6138, and *Lactobacillus buchneri* LN 4637/ATCC PTA-2494 as feed additives for all animal species.

[4]Dossier reference: FEED-2022-009191.

[5]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.

[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[7]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[8]Evaluation report received on 19/09/2022 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) DSM 22501 is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3 | ASSESSMENT

The product consisting of viable cells of *L. buchneri* (formerly *Lactobacillus buchneri*) DSM 22501 is authorised as a technological additive (functional group: silage additive) for use in fresh material for all animal species. This assessment regards the renewal of the authorisation of *L. buchneri* DSM 22501 for all animal species.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive is currently authorised with a minimum content of the active agent (*L. buchneri* DSM 22501) of 5×10^{10} colony forming units (CFU)/g of additive. The applicant declared that the manufacturing process and the formulation have not been changed since the previous authorisation.[10] [redacted][11] [redacted] to reach the minimum concentration specified for *L. buchneri* DSM 22501. The applicant states that no antimicrobial substances are used during the manufacturing.[12]

The analysis of nine samples (obtained from at least five independent batches)[13] showed compliance with the specifications of the active agent (mean 6.4×10^{11} CFU/g and range 5.4×10^{11}–7.2×10^{11} CFU/g).[14]

Four batches of the additive (three independent) were tested for arsenic (As) (range <0.005–0.019 mg/kg), lead (Pb) (range 0.015–0.033 mg/kg), cadmium (Cd) (range 0.014–0.029 mg/kg) and mercury (Hg) (range 0.0124–0.0354 mg/kg). Aflatoxin B1 tested in the same batches was below the limit of quantification (LOQ) of the analytical method.[14]

Specifications are set for coliforms (<10^3 CFU/g), *Escherichia coli* (<10 CFU/g), *Salmonella* spp. (no detection in 5/25 g) and yeasts and filamentous fungi (<10^3 CFU/g). The analysis of the same batches showed compliance with these specifications.[15,16] In addition, the applicant provided data on Enterobacteriaceae for three batches showing <10 CFU/g.[17]

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

The additive appears as a freeze-dried powder, with an approximate density of 0.5 g/mL.

A new analysis of the dusting potential of three batches of the additive was provided, using the Stauber–Heubach method.[18] The values showed an average of 11,543 mg/m^3 (range 9745–12,575 mg/m^3). Three batches were tested for particle size distribution by laser diffraction. Results showed that 3% of the particles of the additive have a diameter below 10 µm, 16% below 50 µm, 28% below 100 µm and the vast majority is between 400 and 2000 µm.[19]

No new data were provided regarding the shelf life and solubility in water of the additive. Since no changes were introduced in the additive manufacturing process, the data described in the previous opinion (EFSA FEEDAP Panel, 2013) still apply.

3.1.2 | Characterisation of the active agent

The active agent was isolated from tomato pulp and is deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) with the accession number DSM 22501.[20] It has not been genetically modified.

[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]Sect_II_Identity_L.buch_DSM22501_ID+Charact_V2.

[11]Annex_II_3.1d.

[12]Statement_DSM22501.

[13]Data of all the samples provided by the applicant were used to calculate the final average.

[14]Annex_II_1.4.2 and Annex_II_1.4.2_V2; LOQ: As (0.005 mg/kg), Pb (<0.01 mg/kg), and aflatoxin B1 (0.46 µg/kg).

[15]Annex_II_1.3a, Annex_II_1.3a_New_CoAs_DSM22501_V2 and LOQ: coliforms (<17 CFU/g), *Escherichia coli* (<10 CFU/g), yeasts and filamentous fungi (<50 CFU/g).

[16]*Salmonella* spp. was analysed in 5 g samples.

[17]Annex_II_1.4.2a.

[18]Annex_II_1.5a.

[19]Annex_II_1.5.

[20]Annex_II_2.1.2.a.

The taxonomical identification of the strain DSM 22501 was confirmed by average nucleotide identity (ANI) based on the whole genome sequence (WGS) data. The results showed an ANI value of 99.9% with the type strain *L. buchneri* DSM 20057^T.[21]

The susceptibility of *L. buchneri* DSM 22501 to antibiotics was tested using broth dilution method and including the set of antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018). All the minimum inhibitory concentration (MIC) values were below the cut-off values, except for chloramphenicol, which was exceeded by one dilution higher. Exceeding the cut-off by one dilution is considered within the variation of the method, and therefore the strain is considered to be susceptible to all the relevant antibiotics.[22]

The WGS data of the strain were interrogated for the presence of antimicrobial resistance (AMR) genes against the NCBI Bacterial Antimicrobial Resistance Reference Gene and ResFinder databases. The search in ResFinder was performed at nucleotide level while the second database was queried at protein level, applying [redacted] identity and [redacted] coverage as thresholds. No hits of concern were identified.[23]

3.1.3 | Conditions of use

The additive is currently authorised for use in fresh material for all animal species with no maximum content. Under other provisions of the authorisation, it is specified that:

- In the directions for the use of the additive, indicate the storage temperature and storage life.
- Minimum dose of the additive when it is not used in combination with other microorganisms as a silage additive: 1×10^8 CFU/kg of fresh material.
- For safety: It is recommended to use breathing protection and gloves during handling.

The additive should be incorporated directly into the forage after dissolving in water.
The applicant has requested to maintain the same conditions of use.[24]

3.2 | Safety

The applicant declared that no adverse effects for target animals, consumers, users and/or the environment have been reported since the approval of the additive.[25]

In its previous opinion, the FEEDAP 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, 2013). In the context of the current application, the identity of the strain as *L. buchneri* was confirmed, and evidence was provided that the strain does not show antimicrobial resistance genes for antibiotics of human and veterinary importance. Consequently, the conclusions previously reached are still valid, and the Panel considers that *L. buchneri* NCIMB 22501 remains safe for the target species, consumers and the environment.

As supporting evidence, a literature search has been performed (May 2022) in order to provide evidence that in the light of the current knowledge the additive remains safe under the approved conditions for target species, consumers, users and the environment. Four databases were searched (Academic Onefile, Food Science Source, AGRIS and Pubmed).[26] The search terms included the species and strain name of the active agent, and covered the safety for the target species, consumer, user and the environment. A total of 156 references were retrieved that were screened for relevance. From those, three publications were selected as potentially relevant, but after reviewing the full text none regarded any safety issue related to the use additive under assessment in animal nutrition.[27]

The safety for the user was evaluated by the FEEDAP Panel in a previous assessment (EFSA FEEDAP Panel, 2013). The Panel concluded that 'given the proteinaceous nature of the active agents and the high dusting potential of the products tested, the FEEDAP Panel considers it prudent to treat these additives as skin and respiratory sensitisers. They are also considered irritants'.

In the current dossier, no specific studies investigating the effects of the additive on the respiratory system were submitted. The dusting potential reported was up to 12,575 mg/m^3, therefore, the FEEDAP Panel considers that the exposure by inhalation is likely. Owing to the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser.

[21] Annex_II_2.1.2b.
[22] Annex_II_2.2.2c.
[23] Annex_II_2.2.2b.
[24] Sect_II_Identity_L.buch_DSM22501_Cond_of_use.
[25] Statement_DSM22501_2023.
[26] Annex_III.1a-d.
[27] Sect_III_Safety_L.buch_DSM22501_User.

To support the safety of the additive for the users, the applicant has submitted an *in vivo* skin irritation study, an *in vitro* eye irritation study and an *in vivo* dermal sensitisation study. All the trials were conducted with the additive with maltodextrin as a carrier.

The acute skin irritation study was performed according to OECD Test Guideline (TG) 404. The results showed that the additive is not irritant to the skin.[28]

The eye irritation study was performed according to OECD TG 405. The results showed that the additive is not irritant to the eye.[29]

A local lymph node assay was performed to assess the skin sensitisation potential of the additive according to OECD TG 429[30] and the Method B42 Skin sensitisation of Commission Regulation (EC) No 440/2008.[31] The study was claimed to be conducted in compliance with GLP principles. The test results would indicate that the product should be considered to be a dermal sensitiser. However, 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.[32] Therefore, no conclusion can be drawn on the skin sensitisation potential of the additive.

Although the applicant has described only one preparation for this additive (using maltodextrin as a carrier), the Panel notes that once an active agent has been authorised as a technological additive, different preparations can be placed on the market with reference to that authorisation. Consequently, not all preparations can be assessed for user safety. The Panel can only conclude on the final preparation tested.

3.2.1 | Conclusions on safety

The FEEDAP Panel concludes that there is no new evidence that would lead to reconsider the previous conclusions that *Lentilactobacillus buchneri* DSM 22501 is safe for the target species, consumer and the environment under the authorised conditions of use.

Regarding user safety, the additive with maltodextrin as a carrier is not irritant to skin or eyes but should be considered a respiratory sensitiser. No conclusions can be drawn on the potential of the additive to be a dermal sensitiser.

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 terms of authorisation.

The Panel concludes that *Lentilactobacillus buchneri* DSM 22501 remains safe for all animal species, consumer and the environment under the authorised conditions of use.

The additive containing maltodextrin as a carrier tested is not irritant to skin or eyes but should be considered a respiratory sensitiser. No conclusions can be drawn on the potential of the additive to be a dermal sensitiser.

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

ABBREVIATIONS

ANI	average nucleotide identity
CFU	colony forming unit
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
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
TG	Test Guidelines
WGS	whole genome sequence

[28] Annex_III_3.1c.

[29] Annex_III_3.1a.

[30] Annex_III_3.1b.

[31] Available online: https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf

[32] https://www.efsa.europa.eu/sites/default/files/2022-07/feedap20220629-30_m.pdf

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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REFERENCES

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Aquilina, G., Bach, A., Bampidis, V., Bastos, M., Flachowsky, G., Gralak, M., Hogstrand, C., Leng, L., López Puente, S., Martelli, G., Mayo, B., Renshaw, D., Rychen, G., Saarela, M., Sejrsen, K., Van Beelen, P., Wallace, R., & Westendorf, J. (2013). Scientific opinion on the safety and efficacy of *Lactobacillus brevis* (DSM 23231), *Lactobacillus buchneri* (DSM 22501), *Lactobacillus buchneri* (NCIMB 40788—CNCM I-4323), *Lactobacillus buchneri* (ATCC PTA-6138) and *Lactobacillus buchneri* (ATCC PTA-2494) as silage additives for all species. *EFSA Journal*, *11*(4), 3168. https://doi.org/10.2903/j.efsa.2013.3168

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Galobart, J. (2018). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Dusemund, B., Fašmon Durjava, M., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Anguita, M., … Innocenti, M. L. (2021). Guidance on the renewal of the authorisation of feed additives. *EFSA Journal*, *19*(1), 6340. https://doi.org/10.2903/j.efsa.2021.6340

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