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SCIENTIFIC OPINION

Assessment of the feed additive consisting of *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) NCIMB 30139 for all animal species for the renewal of its authorisation (Volac International Ltd)

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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 *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) NCIMB 30139 as a technological additive for use in easy to ensile 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 should be considered as a respiratory sensitiser. The additive is not skin irritant, but no conclusions can be drawn on the skin sensitisation or eye irritation 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 NCIMB 30139, QPS, renewal, silage additives, technological additives

CONTENTS

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 one year before the expiry date of the authorisation.

The European Commission received a request from Volac International Ltd represented in the EU by Volac Feeds Ltd[2] for the renewal of the authorisation of the additive consisting of *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) NCIMB 30139, 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). The dossier was received on 9 March 2022 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00198. The particulars and documents in support of the application were considered valid by EFSA as of 8 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* NCIMB 30139, 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*) NCIMB 30139. EFSA issued an opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2012).

The additive is currently authorised for use in feed for all animal species (1k20734).[3]

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 *L. buchneri* NCIMB 30139 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,[2] EFSA carried out a public consultation on the non-confidential version of the application from 10 January 2023 to 31 January 2023 for which no comments were received.

In addition, the confidential version of the technical dossier was subject to a target consultation of the interested Member States from 24 August 2022 to 24 November 2022; the comments received were considered for the assessment.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA, to deliver the present output.

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 *L. buchneri* NCIMB 30139 in animal feed are valid and applicable for the current application.[7]

[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]Volac International Ltd, United Kingdom represented in the EU by Volac Feeds Ltd, Feagh Mullagh Kells Co. Meath, Ireland

[3]Commission Implementing Regulation (EU) No 96/2013 of 1 February 2013 concerning the authorisation of a preparation of *Lactobacillus buchneri* NCIMB 30139 and of a preparation of *Lactobacillus casei* ATTC PTA 6135 as feed additives for all animal species. OJ L 268, 18.10.2003, p. 29.

[4]Dossier reference: FEED-2021-1912

[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]The full report is available on the EU Science Hub at: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0127025202590280_en

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *L. buchneri* NCIMB 30139 is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3 | ASSESSMENT

The additive consisting of a preparation of *L. buchneri* (formerly *L. buchneri*) NCIMB 30139 is currently authorised as a technological additive (functional group: silage additives) in fresh material for all animal species. This assessment regards the renewal of the authorisation of the above-mentioned additive.

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* NCIMB 30139) of 5×10^{10} colony forming units (CFU)/g of additive. The applicant declared that modifications to the manufacturing process compared to the previous application were performed regarding the cryoprotectant agents used, namely the replacement of dextrose/maltodextrin (maximum 64.5%) by sucrose, glycine and sodium erythorbate (maximum 64.9%, with a ratio of one third each).[9] The Panel notes that sodium erythorbate is authorised for use in the EU as a food additive but not as a feed additive, and is considered not to introduce safety concerns. The product consists of dried cells and 2% sodium aluminosilicate.

Analyses of seven batches showed compliance with the specifications regarding the minimum concentration of active agent (mean: 7.3×10^{11} CFU/g additive, range $5.6–8.9 \times 10^{11}$ CFU/g additive).[10]

Specifications are set for coliforms (< 1000 CFU/g), *Salmonella* spp. (no detection in 25 g), *Escherichia coli* (no detection in 10 g), yeasts and filamentous fungi (< 1000 CFU/g). Analysis of the five recent batches of the additive showed compliance with these specifications.[11] No data on *Enterobacteriaceae* concentration was provided.

Five batches of the additive were tested for aflatoxins (B1, B2, G1 and G2), mercury, lead, cadmium and arsenic concentrations. Results showed values for aflatoxins, lead and arsenic below the limit of quantification (LOQ) of the analytical methods.[12] Mercury was quantified in one batch (0.045 mg/kg) and cadmium was quantified in all five batches (mean 0.11 mg/kg, range 0.04–0.32 mg/kg).[13]

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

The applicant provided new data on the bulk density for seven batches of the additive, with results showing a mean value of 366 kg/m^3 (310–440 kg/m^3).[14] The applicant has also provided new data for the dusting potential and particle size distribution by testing three batches of the additive.[15] Results for the dusting potential by Stauber–Heubach method showed a mean of 3880 mg/m^3 (2940–5080 mg/m^3),[16] while particle size distribution was tested by laser diffraction and results showed that approximately 18.4% of the additive consists of particles with diameters below 100 µm, 12.7% below 50 µm and 5.6% below 10 µm.[17]

New data on the stability of the additive and in premixtures was provided.[18] Three batches of the additive were stored at –18°C in sealed aluminium foil sachets for 24 months. Negligible losses (< 0.5 log) were observed in all samples. Three batches of one premixture (containing the active agent and other microorganism) were stored in aluminium foil sachets at 20°C for 6 months.[19] Negligible losses (< 0.5 log) were observed in all samples.

[8]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.

[9]2.1–2.2 - Section II_Identity and Characterisation of the additive

[10]Annex 2–1-3 L. buchneri NCIMB 30139 _Batch variation and media composition and Supplementary Annex 2–1-3 L. buchneri NCIMB 30139_Composition of media and viability

[11]Annex 2–1-6 EFSA-2021-00004644_Lentilactobacillus buchneri NCIMB 30139, Annex 2–1-7 EFSA-2021-00004644_Lentilactobacillus buchneri NCIMB 30139, Annex 2–1-8 EFSA-2021-00004644_L. buchneri NCIMB 30139, Supplementary Annex 2–1-3 L. buchneri NCIMB 30139_Composition of media and viability and Supplementary Annex 2–1-4 - L. buchneri NCIMB 30139_Purity

[12]Limit of Quantification (LOQ): 0.01 µg/kg for aflatoxin B1, B2, G1 and G2; 0.1 mg/kg for arsenic; 0.05 mg/kg for lead; 0.005 mg/kg for mecury; 0.01 mg/kg for cadmium.

[13]Annex 2–1-10 EFSA-2021-00004650_L. buchneri NCIMB 30139, Annex 2–1-11 EFSA-2021-00004650_L. buchneri NCIMB 30139, Annex 2–1-12 EFSA-2021-00004650_L. buchneri NCIMB 30139 and Supplementary Annex 2–1-4 - L. buchneri NCIMB 30139_Purity

[14]Annex 2–1-3 L. buchneri NCIMB 30139_Batch variation and media composition, Supplementary Annex 2–1-3 L. buchneri NCIMB 30139_Composition of media and viability and Annex 2–1-18 L. buchneri NCIMB 30139_Bulk Density

[15]Annex 2–1-14 L. buchneri NCIMB 30139_Particle Size Distribution, Annex 2–1-16 L. buchneri NCIMB 30139_Dusting Potential and Annex 2–1-15 IFF Report No. 3.814_EFSA-2021-00004643

[16]Annex 2–1-16 L. buchneri NCIMB 30139_Dusting Potential and Annex 2–1-15 IFF Report No. 3.814_EFSA-2021-00004643

[17]Annex 2–1-14 L. buchneri NCIMB 30139_Particle Size Distribution and Annex 2–1-15 IFF Report No. 3.814_EFSA-2021-00004643

[18]2.4 – Section II_stability

[19]Annex 2–4-1 L. buchneri NCIMB 30139 Stability

The stability in water was studied during storage of a suspension containing the active agent at 6.24×10^{11} CFU/L for 48 h at 20°C.[20] Negligible losses (< 0.5 log) were observed in all samples.

3.1.2 | Characterisation of the active agent

The active agent was originally isolated from maize silage and is deposited in the National Collections of Industrial, Food and Marine Bacteria under the collection number NCIMB 30139.[21]

The taxonomical identification was confirmed by Average nucleotide identity (ANI) calculation based on the whole genome sequence (WGS) using fastANI tool (version 1.32). The results of this analysis showed an ANI value of 99.84% with *L. buchneri* ssp. *buchneri* DSM 20057^T.[22]

The susceptibility of the strain to antimicrobials was tested using a broth microdilution method and including the set of antimicrobials recommended by EFSA (EFSA FEEDAP Panel, 2018).[23] All the minimum inhibitory concentration values were equal or fell below the relevant EFSA cut-off values for lactobacilli obligate heterofermentative. Therefore, the strain is considered to be susceptible to all the relevant antimicrobials.

The WGS of the strain was interrogated for the presence of antimicrobial resistance genes in the CARD and NCBI databases using ABRIcate (version 1.0.1) and also in AMRfinderPlus (version 3.10.18).[24] Thresholds used in ABRIcate included 80% identity (at protein level) and no threshold for coverage; the search in AMRfinderPlus was performed at protein level using Hidden-Markov model.[25] No hits of concern were identified.

3.1.3 | Conditions of use

The additive is currently authorised for use in easy to ensile fresh material and under other provisions of the authorisation,[26] it is specified that:

1. In the directions for use of the additive and premixture, indicate the storage temperature and storage life.
2. Minimum dose of the additive when used without combination with other microorganisms as silage additives: 1×10^8 CFU/kg fresh material.
3. The additive shall be used in easy to ensile material.[27]
4. For safety: it is recommended to use breathing protection and gloves during handling.

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

3.2 | Safety

The applicant declared that no adverse effects have been reported since the authorisation of the additive.

In its previous opinion (EFSA FEEDAP Panel, 2012), the Panel concluded that following the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007), the use of this strain in the production of silage was considered safe for target species, consumers and the environment. Regarding user safety, the Panel concluded: '*Although users at the farm level are exposed to silage additives only for a short period of time when preparing the aqueous suspension, their potential to be irritants and/or to act as skin sensitisers cannot be fully excluded. The indicative data on particle size distribution and the proteinaceous nature indicate a possible hazard for those handling the additive of exposure by a respiratory route with a consequent risk of sensitisation*'.

In the context of the current application, the identity of a strain as *L. buchneri* was confirmed and evidence was provided that the strain does not show acquired antimicrobial determinants for antibiotics of human and veterinary importance (EFSA, 2007; EFSA BIOHAZ Panel, 2023). Consequently, the conclusions already reached are still valid and *L. buchneri* NCIMB 30139 is considered safe for the target species, consumers and the environment.

Regarding the user safety, owing to its proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser.

[20]Annex 2–4-2 L. buchneri NCIMB 30139 stability in water

[21]L. buchneri_Updated certificate of deposition

[22]Annex 2–2-3 Summary WGS report Lentilactobacillus buchneri NCIMB 30139

[23]MIC Report L. buchneri NCIMB 30139_Jan 2023

[24]Annex 2-2-3 Summary WGS report Lentilactobacillus buchneri NCIMB 30139

[25]Annex 2-2-3 suplementary information_L. Buchneri NCIMB 30139_AMR, Lentilactobacillus buchneri NCIMB 30139 ARGANNOT, Lentilactobacillus buchneri NCIMB 30139 CARD and Lentilactobacillus buchneri NCIMB 30139 NCBI

[26]Commission Implementing Regulation (EU) No 1119/2012 of 29 November 2012 concerning the authorisation of preparations of *Pediococcus acidilactici* CNCM MA 18/5M DSM 11673, *Pediococcus pentosaceus* DSM 23376, NCIMB 12455 and NCIMB 30168, *Lactobacillus plantarum* DSM 3676 and DSM 3677 and *Lactobacillus buchneri* DSM 13573 as feed additives for all animal species, OJ L 268, 18.10.2003, p. 29.

[27]Easy to ensile forage: >3% soluble carbohydrates in fresh material. As defined in Commission Regulation (EC) No 429/2008 (OJ L 133, 22.5.2008, p. 1).

The applicant has submitted skin and eye irritation studies with the additive under assessment,[28] performed in vitro according to OECD guidelines 404 and 492, respectively, showing that the test item is not a skin irritant. Results were inconclusive for the eye irritation potential. For technological additives, 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 product tested. 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. Therefore, no conclusions can be drawn regarding skin sensitisation potential of the additive.

3.2.1 | Extensive literature search

The applicant performed a literature search to provide evidence that the additive remains safe under the approved conditions for target species, consumers, users and the environment.[29] The literature covered the period 2010–February 2022 and involved seven databases (Google Scholar, Pubmed, AGRIS, USDA, Science Direct, Ingenta and The World Cat Library).[30] The search terms used were *L. buchneri* 'NCIMB 30139' and the strategy followed was very briefly reported. A total of 11 hits were retrieved from Google Scholar and 12 hits from The World Cat Library. Upon deduplication and assessment none were considered relevant because they referred either to the previous FEEDAP opinion on the additive, to other products, to the authorisation of the additive or to its efficacy.

3.2.2 | Conclusions on safety

The FEEDAP Panel concludes that there is no new evidence that would lead it to reconsider the previous conclusions that *L. buchneri* NCIMB 30139 is safe for the target species, consumers and the environment under the authorised conditions of use. The additive, preparation of *L. buchneri* NCIMB 30139, is not irritant to skin, is considered a respiratory sensitiser, but no conclusions can be drawn regarding skin sensitisation or eye irritation potential.

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.

The Panel concludes that *L. buchneri* NCIMB 30139 remains safe for the target species, consumers and the environment under the authorised conditions of use.

Regarding user safety, the additive is not skin irritant, but is considered as a respiratory sensitiser. No conclusions could be drawn on the eye irritancy or 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.

ABBREVIATIONS

ANI	average nucleotide identity
CFU	colony forming unit
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
QPS	qualified presumption of safety
WGS	whole genome sequence

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.

[28] Skin and Eye Irritation Report L. buchneri NCIMB 30139_CONFIDENTIAL.

[29] Section III_Safety.

[30] Annex 3–3-1, Annex 3–3-2 and Annex 3–3-3.

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