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Assessment of the efficacy of a feed additive consisting of *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) DSM 32203 for dogs (NBF LANES)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the efficacy of *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) DSM 32203 as a zootechnical additive for dogs. The additive is a preparation of viable cells of *L. reuteri* DSM 32203 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 dogs at a minimum use 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 32203 for dogs. The applicant has provided supplementary information to support the efficacy of the additive to dogs. Based on the data provided, the FEEDAP Panel concludes that *L. reuteri* DSM 32203 has the potential to improve faecal consistency by reducing the moisture content of stools from dogs receiving the additive at 1×10^{10} CFU/kg feed. However, the Panel has some reservations on the effects in the moisture content of stools, 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.

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1. Introduction

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

Regulation (EC) No 1831/2003¹ 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, NBF LANES,² is seeking a Community authorisation of *Lactobacillus reuteri*³ DSM 32203 as a feed additive to be used as a gut flora stabiliser for dogs (Table 1).

Table 1: Description of the substances

Category of additive	Zootechnical additives
Functional group of additive	Gut flora stabilisers
Description	<i>Lactobacillus reuteri</i>
Target animal category	Dogs
Applicant	NBF LANES
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 ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the efficacy of *L. reuteri* DSM 32203 for dogs.

During the discussions 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 the efficacy of the additive.

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 13 April 2021 and the applicant has been requested to transmit them to EFSA as well.

In view of the above, the Commission asks the Authority to deliver a new opinion on *Lactobacillus reuteri* DSM 32203 as a feed additive for dogs 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 additive is a preparation containing viable cells of *L. reuteri* DSM 32203. 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 dogs (EFSA FEEDAP Panel, 2019). In this latter, the FEEDAP Panel could not conclude on the efficacy of *L. reuteri* NBF-1 (DSM 32203) for dogs.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information⁴ to previous application on the same product.⁵

In accordance with Article 38 of the Regulation (EC) No 178/2002⁶ and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the

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 NBF LANES srl, Corso di Porta Vittoria, 14, 20,122 Milano (Italy).

³ The request refers to the synonym *Lactobacillus reuteri*, corresponding to the current taxonomic unit of *Limosilactobacillus reuteri*.

⁴ Dossier reference: EFSA-Q-2021-00534.

⁵ Dossier reference (FEED dossier reference): EFSA-Q-2017-00049 (FAD-2017-0001).

⁶ 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.

same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,⁷ a non-confidential version of the supplementary information has been published on Open.EFSA.⁸

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 429/2008⁹ 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 32203 intended for use as a zootechnical additive (functional group: gut flora stabilisers) in feed for dogs to exert beneficial effects in their gastrointestinal tract leading to an increase in faecal consistency. Since the last opinion, 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 dogs (with a moisture content < 14%) at a level of 6×10^9 CFU per animal and day, which would approximately equate to a range from 0.85– 3×10^{10} CFU/kg complete feedingstuffs for dogs.

In a previous opinion (EFSA FEEDAP Panel, 2019), the FEEDAP Panel could not conclude on the efficacy of *L. reuteri* DSM 32203 for dogs, as none of the efficacy studies could be further considered due to weaknesses in the experimental design and/or reporting. The applicant has provided supplementary information to support efficacy in dogs.

A total of three trials with dogs and 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 healthy adult dogs of both sexes and of different body weight and breeds (French Bulldog,¹⁰ Chihuahua¹¹ and Golden Retriever¹²), fed three different commercial dry extruded dog food once a day based on their energy maintenance requirements.

For each study, dogs were acclimatised to the kennel for 14 days before the start of the study and received unsupplemented feed. At the study start, dogs were randomly allocated to the two dietary treatments: control and *L. reuteri* DSM 32203 at 1×10^{10} CFU/kg feed (not confirmed by analyses).¹³ The additive was mixed with the food directly in the bowl of the animals using a maltodextrin carrier before consumption. Control animals received the same carrier without the additive. Body weight (BW) and body condition score (BCS) were measured at days 0, 7, 14, 21, 28 and 35. On the same days, faecal samples were collected and assessed for moisture content (FM) and consistency (7-point scoring scale: 1 = very hard and dry, 7 = watery).¹⁴ In addition, microbiological analyses (enumeration of lactobacilli and *Escherichia coli*/coliforms) were performed from the samples collected at days 0, 7, 21 and 35. However, details on the analytical methodology for the enumeration of *E. coli*/coliforms were not provided despite being requested.¹⁵

The statistical analysis of the data was done using a repeated measurements model and means were compared with a t-test.

⁷ Decision available at: <https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements>

⁸ Available at: <https://open.efsa.europa.eu/questions/EFSA-Q-2021-00534>

⁹ 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.

¹⁰ Technical dossier/Efficacy study Bouledogue Français dogs.

¹¹ Technical dossier/Efficacy study Chihuahua dogs.

¹² Technical dossier/Efficacy study Golden retriever dogs.

¹³ Technical dossier/Supplementary Information February 2022.

¹⁴ Dossier/Efficacy study Bouledogue Français dogs/ANNEX_IV_5, Dossier/Efficacy study Chihuahua dogs/ANNEX_IV_5, Dossier/Efficacy study Golden retriever dogs/ANNEX_IV_5 and Technical dossier/Supplementary Information February 2022.

¹⁵ Technical dossier/Supplementary Information February 2022/Lactobacillus reuteri NBF 1 (DSM 32203) - Supplementary information.

Table 2: Trial design of the efficacy trials performed in dogs

Trial	Total no of animals (replicates × treatment)	Breed, age and mean body weight (duration)
1 ¹⁶	30 (15; both groups 10♂, 5♀)	French Bulldog, 28 months old, 11 kg (35 days)
2 ¹⁷	30 (15; both groups 6♂, 9♀)	Chihuahua, 23 months old, 2 kg (35 days)
3 ¹⁸	40 (20; Control: 9♂, 11♀; Treatment: 12♂, 8♀)	Golden Retriever, 33 months old, 31 kg (35 days)

In all trials, dogs were healthy during the study. BW and BCS did not change over time, nor between groups (Table 3). Overall results showed that faecal moisture and consistency (faecal score, FS) were lower in treatment groups compared to control. The Panel notes that the FM in the treated dogs decreased in a linear way over time, with limited variability, and reaching very low values. This trend, resulting in low values, if maintained overtime, might cast doubts on the benefits on the long-term use since it could lead to constipation.

Table 3: Effects of *L. reuteri* DSM 32203 on dogs' faecal moisture and faecal score¹⁹

Faecal parameter	Moisture, g/g						Consistency (seven-point score)						
	1		2		3		1		2		3		
Trial	0	1 × 10 ¹⁰	0	1 × 10 ¹⁰	0	1 × 10 ¹⁰	0	1 × 10 ¹⁰	0	1 × 10 ¹⁰	0	1 × 10 ¹⁰	
Treatment (CFU/kg feed)													
Day	0	0.67	0.70	0.68	0.67	0.71	0.71	2.97	2.93	2.56	2.66	3.34	3.38
	7	0.68	0.64*	0.71	0.69	0.71	0.68	2.84	2.54	2.54	2.24	3.39	3.15
	14	0.67	0.59*	0.68	0.63*	0.71	0.64*	2.87	2.24*	2.47	2.07	3.34	2.73*
	21	0.66	0.54*	0.68	0.58*	0.71	0.59*	2.87	2.07*	2.61	1.97*	3.31	2.31*
	28	0.65	0.48*	0.67	0.52*	0.71	0.54*	2.84	2.01*	2.44	1.78*	3.36	2.11*
	35	0.67	0.40*	0.69	0.48*	0.70	0.50*	2.97	1.98*	2.61	1.59*	3.31	2.06*
Overall		0.67	0.53*	0.69	0.58*	0.71	0.59*	2.88	2.17*	2.54	1.93*	3.34	2.47*

*: Means in the same row within a trial are significantly different compared to control with $p \leq 0.001$.

Regarding the faecal microbiological analysis, in all studies a statistically significant effect of the additive was observed at the end of the experimental period (35 days), with an increase of lactobacilli counts and a decrease in *E. coli* counts when compared to the control groups. However, the FEEDAP Panel notes that the decrease of *E. coli* was marginal (< 1 log unit), thus the results are questionable from the biological relevance perspective.

4. Conclusions

The additive consisting of *L. reuteri* DSM 32203 has the potential to improve faecal consistency by reducing the moisture content of stools from dogs receiving the additive at 1 × 10¹⁰ CFU/kg feed. However, the Panel has some reservations on 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.

¹⁶ Dossier/Efficacy study Bouledogue Français dogs.

¹⁷ Dossier/Efficacy study Chihuahua dogs.

¹⁸ Dossier/Efficacy study Golden retriever dogs.

¹⁹ Dossier/Efficacy study Bouledogue Français dogs, Efficacy study Chihuahua dogs, Efficacy study Golden retriever dogs and Dossier/Supplementary Information February 2022/Faecal moisture analysis and New Statistical analysis.

5. Documentation provided to EFSA/Chronology

Date	Event
08/04/2021	Dossier received by EFSA. <i>Lactobacillus reuteri</i> DSM 32203. Submitted by NBF LANES
12/07/2021	Reception mandate from the European Commission
01/10/2021	Application validated by EFSA – Start of the scientific assessment
21/12/2021	Request of supplementary information to the applicant in line with Article 7(3) of Regulation (EC) No 1304/2003 – Scientific assessment suspended. <i>Issues: efficacy</i>
21/02/2022	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
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FM	faecal moisture
FS	faecal score