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SCIENTIFIC OPINION

Assessment of the feed additive consisting of *Limosilactobacillus fermentum* NCIMB 30169 for all animal species for the renewal of its authorisation (Microferm 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 of renewal of *Limosilactobacillus fermentum* NCIMB 30169 as a technological feed additive (functional group: silage additives) for all animal species. The applicant has provided evidence that the additive currently on the market complies with the existing terms of the authorisation. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive remains safe for all animal species, consumers, and the environment. Regarding user safety, the additive should be considered a skin and respiratory sensitiser. No conclusions can be drawn on the eye irritancy 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

Limosilactobacillus fermentum NCIMB 30169, QPS, renewal, safety, 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 Microferm Ltd.[2] for the renewal of the authorisation of the additive consisting of *Limosilactobacillus fermentum* NCIMB 30169, when used as a feed additive for all animal species (category: technological additive; 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 16 May 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00363. The particulars and documents in support of the application were considered valid by EFSA as of 19 September 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 *Limosilactobacillus fermentum* NCIMB 30169, when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

The additive *Limosilactobacillus fermentum* (previously *Lactobacillus fermentum*) NCIMB 30169 is currently authorised for use in feed for all animal species (1k20739).[3]

EFSA issued one opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2014).

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 *Limosilactobacillus fermentum* NCIMB 30169 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, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 06 March to 27 March 2024 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 19 September 2023 to 19 December 2023 for which no comments were received.

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 *L. fermentum* NCIMB 30169 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]Microferm Ltd., Spring Lane North Malvern Link Worcestershire - United Kingdom, represented in the EU by Marigot Ltd., Strand Farm, Currabinny Carrigaline, Co. Cork - Ireland.

[3]Commission Implementing Regulation (EU) No 399/2014 of 22 April 2014 concerning the authorisation of the preparations of *Lactobacillus brevis* DSM 23231, *Lactobacillus brevis* DSMZ 16680, *Lactobacillus plantarum* CECT 4528 and *Lactobacillus fermentum* NCIMB 30169 as feed additives for all animal species. OJ L 119, 22.04.2014, p. 40.

[4]Dossier reference: FEED-2023-16208.

[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]Evaluation report of 20 April 2012 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 *Limosilactobacillus fermentum* NCIMB 30169 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 *L. fermentum* NCIMB 30169 is currently authorised as a technological additive (functional group: silage additives) for use in fresh material for all animal species. The assessment regards the renewal of the authorisation.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive currently authorised is a preparation containing *L. fermentum* NCIMB 30169 at a minimum concentration of 2.5×10^{10} colony forming units (CFU)/g.

The applicant declared that the manufacturing process has not been modified since the previous authorisation and that no antimicrobials are used during the manufacturing process.[9] [redacted]. Cryoprotectants (skimmed milk powder, sucrose, glycerine [redacted]) are added [redacted].[10]

Analytical data to confirm the specifications were provided for seven batches of the additive showing an average value of *L. fermentum* counts of 1.7×10^{11} CFU/g (range 4.1×10^{10}–7.5×10^{11} CFU/g).[11]

Microbiological contamination was analysed in three batches of the additive by determination of *Salmonella* spp., *Escherichia coli*, *Enterobacteriaceae*, coliforms, yeasts and filamentous fungi. *Salmonella* spp. was not detected in 25 g of product and the values for the other parameters were <10 CFU/g, except for one batch analysed for *Enterobacteriaceae* (10 CFU/g) and one for yeasts (10 CFU/g).[12]

Three batches of the additive were analysed for the presence of cadmium, lead, mercury and arsenic. The following values were obtained: 0.19 mg/kg for cadmium, 0.11 mg/kg for arsenic, 0.011–0.017 mg/kg for lead; the value of mercury was below the limit of detection (LOD) of the analytical method. The same batches were analysed for mycotoxin levels, showing the following results: 0.4–1 μg/kg aflatoxins, 25.1–54.5 μg/kg zearalenone, 81.8–109 μg/kg fumonisins (B1, B2, B3) and 550.4–657.7 μg/kg citrinin. The values of ochratoxin A and deoxynivalenol were below the LOD of the analytical methods.[13]

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

No information on the dusting potential was made available. The applicant referred to data on commercial premixtures containing the additive under assessment and other bacterial strains.[14] The Panel considers these data not to be relevant for the current assessment.

No new data were provided regarding the particle size distribution and the stability and homogeneity of the additive. Considering that no changes have been introduced in the manufacturing process and composition, the data described in the previous opinion (EFSA FEEDAP Panel, 2014) are still valid.

3.1.2 | Characterisation of the active agent

The active agent is deposited in the National Collection of Industrial, Food and Marine Bacteria (NCIMB, United Kingdom) with the accession number NCIMB 30169.[15] It has not been genetically modified.

The taxonomical identification of the active agent was confirmed by [redacted] determination using the whole genome sequence (WGS) data. [redacted]

[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]Sect_2.3 Manufacturing and Annex 2.3.1.d Antimicrobial declaration.

[10]Sect_2.1–2.2 identification and characterisation and Sect_2.3 Manufacturing.

[11]2.1.3 Batch to batch_microbial impurities and 2.1.3.a Additional CoAs 30,169.

[12]2.1.3 Batch to batch_microbial impurities and 2.1.3.b CoA and Enteros.

[13]2.1.4 heavy metals mycotoxins 135,827. LOD: < 0.5 μg/kg for ochratoxin A, <134 μg/kg for deoxynivalenol.

[14]Sect_2.4 Properties.

[15]2.2.2.2.a Certificate of deposition CON31 QF107 NCIMB 30169_April 2023.

[redacted].[16]

The susceptibility of *L. fermentum* NCIMB 30169 to antimicrobials was tested using a broth microdilution method and including the data set of antimicrobials recommended by EFSA (EFSA FEEDAP Panel, 2018).[17] All the minimum inhibitory concentration values were equal to or below the corresponding cut-off values. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The WGS of the strain NCIMB 30169 was interrogated for the presence of antimicrobial resistance (AMR) genes against the [redacted].[18] No hits were identified exceeding the thresholds recommended by EFSA (EFSA, 2021).

3.1.3 | Conditions of use

The additive is currently authorised for use as a silage additive for all animal species. Under other provisions of the authorisation,[19] it is specified that:

- in the directions for use of the additive and premixture, indicate the storage conditions.
- minimum content of the additive when used without combination with other micro-organisms as silage additives: 1×10^8 CFU/kg fresh material.
- for safety: it is recommended to use breathing protection, eye protection and gloves during handling.

The applicant did not request any change in the current conditions of authorisation.[20]

3.2 | Safety

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 the target species, consumers and the environment (EFSA FEEDAP Panel, 2014). Regarding user safety, the Panel concluded that the additive may cause irritation to skin/eyes and should be considered as a skin and respiratory sensitiser.

The applicant declared that no incidents or safety issues for the target species, consumers, users and the environment have been documented or reported since the approval of the additive.[21]

In the context of the current application, in line with the requirements of the QPS approach for safety assessment (EFSA BIOHAZ Panel, 2023), the identity of the strain as belonging to *L. fermentum* was confirmed, and evidence that the strain is not resistant to antibiotics of human and veterinary importance was provided. Consequently, the conclusions previously reached are still valid, and the Panel considers that *L. fermentum* NCIMB 30169 remains safe for the target species, consumers and the environment.

No specific data have been submitted on the effects of the additive on user safety. Considering the nature of the additive, the FEEDAP Panel concludes that the additive should be considered as a potential skin and respiratory sensitiser, and any exposure through skin and respiratory tract is considered a risk. In the absence of data, no conclusion can be reached on the eye irritation potential of the additive.

3.2.1 | Conclusions on the safety

The FEEDAP Panel concludes that *L. fermentum* NCIMB 30169 remains safe for the target species, consumers and the environment. Considering the nature of the additive, the FEEDAP Panel concludes that the additive should be considered as a potential skin and respiratory sensitiser, and any exposure through skin and respiratory tract is considered a risk. The Panel cannot conclude on the eye irritation potential of the additive.

[16] 2.2.2.2.b Bioinformatic NCIMB 30169 T1473R1493.1_2022.

[17] 2.2.2.2.c1 Microferm Ltd. ID21867 pheno AST and 2.2.2.2.c2 Microferm Ltd. ID21867 pheno AST results.

[18] 2.2.2.2.b Bioinformatic NCIMB 30169 T1473R1493.1_2022.

[19] Commission Implementing Regulation (EU) No 399/2014 of 22 April 2014 concerning the authorisation of the preparations of *Lactobacillus brevis* DSM 23231, *Lactobacillus brevis* DSMZ 16680, *Lactobacillus plantarum* CECT 4528 and *Lactobacillus fermentum* NCIMB 30169 as feed additives for all animal species. OJ L 119, 22.04.2014, p. 40.

[20] Sect_2.5 Conditions of use.

[21] 3.2.a consumer safety, 3.4.a Environmental safety, 3.1.a target animal safety and 3.3.a User safety.

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 *Limosilactobacillus fermentum* NCIMB 30169 remains safe for all animal species, consumers and the environment. Regarding the user safety, the additive should be considered as a potential skin and respiratory sensitiser, and any exposure through the skin and respiratory tract is considered a risk. No conclusions can be drawn on the eye irritancy 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

AMR	antimicrobial resistance
CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
WGS	whole genome sequence

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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.

REQUESTOR

European Commission

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