SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 February 2019

doi: 10.2903/j.efsa.2019.5646

Safety and efficacy of Probiotic Lactina® (*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 chickens for fattening and suckling and weaned rabbits

EFSA Panel on Additives and Products or Substances used in Animal Feed (EFSA FEEDAP Panel), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Andrew Chesson, Pier Sandro Cocconcelli, Guido Rychen, Robert John Wallace, Montserrat Anguita, Rosella Brozzi, Jaume Galobart and Maria Saarela

Abstract

Following a request from the European Commission, the European Food Safety Authority 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® for chickens for fattening and rabbits. The additive is a preparation containing viable cells of six strains of lactic acid bacteria intended for use in feed at the proposed dose of 2.5×10^9 CFU/kg complete feedingstuffs. The identity of all of the component strains of Probiotic Lactina® was established in a previous opinion, five of which qualify for the qualified presumption of safety (QPS) approach to safety assessment. As no antibiotic resistance of concern was detected in these strains, following the QPS approach, the use of these five strains in feedingstuffs is presumed safe for target species; consumers of products from animals fed the additive and the environment. The identity and safety of the sixth strain, *Enterococcus faecium* NBIMCC 8270, was also established in the previous and current opinions. The FEEDAP Panel concludes that Probiotic Lactina® is safe for rabbits (suckling and weaned) at the recommended inclusion level of 2.5×10^9 CFU/kg feed and reiterates its former conclusion that the product is safe for chickens for fattening at 1×10^{10} CFU/kg feed. The FEEDAP Panel considers Probiotic Lactina® to be safe for consumers of products derived from treated animals when used at the conditions proposed. In the absence of data, the FEEDAP Panel cannot conclude on the irritancy of Probiotic Lactina® to skin and eyes or on its dermal sensitisation. Owing to the proteinaceous nature of the active agents, the additive is considered to be a potential respiratory sensitiser. No conclusions can be drawn on the efficacy of Probiotic Lactina® for chickens for fattening and rabbits (suckling/weaned).

Keywords: zootechnical additive, Probiotic Lactina®, chickens for fattening, suckling and weaned rabbits, QPS, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2017-00051

Correspondence: feedap@efsa.europa.eu

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

Acknowledgements: The FEEDAP Panel wishes to thank the following for the preparatory work on this scientific output: Montserrat Anguita, Jaume Galobart, Paola Manini, Konstantinos Sofianidis and Maria Vittoria Vettori.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Chesson A, Cocconcelli PS, Rychen G, Wallace RJ, Anguita M, Brozzi R, Galobart J and Saarela M, 2019. Scientific Opinion on the safety and efficacy of Probiotic Lactina® (*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 chickens for fattening and suckling and weaned rabbits. EFSA Journal 2019;17(3):5646, 13 pp. https://doi.org/10.2903/j.efsa.2019.5646

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of 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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Lactina Ltd.[2] for authorisation of the Probiotic Lactina® (*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), when used as a feed additive for rabbits (suckling and weaned) and chickens for fattening (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 6 July 2017.

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 product Probiotic Lactina® (*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), when used under the proposed conditions of use (see Section 3.1.1).

1.2. Additional information

Probiotic Lactina® is a feed additive consisting of viable cells of six strains of lactic acid bacteria. EFSA has issued one opinion on the use of this additive in chickens for fattening, piglets (suckling and weaned) and pigs for fattening which raised questions on the product characterisation, safety for the target species, consumers and users, and on the efficacy (EFSA, 2008). The issues relating to characterisation of the active agent and of the additive and its safety were satisfactorily addressed in a second opinion for chickens for fattening and weaned and suckling piglets. However, the efficacy for chickens for fattening and weaned piglets could not be demonstrated (EFSA FEEDAP Panel, 2013). In the context of the same application, the request for authorisation of Probiotic Lactina® for pigs for fattening was withdrawn.

The additive is currently authorised as a zootechnical additive (functional group: gut flora stabilisers) only for use in feed for suckling piglets.[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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agents in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Lactina Ltd., Sofia str 101,1320, Banky, 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, p. 3.
[4] FEED dossier reference: FAD-2017-0003.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2017-0003?search&form-return

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], the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012b).

3. Assessment

Probiotic Lactina® is a preparation of viable cells of six strains of lactic acid bacteria (LAB) intended for use as a zootechnical additive (gut flora stabiliser) in feeds for rabbits (suckling and weaned) and chickens for fattening to improve their performance.

3.1. Characterisation

3.1.1. Characterisation of the additive

The product 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 content of 5×10^9 CFU LAB/g. It has the same formulation: 60% bacterial mass, spent medium and cryoprotectants, 20% glucose, 10% inulin and 10% calcium carbonate, with the exception of the replacement of glucose with polydextrose, and method of manufacture as that considered in previous applications (EFSA, 2008; EFSA FEEDAP Panel, 2013). Thus, the data pertaining to impurities, physical properties, shelf life and stability in feed for chickens still apply. However, some new information has been provided in the current dossier which is described below.

In a previous opinion (EFSA FEEDAP Panel, 2013), the susceptibility of all strains to relevant antibiotics was tested according to the provisions of the Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA, 2008). In that instance, the six strains proved to be susceptible to all the relevant antibiotics listed in the guidance. This conclusion is still considered to be valid. However since then, the FEEDAP Panel introduced a new requirement for testing the susceptibility of *E. faecium* NBIMCC 8270 to tylosin (EFSA FEEDAP Panel, 2012b). The minimum inhibitory concentration (MIC) value of the *E. faecium* strain to tylosin was measured by broth microdilution following the method of the Clinical and Laboratory Standards Institute (CLSI).[7] The MIC value found fell below the corresponding FEEDAP cut-off value (MIC: 2 mg/L vs cut-off value: 4 mg/L), consequently, the strain is considered susceptible to tylosin.

Three batches of Probiotic Lactina® were analysed for the heavy metals and arsenic content.[8] Results showed values for lead (0.030, 0.037 and 0.046 mg/kg), mercury (0.0073, 0.0077 and 0.0084 mg/kg), cadmium (< 0.00045, 0.0092 and 0.012 mg/kg), copper (0.625, 0.0635 and 0.779 mg/kg) and arsenic (0.0240, 0.0345 and 0.394 mg/kg) that do not raise safety concerns.

An analysis of the dusting potential of one batch of the additive (four repetitions), using the Stauber–Heubach method, showed a mean value of 0.7 g/m^3.[9]

3.1.2. Stability and homogeneity

The applicant declares that the product is not suitable for use in premixtures and provides an analysis supporting this statement.[10] Losses in counts of total LAB were greater than 0.5 log when the additive was mixed at 3×10^{10} CFU/kg in two vitamins and minerals premixtures, and stored for 1 month at 23°C/70% relative humidity (RH).

[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, p. 1.
[7] Technical dossier/Supplementary information May 2018/Report on the antimicrobial susceptibility of Enterococcus faecium NBIMCC 8270 to tylosin.
[8] Technical dossier/Supplementary information May 2018.
[9] Technical dossier/Section II/Annex II.8.
[10] Technical dossier/Section II/Annex II.15.

Stability to pelleting process was measured in three batches of the additive incorporated in a mash feed for rabbits at 3.6×10^9 CFU/kg feed (700 mg/kg feed) and subject to pelleting (barrel temperature 125°C, moisture content 16% and feed rate 9 Hz).[11] Results showed negligible losses after the pelleting process on total LAB counts and on individual counts, except for *L. bulgaricus* NBICCM 8244 and *L. lactis* NBIMCC 8250 where the losses reached approximately 0.5 log units. The same samples of pelleted feed were stored at 18°C/70% RH for 3 months and subject to total LAB and individual counts. Losses after this period were negligible (< 0.5 log units).

In another study, the stability of the additive (three batches) was tested when incorporated in a pelleted feed (after pelleting) for rabbits at 500 mg/kg feed (9×10^9 CFU/kg feed) and stored at 23°C/70% RH for 3 months.[10] However, in the absence of differential counts, it is not possible to determine whether equal sensitivity was shown by all strains in the additive or whether one or more component strains were particularly sensitive.

A total of 30 subsamples were taken from three batches of mash feed for rabbits (10 subsamples per feed) at regular intervals after mixing with the additive.[12] The total LAB counts showed a coefficient of variation was 2%, demonstrating homogeneous mixing.

3.1.3. Conditions of use

Probiotic Lactina® is intended for rabbits (suckling and weaned) and for chickens for fattening at the minimum dose of 2.5×10^9 CFU/kg complete feedingstuffs.

The applicant declares that the product is not suitable for use in premixtures.

3.2. Safety

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, consumers and the environment (EFSA, 2007; EFSA BIOHAZ Panel, 2017). In previous opinions (EFSA, 2008 and EFSA FEEDAP Panel, 2013) the identification of the strains and compliance with the QPS qualifications were confirmed. Therefore, the Panel concluded 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 target animals, consumers of products derived from animals fed the additive and the environment. This presumption does not extend to the sixth strain (*E. faecium*).

3.2.1. Safety for the target species

In the opinion from 2013, *E. faecium* NBIMCC 8270 was recognised as non-pathogenic for chickens (EFSA FEEDAP Panel, 2013). In addition, oral dosing of these species with the Probiotic Lactina® did not elicit any detectable adverse responses or adversely affect growth of chickens for fattening. Therefore, the additive was considered safe for chickens for fattening to a maximum of 1×10^{10} CFU/kg complete feed.

The applicant produced a tolerance trial involving 12 female rabbits (New Zealand White) distributed based on age and parity into four groups, and their offspring (three replicates per treatment).[13] A basal diet based on lucerne meal/oat/wheat bran/soybean meal/barley and sunflower meal alone (control) or supplemented with increasing amounts of the additive was used. The feed was pelleted after inclusion of the additive and animals were given a coccidiostat (toltrazuril, not authorised for this use in the European Union (EU)) through the water during 2 days after weaning. The use levels of the additive are expressed in g/kg feed and correspond to 0, 2× the recommended dose, 2× the recommended dose and 200x the recommended dose. Compliance of the batch of the additive used with the specifications and concentration of the additive in the supplemented feeds were confirmed by analysis. Measured values were: 5.2×10^9, 2.6×10^{10} and 2.5×10^{11} CFU LAB/kg feed. Animals remained in the same treatment groups until the end of the experiment, at 77 days of age. At birth the treatment groups were composed of 22, 24, 22 and 23 rabbits, respectively. Animals were sexed at weaning (at 35 days), resulting in 12 males and 8 females in the control group, 9 males and

[11] Technical dossier/Supplementary information May 2018/Supplementary information Application for authorization of Probiotic Lactina.

[12] Technical dossier/Supplementary information May 2018/Report on the results of the conducted test of homogeneity of the feed additive Lactina in mash feed for rabbits.

[13] Technical dossier/Section III/Annex III.1 and Supplementary information July 2018.

12 females in the 2× group, 11 males and 7 females in the 20× group and 12 males and 11 females in the 200× group. At weaning, rabbits were also distributed in three cages (of 7/8 rabbits) within treatment groups, representing the replicates. Rabbits were individually weighed on days 1, 7, 35 and 77. Feed consumption was measured on a cage basis and feed to gain ratio was calculated. Morbidity and mortality were monitored in the overall period. Four rabbits per treatment were necropsied for gross pathology. Data are analysed with an analysis of variance (ANOVA).

Mortality was normal and not treatment related (two animals died in the control group, three in the 2× group and three in the 20× group). At the end of the experiment, no significant differences were observed on body weight (control: 1.98 kg, 2× group: 2.01 kg, 20× group: 2.32 kg and 200× group: 2.26 kg) or feed intake (control: 75.4 g, 2× group: 72.3 g, 20× group: 79.6 g and 200× group: 80.1 g). But a significant difference was observed on the feed to gain ratio between the 2× group and the control group, in favour of the treated group (control: 2.96, 2× group: 2.78, 20× group: 2.73 and 200× group: 2.78, $p < 0.05$). No adverse effects were observed during the necropsy.

The tolerance trial described above presented weaknesses, in particular, the use of only three replicates per treatment. The inclusion of a coccidiostat not authorised in the EU would not correspond to the EU farming practices. However, toltrazuril is not an ionophore, and thus, is not expected to interfere with the gut microbiota. Therefore, taking also into consideration that:

- five of the six strains assessed using the QPS approach are presumed safe for target animals,
- the sixth strain, *E. faecium*, is not a recognised pathogen for rabbits, it lacks the marker genes associated with human clinical isolates and is susceptible to relevant antibiotics,
- the end-products of the metabolism of the species are typical of lactic acid bacteria, and do not raise concerns,
- the additive does not contain excipients of concern, and
- the additive at approximately 200 times the recommended dose, did not adversely affect the health or growth of rabbits,

the FEEDAP Panel concludes that Probiotic Lactina® is safe for rabbits at the recommended inclusion level.

3.2.2. Safety for the consumer

In the previous opinion (EFSA FEEDAP Panel, 2013), *E. faecium* NBIMCC 8270 was found to be susceptible to clinically relevant antibiotics and to lack the marker genes typical of hospital-associated isolates responsible for clinical infections. The metabolism of *E. faecium* is well known and when the potential for infection is excluded, no other harmful metabolites or substances are expected to be produced during fermentation. Consequently, as no safety issues relating to the active agents were identified, and as the additive does not contain excipients of concern, the FEEDAP Panel considered Probiotic Lactina® to be safe for consumers when used under the conditions proposed. The new data on the susceptibility of one of the active agents to tylosin provided (see Section 3.1.1), confirm previous conclusions that Probiotic Lactina® can be considered safe for consumers of products derived from treated animals.

3.2.3. Safety for the user

In the opinion from 2008, the Panel concluded that owing to the proteinaceous nature of the active agents, the possibility for the additive to act as a respiratory sensitiser cannot be excluded (EFSA, 2008). No information on inhalation toxicity, skin/eye irritation or skin sensitisation of the additive has been provided. In the absence of data, the FEEDAP Panel cannot conclude on the irritancy of Probiotic Lactina® to skin and eyes or on its dermal sensitisation. Owing to the proteinaceous nature of the active agents, the additive is considered to be a potential respiratory sensitiser.

3.2.4. Safety for the environment

In the opinion from 2008, the Panel concluded that the bacteria present in the product are common species in foods and/or in the intestinal tract of animals, and their use in the product is not likely to increase their presence in the wider environment (EFSA, 2008). Consequently, no risks for the environment are expected from the use of this product. The Panel considers that these conclusions apply also to the current application.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

The applicant provided three studies performed in the same Member State but in two different locations to identify the effective dose of Probiotic Lactina® in improving chickens' performance.

The design of the studies is presented in Table 1 and the results in Table 2. In all cases, 1-day-old birds (Ross 308, males in study 1[14] and females in studies 2[15] and 3[16]) were allocated to four treatment groups: a control group receiving the basal diet alone (control) and other three groups receiving the same basal diet supplemented with the additive at the recommended inclusion level (2.5×10^9 CFU/kg complete feedingstuffs) or at other concentrations (Table 1). The use levels were calculated in grams of additive per kg of feed. Compliance of the batch used with the specifications (5.5×10^9 CFU LAB/g in study 1, 6×10^9 CFU LAB/g in study 2 and 6.5×10^9 CFU LAB/g in study 3) and of the intended concentration of the additive in the supplemented feeds were confirmed by analysis (Table 2). The diets were offered to the animals *ad libitum.* Birds were weighed individually on days 1, at change of feed (on days 12 and 27 in study 1 and 14 and 28 in studies 2 and 3) and at the end of the trial. Feed consumption was measured on a pen basis and feed to gain ratio calculated. Morbidity and mortality were monitored in the overall period. Data were statistically analysed with an ANOVA using the pen as experimental unit for all the parameters tested. Means were separated with Tukey Honestly-significant-difference (HSD), Scheffe, Dunnett t-, Games-Howell and/or Tamhane tests.

Table 1: Details on the study design for the studies performed in chickens for fattening

Study No Duration (days)	Total animals Replicates/ treatment × animals/ replicate	Intended concentration of Probiotic Lactina® in feed (CFU LAB/kg feed)	Analysed concentration of Probiotic Lactina® in feed (CFU LAB/kg feed)	Basal diets (main ingredients) form
1 (40)	120 3 × 10	0 1.5×10^9 2.5×10^9 3.5×10^9	0 1.5×10^9 2.5×10^9 3.6×10^9	Starter, grower and finisher (maize/soybean meal/wheat) mash
2 (42)	120 3 × 10	0 2.5×10^9 5.0×10^{10} 5.0×10^{11}	0 2.6×10^9 5.5×10^{10} 5.4×10^{11}	Starter, grower and finisher (maize/wheat/ soybean groats mash
3 (42)	120 3 × 10	0 2.5×10^9 5.0×10^{10} 5.0×10^{11}	0 2.7×10^9 5.0×10^{10} 5.2×10^{11}	Starter, grower and finisher (maize/soybean groats/ wheat) mash

CFU: colony forming unit; LAB: lactic acid bacteria.

[14] Technical dossier/Section IV/Annex IV.4 and Supplementary information May 2018/Annexes_Statistical analysis chickens and Question 11.
[15] Technical dossier/Section IV/Annex IV.5 and Supplementary information May 2018/Annexes_Statistical analysis_chickens and Question 11.
[16] Technical dossier/Section IV/Annex IV.6 and Supplementary information May 2018/Annexes_Statistical analysis_chickens and Question 11.

Table 2: Effect of Probiotic Lactina® on the zootechnical performance of chickens for fattening

Study No	Intended concentration of Probiotic Lactina® in feed (CFU LAB/kg feed)	Daily feed intake (g)	Final body weight (g)	Daily body weight gain (g/bird)	Feed to gain ratio	Mortality (n)
1	0	99.5	2,112	51.7	1.93	0
	1.5×10^9	99.9	2,194	53.7	1.87	1
	2.5×10^9	98.3	2,205	54.0	1.82*	0
	3.5×10^9	96.1	2,169	53.1	1.81*	0
2	0	81.3	1,844	42.9	1.90	0
	2.5×10^9	81.3	1,989	46.3	1.76	1
	5.0×10^{10}	80.7	1,904	44.3	1.82	1
	5.0×10^{11}	82.0	1,968	45.8	1.79*	0
3	0	74.4	1,744	40.6	1.84	0
	2.5×10^9	74.7	1,834	43.0	1.76	0
	5.0×10^{10}	74.9	1,794	41.7	1.87	0
	5.0×10^{11}	76.8	1,786	41.6	1.91	0

CFU: colony forming unit; LAB: lactic acid bacteria.
*: Means in a column within a given trial are significantly different from control by at least $p < 0.05$.

Mortality was low and not treatment related. The design of the studies is weak due to the small number of replicates included. Feed to gain ratio was significantly improved in one study at the recommended inclusion level and at a greater concentration (study 1) and in another study at a much greater inclusion level (5.0×10^{11} CFU/kg feed, study 2), compared to control. Therefore, there is insufficient evidence to conclude on the efficacy of Probiotic Lactina® at any dose in chickens for fattening.

3.3.2. Efficacy for suckling and weaned rabbits

The applicant provided three studies performed in the same Member State but in two different locations. The studies were aimed at demonstrating the effects of Probiotic Lactina on rabbits' performance.

The design of the studies is presented in Table 3 and the results in Table 4. The trials shared the same experimental design in which 12 female rabbits, and their offspring, were distributed based on age and parity into four or five groups receiving the same basal diet alone (control) or supplemented with the additive at the recommended inclusion level and at other concentrations (Table 3). Compliance of the batch of the additive used with the specifications and concentration of the additive in the supplemented feeds were confirmed by analysis (Table 4). Feed was pelleted after inclusion of the additive. The coccidiostat toltrazuril (not authorised for this use in the EU) was added to drinking water during 2 days after weaning. The diets were offered to the animals *ad libitum*. Animals remained in the same treatment groups until the end of the experiment (at 77 days of age). Each mother and her offspring represented a replicate. Rabbits were sexed at weaning at 35 days and moved to cages. Individual bodyweight was measured on days 1, 7, 35 and at the end of the trial on day 77. Feed consumption was measured on a pen basis and feed to gain ratio calculated. Morbidity and mortality were monitored in the overall period. Data were statistically analysed with an ANOVA using the pen as experimental unit for all the parameters tested. Means were separated with Tukey HSD, Scheffe, Dunnett t-, Games-Howell and/or Tamhane tests.

Table 3: Details on the study design for the studies performed in rabbits

Study No	Breed	Total animals Replicates/ treatment × animals/ replicate	Intended Probiotic Lactina concentration in feed (CFU LAB/kg feed)	Analysed concentration of Probiotic Lactina in feed (CFU/kg feed)	Basal diets (main ingredients) form
1[(a)]	White New Zealand rabbit ♀,♂	74 3 × 4–5	0 1.5×10^9 2.5×10^9 3.5×10^9 5×10^9	0 1.6×10^9 2.5×10^9 3.6×10^9 5×10^9	Lucerne meal/oat/ wheat whole meal/ barley/soybean meal groats Mash and granulated
2[(b)]	Californian rabbit ♀,♂	88 3 × 6–8	0 3.5×10^9 7.0×10^{10} 7.0×10^{11}	0 2.6×10^9 5.0×10^{10} 5.4×10^{11}	Lucerne meal/oat/ wheat bran/barley/ soybean meal Pelleted
3[(c)]	Californian rabbit ♀,♂	85 3 × 6–8	0 3.5×10^9 7.0×10^{10} 7.0×10^{11}	0 2.8×10^9 2.7×10^{10} 2.5×10^{11}	Lucerne meal/oat/ wheat bran/barley/ soybean meal Pelleted

CFU: colony forming unit; LAB: lactic acid bacteria.
(a): Technical dossier/Section IV/Annex IV.1.
(b): Technical dossier/Section IV/Annex IV.2.
(c): Technical dossier/Section IV/Annex IV.3.

Table 4: Effect of Probiotic Lactina® on the zootechnical performance of rabbits

Study No	Intended concentration of Probiotic Lactina in feed (CFU/kg feed)	Daily feed intake (g)	Final weight (g)	Daily body weight gain (g/rabbit)[(a)]	Feed to gain ratio[(b)]	Mortality (n/N)
1	0	118.3	2,200	n.r.	3.38	1/15
	1.5×10^9	107.0	2,242	n.r.	3.02	1/14
	2.5×10^9	117.4	2,333	n.r.	2.92	0
	3.5×10^9	117.4	2,402	n.r.	2.84	0
	5×10^9	112.7	2,401	n.r.	2.72	0
2	0	77.6	1,980	25.0	3.05	2/20
	3.5×10^9	77.3	2,113*	26.7	2.83	3/25
	7.0×10^{10}	78.5	2,165	27.4	2.87	0
	7.0×10^{11}	79.3	2,225*	28.2	2.80	1/22
3	0	76.7	2,006	26.3	2.91	0
	3.5×10^9	75.5	2,057	26.6	2.84	0
	7.0×10^{10}	76.1	2,091	27.1	2.80	0
	7.0×10^{11}	77.5	2,117*	28.0*	2.77	0

CFU: colony forming unit; LAB: lactic acid bacteria; n.r.: not reported.
*: Means in a column within a given trial are significantly different from control by at least $p < 0.05$.
(a): In study 2 not statistically analysed.
(b): In studies 2 and 3 not statistically analysed.

Mortality was not treatment related. The reporting of the studies was poor despite the requests for completion, and the design weak due to the small number of replicates included. The rabbits receiving the additive showed a significantly greater body weight at the end of the trial in one study at an inclusion level close to the intended one (study 2) and at a much greater level (7.0×10^{11} CFU/kg feed) in the same study and in a second one (study 3), compared to control. Therefore, there is insufficient evidence to conclude on the efficacy of Probiotic Lactina® in rabbits.

3.3.2.1. Conclusions on efficacy for the target species

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

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[17] and Good Manufacturing Practice.

4. Conclusions

The identity of all component strains of Probiotic Lactina® was established in a previous opinion, five of which qualify for the QPS approach to safety assessment. As no antibiotic resistance of concern was detected in these strains, following the QPS approach, their use in feedingstuffs is presumed safe for the target species, consumers of products from animals fed the additive and the environment. The identity and safety of the sixth strain, *E. faecium* NBIMCC 8270, was also established in the previous and current opinions.

The FEEDAP Panel concludes that Probiotic Lactina® is safe for rabbits (suckling and weaned) at the recommended inclusion level of 2.5×10^9 CFU/kg feed and reiterates its former conclusion that the product is safe for chickens for fattening at 1×10^{10} CFU/kg feed.

The FEEDAP Panel considers Probiotic Lactina® to be safe for consumers of products derived from treated animals when used at the conditions proposed.

In the absence of data, the FEEDAP Panel cannot conclude on the irritancy of Probiotic Lactina® to skin and eyes or on its dermal sensitisation. Owing to the proteinaceous nature of the active agents, the additive is considered to be a potential respiratory sensitiser.

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

Documentation provided to EFSA

1) Probiotic Lactina®. January 2017. Submitted by Lactina Ltd.
2) Probiotic Lactina®. Supplementary information. May 2018. Submitted by Lactina Ltd.
3) Probiotic Lactina®. Supplementary information. July 2018. Submitted by Lactina Ltd.
4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Probiotic Lactina®.
5) Comments from Member States.

Chronology

Date	Event
04/01/2017	Dossier received by EFSA
19/01/2017	Reception mandate from the European Commission
06/07/2017	Application validated by EFSA – Start of the scientific assessment
24/10/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation and efficacy*
19/09/2017	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
06/10/2017	Comments received from Member States
22/05/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
17/07/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Safety for the target species*
25/07/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
27/02/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[17] 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, p. 1.

References

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. https://doi.org/10.2903/j.efsa.2007.587

EFSA (European Food Safety Authority), 2008. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of Probiotic LACTINA® for chickens for fattening, piglets and pigs. EFSA Journal 2009;7(1):912, 13 pp. https://doi.org/10.2903/j.efsa.2009.912

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlstr€om H, Cocconcelli PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gomez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C and Fernández Esc_amez PS, 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal 2017;15(3):4664, 177 pp. https://doi.org/10.2903/j.efsa.2017.4664

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. https://doi.org/10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. https://doi.org/10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. https://doi.org/10.2903/j.efsa.2012.2740

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of Probiotic Lactina® (Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermophilus and Enterococcus faecium) for chickens for fattening and piglets. EFSA Journal 2013;11(4):3170, 13 pp. https://doi.org/10.2903/j.efsa.2013.3170. Available online: www.efsa.europa.eu/efsajournal

Abbreviations

ANOVA	analysis of variance
CFU	colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CV	coefficient of variation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
HSD	Honestly-significant-difference
IDF	International Dairy Federation
LAB	lactic acid bacteria
MIC	minimum inhibitory concentration
PFGE	pulsed field gel electrophoresis
QPS	qualified presumption of safety
RH	relative humidity

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Probiotic Lactina®

Probiotic lactina® is the trade name of a preparation based on viable cells from acid lactic bacteria (LAB) containing the following six strains: *Enterococcus faecium* NBIMCC 8270, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus delbrueckii* ssp. *lactis* NBIMCC 8250, *Lactobacillus delbrueckii* ssp. *bulgaricus* NBICCM 8244 and *Streptococcus thermophilus* NBIMCC 8253. This feed additive is currently authorised under the category/functional 4(b) 'zootechnical additives'/'gut flora stabilisers' according to the classification system of Annex I of Regulation (EC) No 1831/2003 for sucking piglets. In the current application, authorisation is sought under article 4 (1) of the Regulation (EC) No 1831/2003 for the new use for chickens for fattening and rabbits suckling and weaned. *Probiotic lactina*® is intended to be marketed as a freeze-dried powder containing a minimum total dose of the sum of the six bacterial active substances (LAB) of 5×10^9 Colony Forming Units (CFU)/g. The feed additive is intended to be used directly in *feedingstuffs* with a minimum total LAB content of 2.5×10^9 CFU/kg of complete *feedingstuffs*.

For the identification and characterisation of the different bacterial strains, the Applicant proposed a combination of classical phenotypic tests and modern molecular sequencing analysis. The EURL recommends instead for official control the Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of the sum of the six strains contained in *Probiotic lactina*® *(feed additive)*, the Applicant suggested the use of several International Dairy Federation (IDF) standards and the Bulgarian Standard (BS) 10945, while the ring-trial validated spread plate method developed by CEN (EN 15787) was suggested for the enumeration in *feedingstuffs*. This CEN method was already evaluated and recommended by the EURL in several dossiers. Based on the performance characteristics available, the EURL recommends for official control the EN 15787 spread plate method for the enumeration of the sum of the six strains contained in *Probiotic lactina*® in the *feed additive* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.