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Safety and efficacy of a feed additive consisting of *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) DSM 32264 as a feed additive for cats (NBF Lanes s.r.l.)

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, Giovanna Martelli, Montserrat Anguita, Jaume Galobart, Jordi Ortuño, Joana Revez and Rosella Brozzi

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the efficacy of a product consisting of *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) DSM 32264 as a zootechnical additive for cats. The additive is a preparation of viable cells of *L. reuteri* DSM 32264 and it has not been previously authorised as a feed additive in the European Union. The additive is intended for use in complete feed for cats at a minimum inclusion level of 6×10^9 colony forming units (CFU) per animal and day. In a previous opinion, the FEEDAP Panel could not conclude on the efficacy of *L. reuteri* DSM 32264 for cats. The applicant has provided supplementary information to support the efficacy of the additive for cats. Based on the data provided, the FEEDAP Panel concluded that *L. reuteri* DSM 32264 has the potential to improve faecal consistency by reducing the moisture content of stools from cats receiving the additive at 1×10^{10} CFU/kg feed. However, the Panel had some reservations on the effects in the moisture content, which if maintained overtime, might cast doubts on the benefits on the long-term use of the additive since it could lead to constipation.

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Keywords: zootechnical additives, gut flora stabilisers, *Limosilactobacillus reuteri* DSM 32264, cats, efficacy, NBF-2

Requestor: European Commission

Question number: EFSA-Q-2021-00633

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

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

The applicant NBF Lanes s.r.l., is seeking a Community authorisation of *Lactobacillus reuteri*[1] DSM 32264 as a feed additive to be used as a gut flora stabiliser for cats (Table 1).

Table 1: Description of the substances

Category of additive	**Zootechnical additives**
Functional group of additive	Gut flora stabilisers
Description	*Lactobacillus reuteri* DSM 32264
Target animal category	Cats
Applicant	NBF Lanes s.r.l.
Type of request	New opinion

On 27 November 2018, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ('EFSA'), in its opinion on the safety and efficacy of the product, could not conclude on the efficacy of *Lactobacillus reuteri* DSM 32264 in cats.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 18 February 2021 and the applicant has been requested to transmit them to EFSA as well.

In view of the above, the Commission asks EFSA to deliver a new opinion on *Lactobacillus reuteri* DSM 32264 as a feed additive for cats based on the supplementary data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2. Additional information

The additive is a preparation containing viable cells of *L. reuteri* DSM 32264. It has not been previously authorised as a feed additive in the European Union.

EFSA issued one opinion on the safety and efficacy of this product when used in feed for cats (EFSA FEEDAP Panel, 2019).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a supplementary information[2] to a previous technical dossier on the same product.[3]

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] 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,[5] a non-confidential version of the supplementary information has been published on Open.EFSA.[6]

[1] The request refers to the synonym *Lactobacillus reuteri,* corresponding to the current taxonomic unit of *Limosilactobacillus reuteri*.
[2] FEED dossier reference: EFSA-Q-2021-00663.
[3] FEED dossier reference: FAD-2017-0002.
[4] 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.
[5] Decision available online: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[6] Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2021-00633

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[7] and the relevant guidance document: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The additive is a preparation of viable cells of *L. reuteri* DSM 32264 intended for use as a zootechnical additive (functional group: gut flora stabilisers) in feed for cats to exert beneficial effects in their gastrointestinal tract leading to an increase in faecal consistency. Since the last opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2019), the taxonomic unit of the species under assessment has been updated from the basonym *Lactobacillus reuteri* to *Limosilactobacillus reuteri* (Zheng et al., 2020) and considered in the list of QPS-recommended biological agents (EFSA BIOHAZ Panel, 2020). The current taxonomic unit is used hereafter in the opinion.

The additive is intended for use in complete and complementary feed for cats (with a moisture content $< 14\%$) at a daily dose of 6×10^9 CFU/animal, which would approximately equate to a range from 0.6–4.5 $\times$ 10^{11} CFU/kg complete feed for cats.

In a previous opinion (EFSA FEEDAP Panel, 2019), the FEEDAP Panel could not conclude on the efficacy of *L. reuteri* DSM 32264 for cats, as none of the five efficacy studies could be further considered due to weaknesses in the experimental design and/or reporting. The applicant has provided supplementary information to support the efficacy of the additive.

A total of three trials with cats sharing a common design were submitted. The details on the study design are provided in Table 2 and the main results in Table 3. The studies were conducted with adult cats of both sexes and of different breeds (Chartreux, Persian and Scottish Fold cat) and body weight (BW), fed with a commercial dry extruded food once a day based on their maintenance energy requirements. Cats were daily examined by a veterinarian.

For each study, cats were acclimatised to individual housing for 14 days before the start of the study, during this period they received unsupplemented feed. At the study start, cats were randomly allocated to the two dietary treatments: control and *L. reuteri* DSM 32264 at 1×10^{10} CFU/kg feed (not confirmed by analysis).[8] The additive was mixed with the food using a maltodextrin carrier before consumption. Control animals received the same carrier without the additive.

Feed intake was measured daily. BW and body condition score (BCS) were recorded at days 0, 7, 14, 21, 28 and 35. BCS assessment was carried out by visual examination and palpation of the animal (nine points scale). On the same days, faecal samples were collected and assessed for moisture content (FM) and consistency (faecal score (FS), using a 7-point scoring scale: 1 = very hard and dry, 7 = watery). In addition, microbiological analyses (enumeration of lactobacilli and *Escherichia coli*/coliforms) were performed on the samples collected at days 0, 7, 21 and 35. However, details on the analytical methodology for the enumeration of *Escherichia coli/* coliforms were not provided despite the request.[8]

According to the information provided, the statistical analysis of the data was done using a repeated measurements model and means were compared with a t-test.

Table 2: Design of the efficacy trials performed in cats

Trial	Total no of animals (animals × gender × treatment)	Breed, age and mean body weight (duration)
1[9]	16 (Control: 4♂, 4♀; Treatment: 2♂, 6♀;)	Chartreux (40 months old, 4 kg (35 days)
2[10]	12 (Control: 3♂, 3♀; Treatment: 1♂, 5♀;)	Persian, 43 months old, 4 kg (35 days)
3[11]	10 (Control: 2♂, 3♀; Treatment: 3♂, 2♀;)	Scottish Fold, 40 months old, 3.8 kg (35 days)

[7] 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.
[8] Technical dossier/Supplementary Information April 2022.
[9] Technical dossier/Efficacy study_Certosino cats/Supplementary Information April 2022.
[10] Technical dossier/Efficacy study_Persian cats/Supplementary Information April 2022.
[11] Technical dossier/Efficacy study_Scottish cats/Supplementary Information April 2022.

Cats were healthy during the study. BW and BCS of cats did not change over time, and there were no differences between groups. Overall results showed a lower FM and FS in treated cats compared to control. For FM, a significant decrease was observed in all trials from day 21 onwards. Regarding the FS, a significant decrease of the scoring was observed from day 28 onwards in studies 1 and 2 but only in the overall value (average of all sampling days) in study 3. The Panel notes that the FM in the treated cats decreased linearly over time, with limited variability, and reaching considerably low values. Should this linear decrease in moisture content be extended/maintained with the prolonged use of the additive, it might lead to constipation, questioning the benefits of its use in cats.

Table 3: Effects of *L. reuteri* DSM 32264 on faecal moisture and faecal score of stools from cats

Faecal parameter		**Moisture (g/g)**						**Consistency (seven-point score)**					
Trial		1		2		3		1		2		3	
Treatment (CFU/kg feed)		0	1×10^{10}	0	1×10^{10}	0	1×10^{10}	0	1×10^{10}	0	1×10^{10}	0	1×10^{10}
Day	0	0.46	0.46	0.45	0.46	0.47	0.48	3.44	3.61	3.42	3.53	3.39	3.61
	7	0.45	0.45	0.45	0.45	0.46	0.46	3.56	3.37	3.53	3.45	3.59	3.31
	14	0.45	0.43	0.45	0.44	0.46	0.44	3.50	3.19	3.45	3.20	3.49	3.11
	21	0.44	0.41*	0.45	0.41*	0.47	0.41*	3.44	3.00	3.45	3.03	3.69	2.91
	28	0.45	0.39*	0.45	0.39*	0.46	0.39*	3.56	2.87*	3.61	2.95*	3.39	2.81
	35	0.44	0.37*	0.45	0.38*	0.47	0.36*	3.56	2.81*	3.53	2.86*	3.39	2.71
Overall		0.45	0.41*	0.45	0.41*	0.46	0.42*	3.56	3.19*	3.51	3.10*	3.49	3.07*

*: Means in the same row within a trial are significantly different compared to control with $p < 0.1$.

Regarding the faecal microbiological analysis, in all studies at the end of the experimental period, lactobacilli counts were greater and *E. coli* counts lower in samples from animals receiving the additive compared to those from control animals. However, the FEEDAP Panel notes that this difference is marginal (< 1 log), and thus of questionable biological relevance.

4. Conclusions

The additive consisting of *L. reuteri* DSM 32264 has the potential to improve faecal consistency by reducing the moisture content of stools from cats receiving the additive at 1×10^{10} CFU/kg feed. However, the Panel has some reservations on the linear decrease in the moisture content, which, if maintained over time, might cast doubts on the benefits on the long-term use of the additive since it could lead to constipation.

5. Documentation provided to EFSA/Chronology

Date	**Event**
17/03/2021	Dossier received by EFSA. *Lactobacillus reuteri* DSM 32264. Submitted by NBF Lanes s.r.l
12/07/2021	Reception mandate from the European Commission
29/10/2021	Application validated by EFSA – Start of the scientific assessment
04/02/2022	Request of supplementary information to the applicant in line Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. *Issues: efficacy*
13/04/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
29/06/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

BCS	body condition score
BW	body weight
CFU	colony forming unit
FM	faecal moisture
FS	faecal score
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed