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SCIENTIFIC OPINION

Assessment of the feed additive consisting of *Enterococcus lactis* NCIMB 10415 (Cylactin®) for cats and dogs for the renewal of its authorisation (DSM Nutritional Products Ltd.)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) | **Vasileios Bampidis** | **Giovanna Azimonti** | **Maria de Lourdes Bastos** | **Henrik Christensen** | **Mojca Durjava** | **Birgit Dusemund** | **Maryline Kouba** | **Marta López-Alonso** | **Secundino López Puente** | **Francesca Marcon** | **Baltasar Mayo** | **Alena Pechová** | **Mariana Petkova** | **Fernando Ramos** | **Roberto Edoardo Villa** | **Ruud Woutersen** | **Paul Brantom** | **Natalia Alija-Novo** | **Montserrat Anguita** | **Rosella Brozzi** | **Jaume Galobart** | **Matteo Lorenzo Innocenti** | **Fabiola Pizzo** | **Joana Revez** | **Nicole Bozzi Cionci**

Correspondence: feedap@efsa.europa.eu

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 Cylactin® as a zootechnical feed additive for cats and dogs. The active agent of the additive is *Enterococcus lactis* NCIMB 10415 and the micro-encapsulated formulation, Cylactin® LBC ME5 PET, was assessed. The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation. The Panel concluded that the additive remains safe for cats and dogs. Regarding user safety, the additive was not shown to be skin and eye irritant, but it should be considered a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation. There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

KEYWORDS

Cylactin®, *Enterococcus lactis* NCIMB 10415, gut flora stabilisers, other zootechnical additives, zootechnical 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 1 year before the expiry date of the authorisation.

The European Commission received a request from DSM Nutritional Products Ltd.[2] for the renewal of the authorisation of the additive consisting of *Enterococcus lactis*[3] NCIMB 10415 (Cylactin®), when used as a feed additive for cats (category: zootechnical additive; functional group: gut flora stabilisers) and dogs (category: zootechnical additive; functional group: other zootechnical additives (improvement of gut conditions)).

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). 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 03 March 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 *E. lactis* NCIMB 10415 (Cylactin®), when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

The additive is a preparation containing *E. lactis* (previously identified as *Enterococcus faecium*) NCIMB 10415 (Cylactin®).[4] EFSA has issued several opinions on the safety and efficacy of this product (EFSA, 2004; EFSA FEEDAP Panel, 2010a, 2010b, 2013a, 2013b, 2014, 2015, 2018a, 2023). Additionally, EFSA has issued one opinion on the preparation of viable cells of *Enterococcus lactis* NCIMB 10415 as a silage additive for all animal species (EFSA FEEDAP Panel, 2013c).

The additive Cylactin® is authorised in different formulations, and herein one of the micro-encapsulated formulation, Cylactin® LBC ME5 PET, is assessed.

The additive is authorised for use in feed for chickens for fattening,[5] chickens reared for laying, minor poultry species for fattening and minor poultry species reared for laying,[6] sows, suckling piglets, weaned piglets and pigs for fattening[7] and calves, kids, cats and dogs[8] (4b1705). In addition, the additive is authorised for use for all animal species as a technological additive (functional group: silage additives) (1k20601).[9]

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[10] in support of the authorisation request for the use of *E. lactis* NCIMB 10415 (Cylactin®) as a feed additive. The dossier was received on 21 November 2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00817.

[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]DSM Nutritional Products Ltd, Wurmisweg 576, CH-4303 Kaiseraugst. Switzerland.

[3]Formerly identified as *Enterococcus faecium*.

[4]The additive has also the trade name of Cernivet®.

[5]Commission Implementing Regulation (EU) No 361/2011 of 13 April 2011 concerning the authorisation of *Enterococcus faecium* NCIMB 10415 as a feed additive for chickens for fattening (holder of authorisation DSM Nutritional products Ltd represented by DSM Nutritional Products Sp. Z.o.o) and amending Regulation (EC) No 943/2005. OJ L 100, 14.4.2011, p. 22.

[6]Commission Implementing Regulation (EU) 2015/518 of 26 March 2015 concerning the authorisation of the preparation of *Enterococcus faecium* NCIMB 10415 as a feed additive for chickens reared for laying, minor poultry species for fattening and minor poultry species reared for laying and amending Implementing Regulation (EU) No 361/2011 as regards the compatibility with coccidiostats (holder of the authorisation DSM Nutritional Products Ltd represented by DSM Nutritional products Sp. z o.o). OJ L 82, 27.3.2015, p. 75.

[7]Commission Implementing Regulation (EU) 2019/11 of 3 January 2019 concerning the authorisation of the preparation of *Enterococcus faecium* NCIMB 10415 as a feed additive for sows, suckling piglets, weaned piglets, pigs for fattening and amending Regulations (EC) No 252/2006, (EC) No 943/2005 and (EC) No 1200/2005 (holder of authorisation DSM Nutritional products Ltd, represented by DSM Nutritional Products Sp. z o.o.). OJ L 2, 04.01.2019, p. 17.

[8]Commission Implementing Regulation (EU) No 1061/2013 of 29 October 2013 concerning the authorisation of a preparation of *Enterococcus faecium* NCIMB 10415 as a feed additive for calves, kids, cats and dogs and amending Regulation (EC) No 1288/2004 (holder of the authorisation DSM Nutritional Products Ltd represented by DSM Nutritional products Sp. Z o.o). OJ L 289, 31.10.2013, p. 38.

[9]Commission Implementing Regulation (EU) No 304/2014 of 25 March 2014 concerning the authorisation of the preparations of *Enterococcus faecium* NCIMB 10415, *Enterococcus faecium* DSM 22502 and *Pediococcus acidilactici* CNCM I-3237 as feed additives for all animal species. OJ L 90, 26.03.2014, p. 8.

[10]Dossier reference: FEED-2022-11290.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 3 March 2023 to 3 June 2023 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[11] 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,[12] 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 11 September to 02 October 2023 for which no comments were received.

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

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety of *E. lactis* NCIMB 10415 (Cylactin® LBC ME5 PET) is in line with the principles laid down in Regulation (EC) No 429/2008[13] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3 | ASSESSMENT

The product Cylactin® LBC ME5 PET, consisting of viable cells of *E. lactis* NCIMB 10415, is currently authorised as a zootechnical additive for use in feed for cats (functional group: gut flora stabilisers) and dogs (functional group: other zootechnical additives (improvement of gut conditions)). This assessment regards the renewal of the authorisation of *E. lactis* NCIMB 10415 for its use in cats and dogs.

3.1 | Characterisation

3.1.1 | Characterisation of the active agent

The active agent *E. lactis* NCIMB 10415 has been characterised and described in a recent opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2023). The strain was unambiguously identified, and the applicant submitted all the necessary data to characterise the strain in all aspects required as per the Guidance (EFSA FEEDAP Panel, 2018b), including the absence of genes of concern and antibiotic susceptibility. The identity of NCIMB 10415 strain was reassigned to *E. lactis*, and evidence was provided that the strain does not harbour acquired antimicrobial resistance (AMR) genes or is virulent.

3.1.2 | Characterisation of the additive

The additive Cylactin® LBC ME5 PET currently authorised is specified to contain *E. lactis* NCIMB 10415 at a minimum concentration of 5×10^9 colony forming unit (CFU)/g, available in a microencapsulated form with shellac. The analysis of five recent independent batches showed compliance with the specifications of the active agent (mean 4.8×10^{10} CFU/g and range $4.2–5.3 \times 10^{10}$ CFU/g).[14]

The additive contains *E. lactis* NCIMB 10415 (1.8%) with 76.5% saccharose, as a carrier, and 8.7% hydroxypropyl methylcellulose and 13% '[redacted] shellac' (E 904),[15] used as coating materials.[16] The Panel notes that shellac is not authorised as a feed additive.[17]

Specifications were set by the applicant for *Enterobacteriaceae* (≤ 10 CFU/g), *Salmonella* spp. (no detection in 25 g), total aerobic microbial count (≤ 10^3 CFU/g), total filamentous fungi and yeasts (≤ 10^2 CFU/g), *Escherichia coli* (no detection in 1 g)

[11]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.

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

[13]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.

[14]Annex 2.1–3 CoA 5 batches variation and Annex ADR1.2 CoA C02227_batch variation ME5 PET_2022.

[15]Annex 2.1–5 EP_Resin_2019, Annex 2.1–6 EU_FA_Resin_2020, ADR2-2 Jasti_Shellac_HPE_Not public_Paid access, Annex 2.3–4 MSDS materials_ME5 PET_2022, ADR3_2_CoA Shellac.

[16]Annex 2.1–1 Comp LBC ME5 PET_2016_non_conf, Annex 2.1–4 EP_Cellulose derivative_2019.

[17]The FEEDAP Panel notes that shellac is currently under re-evaluation by the Panel on Food Additives and Flavourings (FAF) as a food additive.

and *Pseudomonas aeruginosa* (no detection in 1 g).[18] The analysis of seven independent batches showed compliance with these specifications.

Three recent and independent batches were analysed for arsenic, lead, cadmium, mercury, aflatoxins B1, B2, G1 and G2, deoxynivalenol and zearalenone concentration. All the analyses showed values below the limit of detection (LOD) of the analytical methods, except for those on lead, ranging between 0.012 and 0.024 mg/kg.[19]

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

The composition and the manufacturing process of the additive have not been changed since its last authorisation (EFSA FEEDAP Panel, 2013a), [redacted][20] Therefore, the data on physical–chemical properties, shelf-life and stability previously submitted are considered still valid. However, the applicant provided a new study to investigate the capacity of the additive to homogeneously distribute in feed that is described below.[21]

The additive (one batch) was mixed with a dry complementary feed for pets at an inclusion rate of 2.5% and 32 subsamples were collected and analysed for *E. lactis* counts. The coefficient of variation was 25%.[22]

3.1.3 | Conditions of use

The additive is authorised for use in complete feedingstuffs for cats and dogs at a minimum content of 7×10^9 and 2.5×10^9 CFU/kg, respectively.

Under other provisions of the authorisation,[23] it is specified that:

- In the directions for use of the additive and premixture, indicate the storage conditions and stability to pelleting.

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

3.2 | Safety

In the previous opinion (EFSA FEEDAP Panel, 2013a), it was concluded that Cylactin® LBC ME5 PET is safe for cats and dogs. In regard to the user safety, the FEEDAP Panel concluded: 'Cylactin® LBC ME20, a more concentrated formulation of Cylactin® (2×10^{10} CFU/g additive) containing the same excipients, was shown not to be a skin/eye irritant or a skin sensitiser in tests performed following OECD Guidelines. The FEEDAP Panel does not expect the formulation under assessment to behave differently. As this formulation has a large particle size and the dusting potential is low, the potential for exposure via respiratory routes is considered minimal'.

The applicant declared that no incidents or safety issues for target animals and users have been documented or reported regarding the active agent since its first authorisation.[24]

In the context of a recent application (EFSA FEEDAP Panel, 2023), the identity of NCIMB 10415 strain was reassigned to *E. lactis*, and evidence was provided that the strain does not harbour acquired AMR genes or is virulent. The FEEDAP Panel considers the criteria to assess the safety of *E. faecium* applicable also to *E. lactis* strains. In addition, the manufacturing process of the additive, its composition and the conditions of use for the target species have not been modified.

The additive contains 13% shellac, which is not authorised as a feed additive, but as a food additive (E 904). The Panel notes that the tolerance studies originally assessed in 2013 (EFSA FEEDAP Panel, 2013a) included groups supplemented with the additive containing shellac at levels of 70-fold (cats) or 100-fold (dogs) the originally proposed maximum use levels. No adverse effects were observed in any of the studies, which support the safety of shellac in the target species.

In line with the requirements established in the EFSA guidance on the renewal of authorisation of feed additives (EFSA FEEDAP Panel, 2021), the applicant also performed a literature search in order to provide evidence that, in the light of the current knowledge, the additive remains safe under the approved conditions for the target species and users. The literature search covered the period from 2013 to 2022. Three databases were searched (MEDLINE, TOXCENTER, EMBASE). The search terms included the species of the active agent, the strain name, trade name and covered the safety for the target species and user. A total of 52 references were retrieved and screened for relevance. Exclusion and inclusion criteria were

[18] Annex 2.1–3 CoA 5 batches variation and Annex 2.1–7 CoA 3 batches impurities_ME5 PET_2022

[19] Annex 2.1–7 CoA 3 batches impurities. LOD: arsenic (0.0067 mg/kg), lead, cadmium and mercury (0.0017 mg/kg), aflatoxins B1, B2, G1 and G2 (0.001 mg/kg), deoxynivalenol (0.02 mg/kg) and zearalenone (0.01 mg/kg).

[20] Annex 2.3_Sect II_Manufacturing process and Annex ADR1.1_Reply to ADR Q1_Manufprocess_2023.

[21] Annex 2.4–6 Nestle_SF68 homogeneity complemfeed_2022_boxmarked.

[22] Annex 2.4–6 Nestle_SF68 homogeneity complemfeed_2022_boxmarked.

[23] Commission Implementing Regulation (EU) No 1061/2013 of 29 October 2013 concerning the authorisation of a preparation of *Enterococcus faecium* NCIMB 10415 as a feed additive for calves, kids, cats and dogs and amending Regulation (EC) No 1288/2004 (holder of the authorisation DSM Nutritional Products Ltd represented by DSM Nutritional products Sp. Z o.o). OJ L 289, 31.10.2013, p. 38.

[24] Annex 3.3–3 Cerbios_Physician assessment_2022 and Annex ADR1.3 Signed statement_safety cats dogs_2023.

reported. From these, 25 publications were selected and scrutinised but none was further considered since they were either EFSA opinions or did not regard any safety assessment related to the use of the additive in animal nutrition.[25]

Considering all the above, the Panel concludes that Cylactin® LBC ME5 PET remains safe for cats and dogs.

Regarding the safety for the user, no new data has been provided for the irritancy potential, and therefore, the FEEDAP Panel reiterates its previous conclusion that the additive is not irritant to skin or eyes. Owing to the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser. 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 conclusion can be drawn on the skin sensitisation potential of the additive.

The additive is authorised for use in feed for cats and dogs, and therefore, there is no need to perform an assessment of the safety for the consumer and the environment.

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 Cylactin® LBC ME5 PET is safe for the target species. The additive is not considered to be irritant to the skin and eyes. No conclusions can be drawn on the skin sensitisation potential of the additive, but it should be considered a respiratory 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.

There is no evidence that would lead the FEEDAP Panel to reconsider its previous conclusions. Thus, the Panel concluded that the additive remains safe for cats and dogs. Regarding user safety, the additive is not a skin or eye irritant, but it should be considered a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation.

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

ABBREVIATIONS

CFU	colony forming unit
FAF	Food Additives and Flavourings
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
OECD	Organisation for Economic Co-operation and Development

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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

QUESTION NUMBER

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[25]Annex 3.3–4 ExtLitSearch_Cylactin_cats dogs_2022.

PANEL MEMBERS

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

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