SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of *Enterococcus faecium* NBIMCC 8270, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBIMCC 8244 and *Streptococcus thermophilus* NBIMCC 8253 (Probiotic Lactina®) for cats and dogs (Lactina Ltd.)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Maria Saarela, Montserrat Anguita, Jaume Galobart, Elisa Pettenati, Joana Revez, Jordi Ortuño, Jordi Tarrés and Rosella Brozzi

Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of the feed additive consisting of *Enterococcus faecium* NBIMCC 8270, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBIMCC 8244 *and Streptococcus thermophilus* NBIMCC 8253 (Probiotic Lactina®) when used as a zootechnical additive for cats and dogs. The Panel concluded that Probiotic Lactina® is safe for the target species at the proposed conditions of use. The Panel also concluded that Probiotic Lactina® is irritant to skin and eyes and a respiratory sensitiser, but in the absence of data, no conclusions could be reached on its skin sensitisation potential. No conclusions could be drawn on the efficacy of Probiotic Lactina® for dogs and cats based on the data available.

Keywords: zootechnical additives, gut flora stabiliser, Probiotic Lactina®, safety, efficacy, cats, dogs

Requestor: European Commission

Question number: EFSA-Q-2020-00690

Correspondence: feedap@efsa.europa.eu

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

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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 Lactina Ltd.[2] for the authorisation of the additive consisting of *Enterococcus faecium* NBIMCC 8270, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. bulgaricus NBIMCC 8244 and *Streptococcus thermophilus* NBIMCC 8253 (Probiotic Lactina®) for cats and dogs (category: Zootechnical additives; functional group: Gut flora stabilisers).

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 particulars and documents in support of the application were considered valid by EFSA as of 19 March 2021.

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. faecium* NBIMCC 8270, *L. acidophilus* NBIMCC 8242, *L. helveticus* NBIMCC 8269, *L. delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBIMCC 8244 and *S. thermophilus* NBIMCC 8253 (Probiotic Lactina®), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The subject of the assessment is the feed additive consisting of viable cells of six strains of lactic acid bacteria (LAB) intended for use as a zootechnical additive (functional group: gut flora stabilisers) for cats and dogs.

EFSA has issued several opinions on the use of this additive in chickens for fattening, piglets (suckling and weaned), pigs for fattening and suckling and weaned rabbits (EFSA FEEDAP Panel, 2008, 2013, 2019, 2022).

The additive is currently authorised for use in feed for suckling piglets (4b1891).[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 Probiotic Lactina® as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports, 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 active agents in animal feed are valid and applicable for the current application.[5]

[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, pp. 29.
[2] Lactina Ltd., Sofia str. 101, 1,320 Bankya, Bulgaria.
[3] Commission Implementing Regulation (EU) No 1077/2013 of 31 October 2013 concerning the authorisation of a preparation of *Enterococcus faecium* NBIMCC 8270, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus delbrueckii* ssp. *lactis* NBIMCC 8250, *Lactobacillus delbrueckii* ssp. *bulgaricus* NBIMCC 8244, and *Streptococcus thermophilus* NBIMCC 8253 as a feed additive for suckling piglets (holder of authorisation Lactina Ltd). OJ L 292, 1.11.2013, pp. 3.
[4] FEED dossier reference: FAD-2019-0084.
[5] The full report is available on the EU Science Hub website: https://joint-research-centre.ec.europa.eu/publications/fad-2017-0003_en

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Probiotic Lactina® is in line with the principles laid down in Regulation (EC) No 429/2008[6] 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 identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).

3. Assessment

The subject of the assessment is a product consisting of viable cells of six strains of LAB, *L. acidophilus* NBIMCC 8242, *L. delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBICCM 8244, *L. helveticus* NBIMCC 8269, *S. thermophilus* NBIMCC 8253 and *E. faecium* NBIMCC 8270, tradename Probiotic Lactina®, intended for use as a zootechnical additive (gut flora stabiliser) in feeds for cats and dogs. The additive will be referred to as Probiotic Lactina®.

3.1. Characterisation

3.1.1. Characterisation of the active agents

The additive Probiotic Lactina® consists of six non-genetically modified strains of LAB deposited at the Bulgarian National Bank of Industrial Microorganisms and Cell Cultures (NBIMCC) as follows: *L. acidophilus* NBIMCC 8242, *Lactobacillus delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBIMCC 8244, *L. helveticus* NBIMCC 8269, *S. thermophilus* NBIMCC 8253 and *E. faecium* NBIMCC 8270[7].

The taxonomical identification of the strains was confirmed with whole genome sequence (WGS)-based analyses. The average nucleotide identity (ANI) values of the active agents were 99.98% for NBIMCC 8242[8], 98.38% for NBIMCC 8250[9], 99.21% for NBIMCC 8244[10], 99.07% for NBIMCC 8269[11], 98.28% for NBIMCC 8253[12] and 99.2% for NBIMCC 8270[13] with the type strains of the respective species. In addition, Average Amino Acid Identity (AAI) and single nucleotide polymorphism (SNP) analysis were also performed for strains NBIMCC 8242, 8,250, 8,244 and 8,269, corroborating the previous results.

The antimicrobial susceptibility of the bacterial strains to the antibiotics recommended by EFSA (EFSA FEEDAP Panel, 2018b) was assessed by broth microdilution method. With two exceptions, all the minimum inhibitory concentration (MIC) values found were below the FEEDAP cut-off values for the respective species (EFSA FEEDAP Panel, 2018b).[14] The exceptions were the MICs for kanamycin (32 vs. 16 μg/ml) and erythromycin (2 vs. 1 μg/ml) in strain NBIMCC 8244, which were both one dilution higher than the cut-off value. Exceeding the cut-off values by one dilution is considered to fall

[6] 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, pp. 1.

[7] Technical dossier/Section II/Annex II-10 and Supplementary information December 2021/Certificates of deposit: Annex II-10.

[8] Technical dossier/Supplementary information December 2021/Annex II_44_whole genome sequence_L. bulgaricus NBICCM 8244 Annex II_43 L. bulgaricus NBICCM 8244 and Annex II_45 and Annex II_46.

[9] Technical dossier/Supplementary information December 2021/Annex II_52_whole genome sequence_L. lactis NBICCM 8250, Annex II_51 L. lactis NBICCM 8250, and Annex II_53 and Annex II_54.

[10] Technical dossier/Supplementary information December 2021/Annex II_56_whole genome sequence_L. bulgaricus NBICCM 8244, Annex II_55_ *L. bulgaricus*_NBICCM 8244 and Annex II_57 (fastq) and Annex II_58.

[11] Technical dossier/Supplementary information December 2021/Annex II_48_whole genome sequence_*L. helveticus*_NBICCM 8269, Annex II_47_ *L. helveticus*_NBICCM 8269 (fasta), and Annex II_49 and Annex II_50.

[12] Technical dossier/Supplementary information December 2021/Annex II_60_whole genome sequence_*S. thermophilus* NBIMCC 8253, Annex II_59 *S. thermophilus* NBIMCC 8253 and Annex II_61 and Annex II_62.

[13] Technical dossier/Supplementary information December 2021/Annex II_40_whole genome sequence *E. faecium* NBIMCC 8270, Annex II_39 *E. faecium* NBIMCC 8270, Annex II_41, Annex II_42 and FAD-2019-0084_SIn_270122/Enterococcus faecium 8,270.

[14] Technical dossier/Section II/ Annex_II_23_report_antimicrobial_susceptibility_strains and Annex_II_24_report_antimicrobial_susceptibility_enterococcus_faecium.

within the normal variation of the method, and thus, does not raise concerns for safety. Therefore, all bacterial strains are considered to be susceptible to all the relevant antimicrobials.

The WGS data of the strains were screened for antimicrobial resistance (AMR) genes using ABRicate tool with the NCBI Bacterial Antimicrobial Resistance Reference Gene Database.[15] The thresholds used were 70% identity and 60% coverage, at nucleotide level. No hits of concern were identified in any of the strains. Although only one database was searched, considering the susceptibility of these strains to the tested antibiotics, and the fact that the interrogation did not identify relevant hits, the overall data seem to indicate that strains under assessment are free from AMR genes.

The strain *E. faecium* NBIMCC 8270 is susceptible to ampicillin and the genetic markers IS*16*, *esp*, *hyl*Efm were not detected by polymerase chain reaction.[16] The Panel notes that the analyses were not carried out using the WGS data of the strain, however, the results indicate the absence of the genetic traits associated with virulence in human clinical isolates.

3.1.2. Characterisation of the additive

The additive under assessment is a mixture of *L. acidophilus* NBIMCC 8242, *L. delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBICCM 8244, *L. helveticus* NBIMCC 8269, *S. thermophilus* NBIMCC 8253 and *E. faecium* NBIMCC 8270 in a 1:1:1:1:1:1 ratio on a colony forming units (CFU)/g basis, with a total minimum content of 5×10^9 CFU/g. It has the same manufacturing process as that considered in previous applications (EFSA FEEDAP Panel, 2013, 2019). Since then, the formulation (60% freeze-dried LAB; spent medium and cryoprotectants; 20% polydextrose; 10% inulin and 10% calcium carbonate) has not changed except for the replacement of glucose with polydextrose (EFSA FEEDAP Panel, 2013, 2019). Thus, the data pertaining to impurities, physical properties are still valid. However, some new information has been provided in the current dossier which is described below.

Analytical data to confirm the specifications (CFU of total LAB) were provided for six recent batches of the additive, showing an average value of 5.3×10^9 CFU/g (range $5.1–5.7 \times 10^9$ CFU/g).[17] No data based on species specific counts were provided.

Microbiological contamination was analysed in three batches by the determination of Enterobacteriaceae, *Staphylococcus aureus*, yeasts and filamentous fungi with no detection in 1 g and of *Salmonella* spp., with no detection in 25 g of additive.[18]

Three batches of the additive were tested for the presence of mercury (Hg), lead (Pb), arsenic (As), cadmium (Cd), and copper (Cu). Results showed the following value ranges: 0.0073–0.0084 mg Hg/kg, 0.030–0.046 mg Pb/kg, 0.240–0.394 mg As/kg, < 0.00045[19]–0012 mg Cd/kg, and 0.625–0.779 mg Cu/kg.[20] The analysis of aflatoxin M1 in one additional batch showed a value of < 0.05 μg/kg.[21,22]

The detected amounts of the aforementioned impurities do not raise safety concerns.

The dusting potential of the additive was determined in three recent batches using the Stauber–Heubach method and showed values on average of 3,155 mg/m^3 (range 2,810–3,515 mg/m^3).[23]

[15] Technical dossier/Supplementary information December 2021/Annex II_44_whole genome sequence_L. bulgaricus NBICCM 8244, Annex II_52_whole genome sequence_L. lactis NBICCM 8250, Annex II_56_whole genome sequence_L. bulgaricus NBICCM 8244, Annex II_48_whole genome sequence_L. helveticus_NBICCM 8269, Annex II_60_whole genome sequence_ *S. thermophilus* NBIMCC 8253 and Annex II_40_whole genome sequence *E. faecium* NBIMCC 8270.
[16] Technical dossier/Section II/Annex II-13.
[17] Technical dossier/Section II/Annex_II_20_COA_third_trial, Annex_II_18_COA_first_trial and Annex_II_19_COA_second_trial_ homogeneity/Supplementary information December 2021/EFSA cats and dogs/Annex_II_29_COA_first_trial_homogeneity_ cats, Annex_II_30_COA_second_trial_homogeneity_cats and Annex_II_31_COA_third_trial_homogeneity_cats.
[18] Technical dossier/Section II/Annex_II_18_COA_first_trial, Annex_II_19_COA_second_trial_homogeneity and Annex_II_20_ COA_third_trial.
[19] No information has been provided on whether 0.00045 mg/kg refers to the limit of detection (LOD) or quantification (LOQ) of the analytical method.
[20] Technical dossier/Section II/Annex_II_1_heavy_metals_contaminants. No information on the LOD/LOQ of the methods to determine the of Hg, Pb, As, Cd, and Cu concentrations was provided.
[21] Technical dossier/Section II/Annex_II_3_microbiological_purity_toxins.
[22] Not specified whether it is the limit of detection or quantification.
[23] Technical dossier/Section II/Annex_II_8_dusting_potential.

3.1.3. Stability and homogeneity

The shelf life of the additive (three batches from 2017–2018) was studied when stored at 18°C/30% relative humidity (%RH) in a multilayer packaging containing oriented polypropene, aluminium and polythene up to 12 months. Bacterial counts based of the individual strains and total LAB were identical in all samples and at all times.[24]

The stability of the same three batches of the additive in a commercial feed for dogs (composition provided) was studied at month intervals when supplemented at 2.5–2.6 $\times$ 10^9 CFU/kg feed and stored at 23°C/70% RH (packaging not described) for up to 6 months. LAB counts were identical at all the sampling times for the three batches.[25]

The homogeneous distribution of the additive (three batches) in feed for dogs (inclusion level 0.5%) was studied in thirty subsamples (10 per batch). The coefficient of variation was < 2%.[26]

3.1.4. Conditions of use

The additive is intended for use in feed for dogs and cats at a proposed minimum use level of 2.5 $\times$ 10^9 CFU/kg feed, equivalent to 500 mg additive/kg feed.[27] The applicant states that Probiotic Lactina should not be used in premixtures.

3.2. Safety

3.2.1. Safety for the target species

The species *L. acidophilus*, *L. delbrueckii*, *L. helveticus* and *S. thermophilus* are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establishing safety for the target species (EFSA, 2007; EFSA BIOHAZ Panel, 2020). In the view of the FEEDAP Panel, the identification of the strains and compliance with the QPS qualifications were confirmed. Therefore, the Panel concludes that *L. acidophilus* NBIMCC 8242, *L. helveticus* NBIMCC 8269, *L. delbrueckii* ssp. *lactis* NBIMCC 8250, *L. delbrueckii* ssp. *bulgaricus* NBIMCC 8244 and *S. thermophilus* NBIMCC 8253 can be presumed safe for the target animals. This presumption does not extend to the sixth strain (*E. faecium*). However, *E. faecium* is not a recognised pathogen for cats and dogs and the end-products of the metabolism of the species are typical of LAB, and do not raise concerns. Moreover, *E. faecium* NBIMCC 8270 lacks the marker genes associated with human clinical isolates and is susceptible to all relevant antibiotics.

Considering the above and the fact that the additive does not contain excipients of concern, the FEEDAP Panel concludes that Probiotic Lactina® is safe for the target animals.

The applicant submitted tolerance trials with dogs[28] and cats[29] to support the safety of the additive. However, none could be further considered for the assessment due to substantial flaws in the experimental design and reporting: no individual data provided regarding the animals involved in the study (breed, initial body weight, sex, physiological condition, age) and on the measures applied to ensure a homogeneous distribution of the animals to the treatment groups; no adequate information on the trial site (experimental facilities and domestic households in the case of dogs and cats, respectively) and husbandry conditions in which the animals were kept; no details on the number of kennels/houses included in the trial and the number of animals per kennel/house; poor description and justification of the study design (use levels included do not match with the conditions of use); incomplete data on the experimental diets (no proximate analysis, no appropriate description of the method of supplementation of the additive); incomplete battery of observations performed; incomplete/not adequate statistical analysis; poor reporting of results.

3.2.2. Conclusions on safety for target species

Probiotic Lactina® is safe for the target species at the proposed conditions of use.

[24] Technical dossier/Section II/Annex_II_16_stability/Supplementary information December 2021/Annex_II_32_packaging_material_description.
[25] Technical dossier/Section II/Annex_ II_15_stability_in_feeding_stuff/Supplementary information December 2021/Annex_II_32_packaging_material_description.
[26] Technical dossier/Section II/Annex_II_17_report_homogeneity.
[27] Technical dossier/Section II and Supplementary information December 2021/FAD-2019-0084_AppSIn_131221.
[28] Technical dossier/Section II/Annex III_1–8.
[29] Technical dossier/Supplementary information December 2021/Annexes_Sect.III_19–26.

3.2.3. Safety for the user

The dusting potential of the additive (highest value measured 3,515 mg/m^3) indicates that users may be exposed via the respiratory route. Owing to the proteinaceous nature of the active agents, the additive is considered a respiratory sensitiser.

The applicant provided two *in vitro* studies to assess the potential of the additive to be irritant to skin and eyes. The studies were claimed to be performed according to the relevant OECD Guidelines, however, the reporting was very poor and GLP compliance was not indicated.

The results of the *in vitro* skin irritation study (OECD Guideline 439) indicated that the product should be classified as irritant to skin.[30]

Although the results of the *in vitro* ocular irritation study (OECD Guideline 492) indicated that the product should be classified as not irritant to the eyes, considering the positive results in skin irritancy, the Panel concludes that the additive should be considered as irritant to eyes.[31]

No data were provided regarding skin sensitisation.

3.2.4. Conclusions on safety for the user

In the opinion on the use of the additive in suckling and weaned rabbits (EFSA FEEDAP Panel, 2022), the Panel concluded that Probiotic Lactina® is irritant to skin and eyes and in the absence of data, no conclusions can be reached on its skin sensitisation potential. The Panel considers that these conclusions apply also to the current application.

3.3. Efficacy

Three studies in dogs[32] and three in cats[33] have been submitted to support the efficacy of the additive for the target species. However, none could be further considered for the assessment due to substantial flaws in the experimental design and reporting, which were partially shared in all studies and were not clarified despite the requests, including: poor description and justification of the study design (selection of endpoints in relation to the efficacy claim); no individual data provided regarding the animals involved in the study (breed, initial body weight, sex, physiological condition, age) and on the measures applied to ensure a homogeneous distribution of the animals to the treatment groups; insufficient/unclear information on the experimental facilities (dogs)/houses (cats) and the husbandry conditions in which the animals were held; no details on the number of kennels/houses included in the trial and the number of animals per kennel/house; incomplete information on the diet composition (no proximate analysis, no appropriate description of the method of supplementation of the additive); no information on the individual feed intake and body weight of animals; incomplete/not adequate statistical analysis; poor reporting of results.

3.3.1. Conclusions on efficacy

The FEEDAP Panel is not in the position to conclude on the efficacy of the additive for dogs or cats.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[34] and Good Manufacturing Practice.

4. Conclusions

Probiotic Lactina® is safe for the target species at the proposed conditions of use.

[30] Technical dossier/Section IV/Annex_III_11_skin_ irritation_test.
[31] Technical dossier/Section IV/Annex_III_10_ocular_ irritation_test.
[32] Technical dossier/Section IV/Annexes_Sect.IV_first_efficacy_trial_dogs, Annexes_Sect.IV_second_efficacy_trial_dogs and Annexes_Sect.IV_third_efficacy_trial_dogs/Supplementary information December 2021/Annexes_IV_1–17, Annexes_IV_2–14 and Annexes_IV_3–13.
[33] Technical dossier/Supplmenetary information December 2021/Annexes_Sect.IV_first_efficacy_trial_cats, Annexes_Sect.IV_second_efficacy_trial_cats and Annexes_Sect.IV_third_efficacy_trial_cats.
[34] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, pp. 1.

Probiotic Lactina® is considered to be irritant to skin and eyes and a respiratory sensitiser. In the absence of data, no conclusions can be reached on its skin sensitisation potential.

No conclusions can be drawn on the efficacy of Probiotic Lactina® for dogs and cats based on the data available.

5. Documentation provided to EFSA/Chronology

Date	Event
12/10/2020	Dossier received by EFSA. Zootechnical feed additive for pets and other non-food producing animals (dogs). Submitted by Lactina Ltd.
26/10/2020	Reception mandate from the European Commission
19/03/2021	Application validated by EFSA – Start of the scientific assessment
13/04/2021	Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 (for 178) – Scientific assessment suspended. *Issues: characterisation and conditions of use*
26/06/2021	Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 (for 178) – Scientific assessment suspended. *Issues: characterisation, conditions of use and efficacy*
28/01/2022	Reception of supplementary information. Scientific assessment re-started
2/3/2022	Comments received from Member States
	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AAI	average amino acid identity
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
LAB	lactic acid bacteria
MIC	minimum inhibitory concentration
NBIMCC	Bulgarian National Bank of Industrial Microorganisms and Cell Cultures
QPS	qualified presumption of safety
RH	relative humidity
SNP	single nucleotide polymorphism