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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of viable cells of *Lactobacillus acidophilus* CNCM I-3231, *Ligilactobacillus salivarius* CNCM I-3233, *Lactiplantibacillus plantarum* CNCM I-3232, *Lacticaseibacillus rhamnosus* CNCM I-4427, and *Bifidobacterium animalis* subsp. lactis CNCM I-3993 (FlorEquilibre® Chien) for dogs (Wamine SAS)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of FlorEquilibre® Chien when used as a zootechnical additive (functional group: gut flora stabilisers) for dogs. The product under assessment is based on viable cells of *Lactobacillus acidophilus* CNCM I-3231, *Ligilactobacillus salivarius* CNCM I-3233, *Lactiplantibacillus plantarum* CNCM I-3232, *Lacticaseibacillus rhamnosus* CNCM I-4427 and *Bifidobacterium animalis* subsp. *lactis* CNCM I-3993. *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232 and *L. rhamnosus* CNCM I-4427 are considered by EFSA to be suitable for the qualified presumption of safety approach. The identity of these strains was established, and no acquired antimicrobial resistance genes were detected. Therefore, the FEEDAP Panel concluded that the use of these four strains is presumed safe for the target species and the environment. However, the Panel notes that the use of *B. animalis* subsp. *lactis* CNCM I-3993 represents a safety concern for the target species, the users and the environment because the strain harbours an acquired antimicrobial resistance gene. The Panel concluded that the additive is eye irritant, and a skin and respiratory sensitiser. Exposure via any route is considered a risk. Based on the data provided, the Panel is not in the position to conclude on the efficacy of the additive for dogs.

KEYWORDS

efficacy, FlorEquilibre® Chien, gut flora stabiliser, other zootechnical, safety, zootechnical additives

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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 Wamine SAS[2] for the authorisation of the additive consisting of viable cells of *Lactobacillus acidophilus* CNCM I-3231, *Ligilactobacillus salivarius* CNCM I-3233, *Lactiplantibacillus plantarum* CNCM I-3232, *Lacticaseibacillus rhamnosus* CNCM I-4427, and *Bifidobacterium animalis* subsp. *lactis* CNCM I-3993 (FlorEquilibre® Chien) when used as a feed additive for dogs (category: zootechnical additives; functional groups: gut flora stabiliser, other zootechnical additives). During the course of the assessment, the applicant decided to withdraw the functional group "other zootechnical additives".[3]

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 dossier was received on 30 June 2023 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00451. The particulars and documents in support of the application were considered valid by EFSA as of 29 April 2024.

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 viable cells of *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232, *L. rhamnosus* CNCM I-4427, and *B. animalis* subsp. *lactis* CNCM I-3993 (FlorEquilibre® Chien), when used under the proposed conditions of use (see **Section 3.1.4**).

1.2 | Additional information

The additive is a preparation containing viable cells of *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232, *L. rhamnosus* CNCM I-4427 and *B. animalis* subsp. *lactis* CNCM I-3993 (FlorEquilibre® Chien), intended to be used in the feed for dogs. It has not been previously authorised as a feed additive in the European Union.

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 a preparation containing viable cells of *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232, *L. rhamnosus* CNCM I-4427 and *B. animalis* subsp. *lactis* CNCM I-3993 (FlorEquilibre® Chien) as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 3 May to 3 August 2024; the comments received were considered for the assessment.

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

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 18 March to 8 April 2025 for which no comments were received.

[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]1 zone industrielle du taillis Champtoceaux Orée d'Anjou – France.
[3]Annex IV_3_0.
[4]Dossier reference: FEED-2023-17004.
[5]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.
[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

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 and experts' (elicitation) knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the agents in animal feed.[7]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232, *L. rhamnosus* CNCM I-4427 and *B. animalis* subsp. *lactis* CNCM I-3993 (FlorEquilibre® Chien) is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2024), EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2024).

3 | ASSESSMENT

The product containing viable cells of *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232, *L. rhamnosus* CNCM I-4427 and *B. animalis* subsp. *lactis* CNCM I-3993 is intended to be used as a zootechnical additive (functional group: gut flora stabilisers) in feed for dogs. It will be hereafter referred to with its trade name FlorEquilibre® Chien.

3.1 | Characterisation

3.1.1 | Characterisation of the active agents

The active agents are strains of lactic acid bacteria deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) of Institut Pasteur: *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232, *L. rhamnosus* CNCM I-4427 and *B. animalis* subsp. *lactis* CNCM I-3993.[9] The applicant declared that the strains were isolated from human (CNCM I-3231, CNCM I-4427 and CNCM I-3993) and plant sources (CNCM I-3233 and CNCM I-3232) and that they have not been genetically modified.[10]

The taxonomic identification of *L. acidophilus* CNCM I-3231 was confirmed by average nucleotide identity (ANI) determination on the whole genome sequence (WGS) data.[11] [redacted] No plasmids were predicted in the interrogation of the WGS data.

The taxonomic identification of *L. salivarius* CNCM I-3233 was confirmed by ANI determination on the WGS data.[12] [redacted] No plasmids were predicted in the interrogation of the WGS data.

The taxonomic identification of *L. plantarum* CNCM I-3232 was confirmed by ANI determination on the WGS data.[13] [redacted] The strain was shown to harbour one plasmid.

[7]Evaluation report received on 29/10/2025 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.

[8]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.

[9]Annex_II_2_2 Deposition cert_LAB.

[10]Section II_Point 2-1_2-2_Identity_Characterisation_CHIEN_REV MARCH 2025.

[11]Annex_II_2_5 WGs_CNCM I-3231.

[12]Annex_II_2_6 WGs_CNCM I-3233.

[13]Annex_II_2_7 WGs_CNCM I-3232.

The taxonomic identification of *L. rhamnosus* CNCM I-4427 was confirmed by ANI determination on the WGS data.[14] [redacted] No plasmids were predicted in the interrogation of the WGS data.

The taxonomic identification of *B. animalis* subsp. *lactis* CNCM I-3993 was confirmed by ANI determination on the WGS data.[15] [redacted] No plasmids were predicted in the interrogation of the WGS data.

The antimicrobial susceptibility of the strains *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232, *L. rhamnosus* CNCM I-4427 and *B. animalis* subsp. *lactis* CNCM I-3993 was tested against the set of antibiotics recommended by the FEEDAP Panel for *L. acidophilus* group, *Lactobacillus* facultative heterofermentative, *Lactobacillus plantarum/pentosus*, *Lactobacillus rhamnosus* and *Bifidobacterium*, respectively (EFSA FEEDAP Panel, 2018).[16] All the minimum inhibitory concentration (MIC) values were equal to or fell below the corresponding EFSA cut-off values. Therefore, the strains are phenotypically susceptible to the relevant antibiotics.

The WGS data of the strains were interrogated for the presence of antimicrobial resistance (AMR) genes by a search against the [redacted] and [redacted] Bacterial Antimicrobial Resistance Reference Gene databases.[17] No hits were identified exceeding the thresholds recommended by EFSA (EFSA, 2021), except for *B. animalis* subsp. *lactis* CNCM I-3993.

For *B. animalis* CNCM I-3993, a hit corresponding to the resistance determinant *tet*(W) was detected. This gene encodes a protection protein that attaches to the ribosome and causes an alteration of the ribosomal conformation to which tetracycline cannot bind, and therefore protein synthesis can proceed (Chopra & Roberts, 2001; Connell et al., 2003). *tet*(W) has been demonstrated to be spread in several bifidobacterial species (Nøhr-Meldgaard et al., 2021; Wang et al., 2017). Further, genes with more than 80% identity to *tet*(W) have also been found in Gram-positive and Gram-negative bacteria and thus it is considered the most widely spread tetracycline resistance gene class (Chopra & Roberts, 2001; Nøhr-Meldgaard et al., 2021). In *B. animalis*, the gene has been shown to be present in most but not all *B. animalis* subsp. *lactis* genomes and absent in all *B. animalis* subsp. *animalis* genomes analysed (Nøhr-Meldgaard et al., 2021).

To investigate the intrinsic/acquired nature of the *tet*(W) gene in the species *B. animalis*, the applicant downloaded 229 genomes from the [redacted] database, provided information on the origin/source of isolation and conducted a phylogenomic tree analysis.[18] The frequency of the *tet*(W) gene among 58 strains selected based on the completeness of the genome sequences was shown to be > 90%.

The Panel notes that considering the long-term world-wide commercial use of *B. animalis* subsp. *lactis* strains in foods and supplements, different geographic locations or sources related to food consumption of isolates cannot be used to rule out clonality. Additionally, the small number of single nucleotide polymorphisms shown in the genome of publicly available *B. animalis* subsp. *lactis* strains suggests a common and clonal origin (Barrangou et al., 2009; Milani et al., 2013).

To assess the non-clonal relationship of the *B. animalis* strains the applicant performed an [redacted] analysis by using a 99.9% threshold. The non-clonal relationship was demonstrated for only [redacted] strains, which means that the minimum number of 30 epidemiologically and/or ecologically unrelated strains was not reached, as per EFSA requirements (EFSA BIOHAZ Panel, 2023a).

Based on the above, the Panel considers that the intrinsic nature of *tet*(W) in *B. animalis* is not demonstrated.

The Panel notes that although *B. animalis* subsp. *lactis* CNCM I-3993 was shown to be phenotypically susceptible to tetracycline (MIC value equal to the EFSA cut-off: 8 mg/L),[19] silent *tet*(W) genes in strains of this species have proven to be activated following exposure to tetracycline (Gueimonde et al., 2010). In the majority of the strains of *B. animalis* subsp. *lactis*, the *tet*(W) gene was found to be flanked by genes annotated as encoding mobile element proteins, which could contribute to its mobilisation within a genome or horizontally (Nøhr-Meldgaard et al., 2021; Rozman et al., 2020; Tóth et al., 2023; Vandecraen et al., 2017). The transferability of *tet*(W) from *B. animalis* subsp. *lactis* to other species and genera has not been demonstrated in vitro (Gueimonde et al., 2010; Shin et al., 2023). However, the mobilisation of genes under natural conditions in the animal gut cannot be excluded (Nielsen et al., 2014).

Based on the above, the Panel concludes that the *tet*(W) gene in *B. animalis* subsp. *lactis* CNCM I-3993 is acquired and therefore represents a risk.

[14]Annex_II_2_8 WGs_CNCM I-4427.

[15]Annex_II_2_9 WGs_B lactis_CNCM I-3993_REV AUGUST 2025.

[16]Annex_II_2_10 MIC assay_CNCM I-3231, Annex_II_2_11 MIC assay_CNCM I-3233, Annex_II_2_12 MIC assay_CNCM I-3232, Annex_II_2_13 MIC assay_CNCM I-4427 and Annex_II_2_14 MIC assay_CNCM I-3993.

[17]Annex_II_2_5 WGs_CNCM I-3231, Annex_II_2_6 WGs_CNCM I-3233, Annex_II_2_7 WGs_CNCM I-3232, Annex_II_2_8 WGs_CNCM I-4427 and Annex_II_2_9 WGs_B lactis_CNCM I-3993_REV AUGUST 2025.

[18]Annex_II_2_9 WGs_B lactis_CNCM I-3993_REV AUGUST 2025; Annex_II_2_9 WGs_B lactis_CNCM I-3993_Annex 3_REV AUGUST 2025; Annex_II_2_9 WGs_B lactis_CNCM I-3993_Annex 4_REV AUGUST 2025.

[19]Annex_II_2_14 MIC assay_CNCM I-3993.

3.1.2 | Manufacturing process

Each of the five strains included in FlorEquilibre® Chien is grown separately. After submerged liquid fermentation, the viable cells/spores are harvested by centrifugation, freeze-dried and homogenised. The resulting individual strain lyophilisates ([redacted] in total in the final product, representing *L. acidophilus* [redacted] w/w of final product; *L. salivarius, L. rhamnosus*, and *B. lactis* [redacted] each and *L. plantarum* [redacted]) are mixed with pre-gelatinised starch from maize ([redacted]) and dolomite-magnesite (1g598; [redacted]) to reach a final minimum total microbial count of 2.0×10^{10} CFU/g product.[20]

3.1.3 | Characterisation of the additive

The minimum total microbial count of FlorEquilibre® Chien is specified as 2.0×10^{10} CFU/g product. The proportion of each strain is 30% for *L. acidophilus* CNCM I-3231, 20% for *L. salivarius* CNCM I-3233, 10% for *L. plantarum* CNCM I-3232, 20% for *L. rhamnosus* CNMC I-4427 and 20% for *B. lactis* CNCM I-3993.

The data provided by the applicant on the batch-to-batch variation (total counts of lactic acid bacteria and proportion of the individual strains),[21] impurities[22] and microbial contamination[23] of the additive are reported in Table 1.

TABLE 1 Data on the batch-to-batch variation, impurities and microbial contamination FlorEquilibre® Chien.

		Analysis		
Parameter	**Specification**	**Average**	**Range**	**No of batches**
Batch-to-batch variation				
Total counts (CFU/g)	2.0×10^{10}		2.0×10^{10}–2.4×10^{10}	5
L. acidophilus CNCM I-3231 (%)	[redacted]		[redacted]	5
L. salivarius CNCM I-3233 (%)	[redacted]		[redacted]	5
L. plantarum CNCM I-3232 (%)	[redacted]		[redacted]	5
L. rhamnosus CNCM I-4427 (%)	[redacted]		[redacted]	5
B. animalis subsp. *lactis* CNCM I-3993 (%)	[redacted]		[redacted]	5
Impurities				3
Lead (mg/kg)			< 0.5	
Mercury (mg/kg)			< 0.02	
Cadmium (mg/kg)			< 0.2	
Arsenic (mg/kg)			< 0.5	
Dioxins and furans (upper bound)[1]				3
PCDD/Fs (ng WHO_{2005}-TEQ/kg)			0.15	
WHO-PCDD/F-PCB (ng TEQ/kg)			0.27	
Non-DL-PCB-TEQ/kg (µg/kg)			1.6	
Mycotoxins (µg/kg)				3
Aflatoxin B1			< 0.3	
Aflatoxin B2			< 0.5	
Aflatoxin G1			< 0.5	
Aflatoxin G2			< 0.5	
Ochratoxin A			< 0.5	
Fumonisins B1 + B2			< 20	
Zearalenone			< 5	
Deoxynivalenol			< 50	
HT-2 toxin			< 10	
T-2 toxin			< 5	
Microbial contamination				3
Salmonella spp. (in 25g)			Not detected	

[20]Section II_Point 2-3_Manufacturing process_CHIEN_REV DEC 2023.
[21]Annex_II_1_2A Strains quantification method; Annex_II_1_2B Strains quantification method_Version REV MARCH 2025.
[22]Annex_II_1_7 HM_Mycotox_dioxins_FlorEquilibre Chien.
[23]Annex_II_1_8 Microbial purity_FlorEquilibre Chien.

TABLE 1 (Continued)

Parameter	Specification	Analysis		
		Average	Range	No of batches
Enterobacteriaceae (CFU/g)			< 10	
Yeasts (CFU/g)			< 10	
Moulds (CFU/g)			< 10	
Escherichia coli (CFU/g)			< 10	
Anaerobic sulfate reducing bacteria (CFU/g)			< 10	
Coagulase-positive staphylococci (CFU/g)			< 10	
Clostridium perfringens (CFU/g)			< 10	

Abbreviations: <, means below the limit of quantification (LOQ) of the analytical method; CFU, colony forming units; nDL-PCBs, non-dioxin-like PCBs; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-p-dioxins; PCDFs, polychlorinated dibenzofurans; TEQ, toxic equivalent factors for dioxins, furans and dioxin-like PCBs established by WHO in 2005 (Van den Berg et al., 2006); WHO, World Health Organization.

[1]Upper bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification. Values are expressed per kg of additive with 88% dry matter content.

The data provided showed compliance with the specifications set by the applicant. Additionally, compliance with the quantitative specifications of each individual strain was demonstrated in five batches of the additive assayed in triplicate, by identifying colonies after culturing by specific quantitative polymerase chain reaction (qPCR).

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

The data provided by the applicant on the physicochemical and technological properties[24] of the additive are reported in Table 2.

TABLE 2 Data on the physicochemical and technological properties of FlorEquilibre® Chien.

Physical properties	Range	No. of batches
Physical form	Powder	
Bulk density (kg/m^3)	605–608	3
Tapped density (kg/m^3)	763–765	3
Dusting potential (mg/m^3)	3285–3895	3
Particle size distribution (%)		
< 100 µm	73.7	
< 50 µm	40.1	
< 10 µm	9.0	3
Shelf-life (losses)		
4/25/30°C for 24 months	≤1 log CFU[1]	3
Stability (losses)		2/feed
Dry kibbles, 25/30°C for 3 months	≤1 log CFU[1]	
Homogeneity (%CV; CFU/g)		3
Dry kibbles	13–13.8	

Abbreviations: CFU, colony-forming units; CV, coefficient of variation.

[1]At all temperatures; viability losses for both active agents were considered as not biologically relevant.

3.1.4 | Conditions of use

The additive is intended for use in feed for dogs at a proposed minimum use level of 8.0×10^{10} CFU/kg complete feed.

[24]Annex_II_1_9 PSD_Dusting_Density.

3.2 | Safety

3.2.1 | Safety of the active agents

The species *L. acidophilus, L. salivarius, L. plantarum, L. rhamnosus* and *B. animalis* are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA BIOHAZ Panel, 2023b). This approach requires the identification of the strain(s) to be conclusively established and evidence that the strain(s) does not show acquired resistance to antibiotics of human and veterinary importance. The identity of the five strains has been unambiguously established. For *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232 and *L. rhamnosus* CNCM I-4427, the assessment of antimicrobial resistance demonstrated compliance with the relevant criteria. Consequently, these strains fulfil the QPS requirements. In contrast, for *B. animalis* subsp. *lactis* CNCM I-3993, the analysis of antimicrobial resistance genes revealed the presence of the acquired AMR gene *tet*(W). The FEEDAP Panel therefore concludes that the presence of the *tet*(W) gene in *B. animalis* subsp. *lactis* CNCM I-3993 constitutes a risk.

3.2.2 | Safety for the target species

L. acidophilus CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232 and *L. rhamnosus* CNCM I-4427 fulfil the QPS requirements and are considered safe for the target species. The presence of the acquired AMR gene in *B. animalis* subsp. *lactis* CNCM I-3993 is considered a risk for the target species. Therefore, the additive is not considered safe for the target species.

3.2.3 | Safety for the user

No studies were conducted to support the safety for the user. Based on the dusting potential measured value (see Section 3.1.3), the FEEDAP Panel considers that the exposure of users through inhalation is likely. The additive contains microorganisms and, therefore, should be considered a skin and respiratory sensitiser. According to the safety data sheet, the additive is an eye irritant.

The FEEDAP Panel concludes that the additive should be considered an eye irritant and a skin and respiratory sensitiser. The presence of the acquired AMR gene in *B. animalis* subsp. *lactis* CNCM I-3993 is considered a risk for non-protected users. Exposure via any route is considered a risk.

3.2.4 | Safety for the environment

The additive under assessment is intended for use in dogs only. No environmental risk assessment is necessary for such use (EFSA FEEDAP Panel, 2019). However, the Panel notes that the use of *B. animalis* subsp. *lactis* CNCM I-3993 represents a safety concern because it harbours an acquired antimicrobial resistance gene.

3.3 | Efficacy

Three trials with dogs (*Canis familiaris*) sharing a common design were submitted. However, two of the trials were conducted in the same location, using the same basal diet, and most (10 out of 14) dogs involved in both trials were the same. Therefore, these studies were not independent, and the data from both trials were pooled. The details on the study design are provided in Table 3, and the main results in Table 4.

In trial 1,[25] the dogs were housed collectively and moved to individual boxes for feeding and faecal sampling. In trial 2,[26] dogs were housed individually. Dogs were randomly allocated to two experimental groups, a control group and a group supplemented with FlorEquilibre® Chien to achieve 8×10^{10} CFU/kg complete feed. The additive was top-dressed onto the basal diet at each feeding; the overall content of the active agents was regularly analysed for each batch of additive used in each study. The experimental diet consisted of dry commercial food in kibbles offered for [redacted] days. In both trials, an additional [redacted] were included, during which the animals in both groups were offered the same basal diet without supplementation.

[25]Annex_IV_3_1 Efficacy trial No 1; Annex_IV_3_1 Q10_Global means.

[26]Annex_IV_3_2 Efficacy trial No 2; Annex_IV_3_3 Efficacy trial No 3; Annex_IV_3_4 Pooled data_Trial 2-3_final report.

TABLE 3 Trial design and use level of the efficacy trials performed in dogs.

Trial	Total no of animals (animals × replicate) replicates × treatment	Breed (sex) age	Duration (monitoring time)	Composition feed (form)	Groups (CFU/kg feed)	
					Intended	Calculated[1]
1	[redacted]	Beagle ([redacted]♀[redacted]♂) [redacted]	[redacted]	Cereals (maize, wheat, rice) and chicken (kibbles)	0 8×10^{10}	0 8.0×10^{10}
2	[redacted]	Beagle [redacted]	[redacted]	Chicken and cereals (rice, maize, wheat) (kibbles)	0 8×10^{10}	0 8.5×10^{10}

Abbreviation: CFU, colony-forming unit.

[1]The total bacteria count in the feed was calculated based on the analytical content in the batch of the additive used and the average feed intake of the animals during the study.

The animals' overall health status was monitored daily. The animals were weighed [redacted]. Feed intake was [redacted] recorded. Faecal samples were collected at different times during the experimental period and additional samples were collected during the monitoring period. At each collection time point, the faecal dry matter content was analysed, and the faecal score was evaluated using a five-point scoring system.[27]

In trial 1, the experimental data were analysed using a repeated-measures analysis of covariance, with the diet, time and interaction diet × time as fixed effects and the time at day 0 as a covariate. In trial 2, the data were analysed using a mixed model, with the diet, time and interaction diet × time as fixed effects and the animal as a random effect. The dog was considered the experimental unit in all cases. The significance level was set at 0.10.

TABLE 4 Effects of FlorEquilibre® Chien on the faecal dry matter and consistency of dogs at the end of the study.

Trial	Groups (CFU/kg feed)	Faecal dry matter (%)	Faecal score
1	0 8×10^{10}	39.1 36.4	2.55 2.76
2	0 8×10^{10}	29.0 28.4	2.58 2.59

Abbreviation: CFU, colony-forming unit.

In both trials, the inclusion of FlorEquilibre® Chien in the diet of dogs at 8×10^{10} CFU/kg complete feed showed no effects on the faecal dry matter content and faecal score in comparison to the control group. The animals' body weight and feed intake (data not shown) did not change throughout the experimental period.

3.3.1 | Conclusions on efficacy

Based on the data provided, the Panel is not in a position to conclude on the efficacy of the additive when included in the feed for dogs.

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[28] and Good Manufacturing Practice.

4 | CONCLUSIONS

The strains *L. acidophilus* CNCM I-3231, *L. salivarius* CNCM I-3233, *L. plantarum* CNCM I-3232 and *L. rhamnosus* CNCM I-4427 fulfil the QPS requirements. Therefore, the FEEDAP Panel concludes that these strains are presumed safe for the target species and the environment. However, the presence of the acquired *tet*(W) gene in *B. animalis* subsp. *lactis* CNCM I-3993 constitutes a risk for the target species, the users and the environment. The additive should be considered eye irritant, and skin and respiratory sensitiser. Exposure via any route is considered a risk.

[27][redacted] Faeces Scoring System: scale from 1 (dry faeces; signs of constipation) to 5 (liquid faeces; diarrhoea) (half points), considered 2.5 the optimal score.

[28]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.

Based on the data provided, the Panel cannot conclude on the efficacy of the additive when included in the feed for dogs at 8×10^{10} CFU/kg complete feed.

ABBREVIATIONS

ANI	average nucleotide identity
AMR	antimicrobial resistance
BW	body weight
CFU	colony forming unit
CNCM	Collection Nationale de Cultures de Microorganismes
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification
MIC	minimum inhibitory concentration
nDL-PCBs	non-dioxin-like polychlorinated biphenyls
PCB	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-p-dioxins
PCDF	polychlorinated dibenzofurans
qPCR	quantitative polymerase chain reaction
QPS	qualified presumption of safety
TEQ	toxic equivalent factors
WGS	whole genome sequence
WHO	World Health Organization

REQUESTOR

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