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 chickens for fattening and suckling and weaned rabbits (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, Montserrat Anguita, Jaume Galobart, Jordi Ortuño 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 Probiotic Lactina®, 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, for chickens for fattening and suckling and weaned rabbits. In a previous opinion, the FEEDAP Panel assessed the safety and the efficacy of the product when used in these target species and concluded that the additive is presumed safe for the target animals, consumers and the environment. Regarding the safety for the user, the Panel could not conclude on the potential of the additive to be irritant to skin and eyes or on its dermal sensitisation potential due to the lack of data. Moreover, the data provided in the previous assessment were not sufficient to conclude on the efficacy of the additive in the target species. In the current assessment, the applicant provided supplementary information to address these flaws. Based on the new studies, the Panel concluded that Probiotic Lactina® is irritant to skin and eyes. In the absence of data, no conclusions could be reached on its sensitisation potential. Due to the absence of appropriate data, no conclusions could be drawn on the efficacy of Probiotic Lactina® for chickens for fattening and suckling/weaned rabbits.

Keywords: zootechnical additives, gut flora stabiliser, Probiotic Lactina®, safety, efficacy, chickens for fattening, suckling/weaned rabbits

Requestor: European Commission

Question number: EFSA-Q-2021-00320

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1. Introduction

1.1. Background as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition, and in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Lactina Ltd., is seeking Community authorisation 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 for chickens for fattening and suckling and weaned rabbits (Table 1).

Table 1: Description of the substances

Category of additive	Zootechnical additive
Functional group of addittive	Gut flora stabiliser
Description	*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
Target animal category	Chickens for fattening and rabbits suckling and weaned
Applicant	Lactina Ltd.
Type of request	New opinion

On 19 March 2019, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the safety for the user and the efficacy of Probiotic Lactina® for chickens for fattening and rabbits (suckling and weaned).

After the discussion with the Member States at a meeting of the Standing Committee on Plants, Animals, Food and Feed (Animal Nutrition section), it was suggested to check for the possibility to demonstrate safety and efficacy of the additive.

The Commission gave the possibility to the applicant to submit complementary information and data in order to complete the assessment and to allow a revision of the Authority's opinion. The new data have been received on 15 June 2020 and were already transmitted to the Authority by the applicant.

In view of the above, the Commission asks the Authority to deliver a new opinion on the preparation 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, as a feed additive for chickens for fattening and suckling and weaned rabbits based on the additional data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2. Additional information

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

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, 2008; EFSA FEEDAP Panel, 2013, 2019).

The additive is currently authorised for suckling piglets (4b1891).[2]

[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] 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, p. 3.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[3] to a previous application on the same product.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of active substance (trade name of the product) is in line with the principles laid down in Regulation (EC) No 429/2008[5] 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 FEEEDAP Panel, 2017) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The additive under assessment is a preparation of viable cells 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 with a total minimum content of 5×10^9 CFU lactic acid bacteria (LAB)/g. The additive is intended to be used as a zootechnical additive (functional group: gut flora stabilisers) in feed for chickens for fattening and suckling and weaned rabbits at the recommended application level of 2.5×10^9 CFU/kg complete feed, to improve performance. It will be hereafter referred to as Probiotic Lactina®.

In previous opinions, the FEEDAP Panel assessed the safety and the efficacy of the product when used in feed for chickens for fattening and weaned and suckling piglets (EFSA, 2013) and suckling and weaned rabbits (EFSA FEEDAP Panel, 2019). From the information/data provided in the assessments, the Panel concluded that the additive is safe for the target species, consumers and the environment. The Panel also considered the product to be a potential respiratory sensitiser but could not conclude on the potential of the additive to be irritant. Moreover, the data provided were not sufficient to conclude on the efficacy of the additive for chickens for fattening, weaned piglets or rabbits.

The applicant has provided new data to fill the gaps identified in the user safety assessment and to complement the information supporting the efficacy of the additive in feed for chickens and rabbits.

3.1. Safety for the user

The applicant provided two *in vitro* studies to assess the potential of the additive to be irritant to skin[6] and eyes.[7] The studies were claimed to be performed according to the relevant OECD test guidelines (TG); however, the reporting was very poor and there was no indication of GLP compliance. The results of the *in vitro* skin irritation study (OECD TG 439) indicated that the product should be classified as irritant to skin. Although the results of the *in vitro* ocular irritation study (OECD TG 492) indicated that the product should be classified as not irritant to the eyes, taking into account the positive results in skin irritancy, the Panel considers that the additive should be considered as an irritant to eyes.

No data were provided regarding skin sensitisation.

Considering the above, the Panel concludes that Probiotic Lactina® is irritant to skin and eyes. In the absence of data, no conclusions can be reached on its skin sensitisation potential.

[3] FEED dossier reference: FAD-2020-0045.
[4] FEED dossier reference: FAD-2017-0003.
[5] 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.
[6] Technical dossier/Section III/Annex_skin_irritation_test and Supplementary information September 2021/ Annex_III_11_skin_ irritation_test.
[7] Technical dossier/Section III/Annex_ocular_irritation_test and Supplementary information September 2021/ Annex_III_10_ocular_ irritation_test.

3.2. Efficacy

3.2.1. Chickens for fattening

In the previous opinion, the Panel assessed three studies in which feed to gain ratio was significantly improved in one study at the recommended inclusion level (2.5×10^9 CFU/kg complete feed) and at 5×10^{11} CFU/kg feed in a second study, compared to control. In the current submission, two new efficacy studies were submitted.[8] However, none could be further considered for the assessment due to substantial flaws in the experimental design and reporting, despite the requests for completion, including: housing conditions (e.g. stocking density, pen dimensions) and experimental design (e.g. number of replicates, distribution of animals, inconsistency in the report of animals' sex) not fully and properly reported; low productive performance not properly justified; and statistical analysis of required outcomes not provided (i.e. average daily gain, feed conversion ratio) or not properly analysed (i.e. the experimental unit used for the body weight analysis was not correct).

Therefore, there is insufficient evidence to conclude on the efficacy of Probiotic Lactina® in chickens for fattening.

3.2.2. Suckling and weaned rabbits

In the previous opinion, the Panel assessed three studies poorly reported and with a weak design due to the small number of replicates included (three does/treatment group). The rabbits receiving the additive showed a significantly greater body weight at the end of the trial in one study at 3.5×10^{11} CFU/kg feed and in another at 7×10^{11} CFU/kg feed, compared to control. In the current submission, two new efficacy studies were submitted.[9] However, none could be further considered for the assessment due to substantial flaws in the experimental design and reporting despite the requests for completion, including: housing conditions (stocking density, pen dimensions) and experimental design (number of replicates, length of experimental phases, mixing of animals from different does) not fully and properly reported; low productive performance not properly justified; and statistical analysis of required outcomes not provided (average daily gain, feed conversion ratio) or not properly analysed (experimental unit used for the body weight analysis was not correct).

Therefore, there is insufficient evidence to conclude on the efficacy of Probiotic Lactina® for suckling and weaned rabbits.

3.2.3. Conclusions on efficacy

No conclusions can be drawn on the efficacy of Probiotic Lactina® for chickens for fattening and rabbits (suckling/weaned) based on the data provided.

4. Conclusions

Based on the new data provided, the Panel concludes that Probiotic Lactina® is irritant to skin and eyes. In the absence of data, no conclusions can be reached on its skin sensitisation potential.

In the absence of appropriate data, no conclusions can be drawn on the efficacy of Probiotic Lactina® for chickens for fattening and suckling/weaned rabbits.

5. Documentation provided to EFSA/Chronology

Date	Event
12/06/2020	Dossier received by EFSA. Probiotic Lactina® for chickens for fattening and suckling and weaned rabbits. Submitted by Lactina Ltd
09/04/2021	Reception mandate from the European Commission
09/04/2021	Application validated by EFSA – Start of the scientific assessment
29/07/2021	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: safety for the user, efficacy*

[8] Technical dossier/20200612/Chickens first trial C1 and second trial C2.
[9] Technical dossier/20200612/Rabbits first trial C1 and second trial C2.

Date	Event
27/09/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
23/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CFU	colony forming unit
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GLP	good laboratory practice
OECD	Organization for Economic Co-operation and Development
TG	technical guidelines