SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of *Lacticaseibacillus casei* IDAC 210415-01, *Limosilactobacillus fermentum* IDAC 210415-02, *Levilactobacillus brevis* IDAC 051120-02 and *Enterococcus faecium* IDAC 181218-03 (K-9 Heritage Probiotic Blend®) for dogs (CanBiocin Inc.)

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 Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Montserrat Anguita, Rosella Brozzi, Jaume Galobart, Matteo Innocenti, Jordi Ortuño, Elisa Pettenati and Yolanda García-Cazorla

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of K-9 Heritage Probiotic Blend® when used as a zootechnical additive (functional group: gut flora stabilisers) for dogs. The product under assessment is based on viable cells of *Lacticaseibacillus casei* IDAC 210415-01, *Limosilactobacillus fermentum* IDAC 210415-02, *Levilactobacillus brevis* IDAC 051120-02 and *Enterococcus faecium* IDAC 181218-03. The FEEDAP Panel was not in the position to conclude on the identification of the strains and, therefore, the safety of the product cannot be based on the presumption of safety of the active agents. The Panel notes that the use of *E. faecium* IDAC 181218-03 represents a safety concern because it harbours an acquired antimicrobial resistance gene. Moreover, the hazard related to the presence of additional antimicrobial resistance genes in the active agents cannot be excluded. No tolerance trials on the target animals have been provided. Therefore, the Panel is not in the position to conclude on the safety of the additive for dogs. Regarding the user safety, the Panel cannot conclude on the irritant potential of the additive for skin or eyes due to the absence of data. Given the proteinaceous nature of the active agents, the additive should be considered a respiratory sensitiser. No conclusions could be drawn on its potential to be a skin sensitiser. The use of K-9 Heritage Probiotic Blend® in animal nutrition represents a safety concern for the environment due to the potential carryover of at least an antimicrobial resistance gene. The FEEDAP Panel is not in the position to conclude on the efficacy of K-9 Heritage Probiotic Blend® for the target species.

Keywords: zootechnical additives, gut flora stabilisers, *Lacticaseibacillus casei* IDAC 210415-01, *Limosilactobacillus fermentum* IDAC 210415-02, *Levilactobacillus brevis* IDAC 051120-02, *Enterococcus faecium* IDAC 181218-03, safety

Requestor: European Commission

Correspondence: feedap@efsa.europa.eu

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Panel members: 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 and Ruud Woutersen.

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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 CanBiocin Inc.[2] for the authorisation of the product containing *Lacticaseibacillus casei* IDAC 210415-01, *Limosilactobacillus fermentum* IDAC 210415-02, *Levilactobacillus brevis* IDAC 051120-02 and *Enterococcus faecium* IDAC 181218-03 (K-9 Heritage Probiotic Blend®), when used as a feed additive for dogs (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 dossier was received on 29 June 2021 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00383. The particulars and documents in support of the application were considered valid by EFSA as of 1 April 2022.

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, users and the environment and on the efficacy of the product containing *Lacticaseibacillus casei* IDAC 210415-01, *Limosilactobacillus fermentum* IDAC 210415-02, *Levilactobacillus brevis* IDAC 051120-02 and *Enterococcus faecium* IDAC 181218-03 (K-9 Heritage Probiotic Blend®), when used under the proposed conditions of use (see **Section 3.1.4**).

1.2. Additional information

The product under assessment is based on viable cells of *Lacticaseibacillus casei* IDAC 210415-01, *Limosilactobacillus fermentum* IDAC 210415-02, *Levilactobacillus brevis* IDAC 051120-02 and *Enterococcus faecium* IDAC 181218-03. It is not 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[3] in support of the authorisation request for the use of the product containing *L. casei* IDAC 210415-01, *L. fermentum* IDAC 210415-02, *L. brevis* IDAC 051120-02 and *E. faecium* IDAC 181218-03 (K-9 Heritage Probiotic Blend®) as a feed additive.

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 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,[5] EFSA carried out a public consultation on the non-confidential version of the application from 4 May to 25 May 2023 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] [redacted]

[3] FEED dossier reference: FEED-2021-0847.

[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

In addition, the confidential version of the technical dossier was subject to a target consultation of the interested Member States from 1 April to 1 July 2022 for which received comments that were considered for the assessment.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the product containing *L. casei* IDAC 210415-01, *L. fermentum* IDAC 210415-02, *L. brevis* IDAC 051120-02 and *E. faecium* IDAC 181218-03 (K-9 Heritage Probiotic Blend®) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) and EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021).

3. Assessment

The product containing a lyophilised blend of viable cells of *L. casei* IDAC 210415-01, *L. fermentum* IDAC 210415-02, *L. brevis* IDAC 051120-02 and *E. faecium* IDAC 181218-03 (K-9 Heritage Probiotic Blend®) 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 K-9 Heritage Probiotic Blend®.

3.1. Characterisation

3.1.1. Characterisation of the active agents

The strains of *L. casei* and *L. fermentum* were isolated from family-owned healthy dogs, while *L. brevis* and *E. faecium* were isolated from free-ranging wolves. They are deposited in the International Depositary Authority of Canada (IDAC), with the accession numbers 210415-01, 210415-02, 051120-02 and 181218-03, respectively.[8] The strains have not been genetically modified. The methodology applied by the applicant does not allow to exclude the presence of plasmids.

The whole genome sequence (WGS) of each active agent[9] was compared by BLASTn against a set of public genomes of the expected species.[10] The Panel notes that the analyses provided are not compliant with the recommendations in relevant documents (EFSA FEEDAP Panel, 2018a,b; EFSA, 2021), including for instance the no use of state-of-the-art bioinformatic methodologies such as dDDH, ANI or phylogenomic analysis for inferring overall genome relatedness; omission in the alignments of reference genomes of the type strains for *L. casei*, *L. fermentum* and *E. faecium*; no reporting, among others parameters, of the thresholds used in the software and proportion of reads/contigs analysed. Overall, the analyses and reports provided do not allow to unequivocally confirm the identity of the strains under assessment.

The antimicrobial susceptibility of the four strains was tested [redacted] against the battery of antibiotics and the cut-off values for the claimed species recommended by the FEEDAP Panel (EFSA FEEDAP Panel, 2018b).[11] All the minimum inhibitory concentration (MIC)

[6] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/feed-2021-0847_en

[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] Annex_II_1_IDAC_certificates_of_deposit_all_4_strains.

[9] Annex_II_5_Strain_identity_confirmation_report.

[10] Appendix-Q2-5-L. casei K9-1 genome sequence alignment to L. casei DSM 20011, Appendix-Q2-6-L. fermentum K9-2 genome sequence alignment to L. fermentum LMT2-75, Appendix-Q2-7-L. brevis WF-1B genome sequence alignment to L. brevis NCTC13768 and Appendix-Q2-8-E. faecium WF-3 genome sequence alignment to E. faecium ATCC 8459.

[11] Annex_II_6_Four_strain_antibiotic_susceptibility_assessment_report.

values for the strains *L. casei* IDAC 210415-01 and *L. fermentum* IDAC 210415-02 were equal or fell below the corresponding cut-off values. The MIC values obtained for *L. brevis* IDAC 051120-02 exceeded the cut-off values for ampicillin (8 vs. 2 mg/L), kanamycin (85 vs. 64 mg/L), clindamycin (8 vs. 4 mg/L), tetracycline (64 vs. 4 mg/L) and chloramphenicol (16 vs. 4 mg/L), while for *E. faecium* IDAC 181218-03 the cut-off values were exceeded only for tetracycline (64 vs. 4 mg/L). Exceeding the cut-off value by one dilution is considered to be within the normal range of variation and, thus, not a matter of concern. Therefore, *L. casei* IDAC 210415-01 and *L. fermentum* IDAC 210415-02 are considered to be susceptible to all the relevant antibiotics; *L. brevis* IDAC 051120-02 is considered to be susceptible to all the relevant antibiotics except to ampicillin, tetracycline and chloramphenicol; and *E. faecium* IDAC 181218-03 is considered to be susceptible to all the relevant antibiotics except to tetracycline. The Panel notes that, owing to the absence of a conclusive identification of the strains, the antimicrobial susceptibility profiles cannot be established based on the data available.

The WGS of each strain was interrogated for the presence of antimicrobial resistance (AMR) genes against three databases, the Panel only considered the results obtained from the two databases that are maintained, ResFinder and CARD.[12] [redacted][13] No hits of concern were identified for the strains *L. casei* IDAC 210415-01, *L. fermentum* IDAC 210415-02 and *L. brevis* IDAC 051120-02. In the genome of the strain *E. faecium* IDAC 181218-03 it was identified the resistance determinant *tet*(M) which is considered an acquired gene in *Enterococcus* and in other diverse genus (Jones et al., 2006; Flórez et al., 2008; León-Sampedro et al., 2016; Lengliz et al., 2022). This resistance determinant codifies for a well-characterised ribosome protection protein known to confer resistance to tetracycline by a direct mechanism of action to dislodge and release tetracycline from the ribosome. The presence of this gene represents a safety concern. The Panel notes that the thresholds used in the WGS interrogation are higher than those recommended by EFSA (2021) and potential hits could have been missed. Therefore, the presence of other antimicrobial resistance genes in the active agents cannot be excluded.

The WGS of the strain *E. faecium* IDAC 181218-03 was additionally interrogated for the presence of the virulence factors IS*16*, *hyl*$_{Efm}$ and *esp* genes by a targeted search using BLASTn.[14] No hits were identified. The presence of these genetic elements was also excluded using conventional PCR (IS*16*, *hyl*$_{Efm}$), dot-blot DNA hybridisation method (*esp*) [redacted][15] Therefore, the active agent *E. faecium* IDAC 181218-03 is free of the virulence factors IS*16*, *hyl*Efm and *esp*.

The four active agents *L. casei* IDAC 210415-01, *L. fermentum* IDAC 210415-02, *L. brevis* IDAC 051120-02 and *E. faecium* IDAC 181218-03 showed inhibitory activity in a deferred agar essay against human and animal pathogenic strains.[16]

3.1.2. Characterisation of the additive

[redacted] The final product (hereby referred to as 'additive') is in a powder form and is standardised by mixing the four freeze-dried cell concentrates (ratio of 1:1:1:1) with maltodextrin as cryoprotectant, to reach a minimum guaranteed concentration of $\sim 2.5 \times 10^8$ colony forming units (CFU) of each strain per gram of additive (total $\geq 1.0 \times 10^9$ CFU/g additive).[17]

Analytical data to confirm the specifications for individual strain counts content were provided for five batches of the additive applying a modified method based on ISO standard 4833-2.[18] The EURL recommends this method for official control and enumeration of the total active agents in the additive.[6] However, the FEEDAP Panel notes that this methodology relies on morphological characteristics and is considered not adequate to individually enumerate and discriminate between the four active agents. A total of [redacted] were further

[12] Annex_III_2_14_Four_strain_antibiotic_susceptability_assessment_report_CanBiocin_20201216.
[13] Appendix-Q5-1-Parameter report on databases (CARD, ResFinder, ARG-ANNOT).
[14] Appendix-Q6-1-Analysis of Enterococcus faecium WF3 genome Summary.
[15] Annex_III_3_1_E_faecium_WF_3_safety_assessment_report_CanBiocin_20200813.
[16] Annex_II_7_Production_of_inhibitory_substances_report_CanBiocin_20210503.
[17] Sect_II.1_and_II.2_Identity_and_characterisation and Sect_II.3_Manufacturing.
[18] Annex_II_2_ Certificates_of_analysis_5_batches and Annex_II_11_Product_composition_QA_and_SL_testing_report.

analysed and identified by a molecular technique able to discriminate between the four active agents.[19] This limited amount is not considered as a representative number of colonies to draw a conclusion. Overall, the analyses and reports provided do not allow to unequivocally confirm the quantitative specifications of each individual strain in the additive under assessment.

The same five batches were analysed for microbial contamination. Enterobacteriaceae, yeasts and filamentous fungi counts were below the corresponding limit of detection (LOD) of the methods,[20] *Escherichia coli* and *Staphylococcus aureus* were not detected in 1 g of the additive, while *Salmonella* spp. was not detected in 10 g of the additive.[18]

No data were provided to support the lack of chemical impurities in the additive.

A set of physico-chemical parameters were determined. The average apparent density [redacted][21] The dusting potential using the Stauber-Heubach method to analyse three batches of the additive showed an average value of 4.80 g/m^3 (range 3.75–5.36 g/m^3).[22] The mean particle size using laser diffraction to analyse three batches of the additive resulted in 57 μm (range 10–108 μm).[23]

3.1.3. Stability and homogeneity

The shelf life of the additive [redacted] was studied [redacted][24] The Panel notes that individual counts of the active agents have not been provided.

3.1.4. Conditions of use

The additive is intended to be supplied by daily top dressing the feed for dogs, with the dose adjusted according to body weight of the animals ranging from ~ 0.75 g of additive per day (corresponding to 0.5×10^9 total CFU for dogs with body weight ≤ 4.5 kg) to 4.50 g of additive (corresponding to 3.0×10^9 total CFU for dogs with body weight 22.6–27.0 kg).[25] A minimum inclusion level based on CFU/kg complete feed has not been specified by the applicant. The additive is intended to be added to the feed by top dressing rather than by mixing with the complete feed with the aim to ensure that the target animals receive the daily dose of the additive regardless of the amount of feed consumed.

3.2. Safety

3.2.1. Safety of the active agents

The species *L. casei, L. brevis* and *L. fermentum* are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establish safety for the target species (EFSA, 2007; EFSA BIOHAZ Panel, 2023). This approach requires the identity of the strains to be conclusively established and evidence that they do not show acquired resistance to antibiotics of human and veterinary importance. The identification of the strains *L. casei* IDAC 210415-01, *L. fermentum* IDAC 210415-02 and *L. brevis* IDAC 051120-02 could not be conclusively established. Consequently, these strains do not qualify for QPS safety assessment, and cannot be presumed safe. Similarly, the identification of the strain IDAC 181218-03 as *E. faecium* was not conclusively established and it harbours an AMR that represents a safety concern. The hazard related to the presence of additional antimicrobial resistance genes in the active agents cannot be excluded.

[19] Appendix-Q3-3-PCR Analysis of Heritage Blend 4 Strains Summary and Appendix Q3-1-QCPRO-1201.00 Heritage Blend 4 PCR Analysis Procedure.
[20] LOD: Enterobacteriaceae, yeasts and filamentous fungi 100 CFU/g.
[21] Annex_II_12_Product_density_and_water_activity.
[22] Annex_II.3_Dusting_potential and Annex_III_4_15_Dusting_potential_CanBiocin_Report_No_3_709.
[23] Annex_III_5_15_Final_report_Particle_size_distribution_CanBiocin_20201110.
[24] Appendix-Q9-1-K-9 Heritage Probiotic Blend - Shelf Life.
[25] Sect_II.5_Conditions_use; for dogs ≤ 4.5 kg body weight 0.75 g of additive per day; from 4.6 to 9.0 kg body weight 1.50 g; from 9.1 to 13.5 kg body weight 2.25 g; from 13.6 to 18.0 kg body weight 3.00 g; from 18.1 to 22.5 kg body weight 3.75 g; and from 22.6 to 27.0 kg body weight 4.50 g.

3.2.2. Safety for the target species

No tolerance trials with the target species have been provided. The safety of the product cannot be based on the safety of the active agents (see Section 3.2.1). Moreover, the Panel notes that the use of *E. faecium* IDAC 181218-03 represents a safety concern because the strain harbours an antimicrobial resistance gene. The hazard related to the presence of additional antimicrobial resistance genes in the active agents cannot be excluded. Therefore, the Panel is not in the position to conclude on the safety of the additive for the target animals.

3.2.3. Safety for the user

No specific studies investigating the effects of the additive on the respiratory system were submitted.[26] The dusting potential of the additive indicates that users may be exposed via the respiratory route. Owing to the proteinaceous nature of the active agents, the additive is considered a respiratory sensitiser.

No data was submitted by the applicant to study the potential of the additive to be irritant to skin/eyes or to be a skin sensitiser. The Panel cannot conclude on the potential of the additive as a skin and eyes irritant.[26] Regarding the skin sensitisation potential, the FEEDAP Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only and that currently no validated assays for assessing the sensitisation potential of microorganisms are available.[27]

The Panel notes that the use of *E. faecium* IDAC 181218-03 represents a safety concern because it harbours an antimicrobial resistance gene. The hazard related to the presence of additional antimicrobial resistance genes in the active agents cannot be excluded.

3.2.4. Safety for the environment

The additive under assessment is intended to be used in dogs only. No environmental risk assessment is necessary for such use (EFSA FEEDAP Panel, 2019). However, the Panel notes that the use of *E. faecium* IDAC 181218-03 represents a safety concern because it harbours an antimicrobial resistance gene. The hazard related to the presence of additional antimicrobial resistance genes in the active agents cannot be excluded.

3.3. Efficacy

Four *in vitro* studies were provided with the aim to assess the mode of action and efficacy of the additive. The objective of the first study[28] was to evaluate the potential anti-inflammatory activity and effects of the additive on the gut barrier functions, testing its effect against a pro-inflammatory pathogenic bacterium (*Salmonella enterica*) and on cytochalasin D, respectively. A second study[29] evaluated the survival of the microbial strains of the additive in the stomach and small intestine, using an *in vitro* gastrointestinal model to simulate digestion conditions in the dog. In the third study[30] the microbial strains of the additive were incubated with different substrates (maltodextrin, humic and fulvic acids) evaluating the effects on short-chain fatty acids and microbial community profiles. The last *in vitro* study[31] examined the capacity of the microbial strains of the additive to produce substances with potential inhibitory effects against some common spoilage and pathogenic bacteria, including *E. coli*, *S. enterica*, *Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* spp., *Clostridium perfringens* and *Clostridioides difficile*. For zootechnical additives, *in vivo* trials are needed to support the efficacy of the additive (EFSA FEEDAP Panel, 2018a). Therefore, the *in vitro* studies will not be further considered for the current assessment.

Three long-term studies (trials 1–3) sharing a common design were submitted in order to assess the effect of the additive on the faecal moisture and consistency of Beagle dogs. All trials had a duration of 28 days. Details on the study design are provided in Table 1 and the main results in Table 2.

[26] Section III_Safety of use of the additive for users.
[27] https://www.efsa.europa.eu/sites/default/files/2022-07/feedap20220629-30_m.pdf
[28] Annex_IV_1–8 Report_TNO_InTESTine_CanBiocin_final_V2_23JUL20.
[29] Annex_IV_2–8_TNO_R21341_TIM_Final_report v2_16Oct2020.
[30] Annex_IV_3_8_TNO_i screen_ 2020_R11249_Amendement_1_FINAL_02Sep20.
[31] Annex_IV_4_12_Production_of_inhibitory_substances_report.

Table 1: Design of the efficacy trials performed in dogs

Trial	Total no of animals (animals of each gender per group)	Breed (age) Mean body weight
1[32]	[redacted]	Beagle [redacted]
2[33]	[redacted]	Beagle [redacted]
3[34]	[redacted]	Beagle [redacted]

[redacted] All dogs received a dry commercial [redacted] diet [redacted] and water offered *ad libitum*. [redacted] the dogs were fed [redacted] wet commercial diet [redacted] with the additive K-9 Heritage Probiotic Blend® (additive group) or with an equivalent amount of the carrier maltodextrin without the additive (control group). The amount of additive/maltodextrin was calculated from each dog bodyweight according to the conditions of use.

[redacted]

General health of the animals was examined daily (trials 1 and 3) or every 2 days (trial 2), and any veterinary medical intervention was recorded. In trial 1, dogs were weighed weekly, while in trials 2 and 3 the bodyweight and body condition score were measured at the beginning (day 1) and end (day 28) of the experimental period. The basal dry feed intake was recorded daily in all trials. The faecal consistency was visually evaluated daily (trial 3) or every 2 days (trials 1 and 2) using a different scoring system for each trial.[35] In all trials, faecal samples were collected every 2 days and analysed for the moisture content.

Additionally, faecal samples collected at [redacted] (trial 1)[32] or [redacted] (trial 3)[36] were further analysed for the concentration of short-chain fatty acids ([redacted] trial 3) and the relative abundance of the four active agents ([redacted] trial 1; [redacted] trial 3). These two end-points are not considered suitable to demonstrate the efficacy of the additive and, therefore, the data will not be further considered.

In trial 1, faecal consistency and moisture were evaluated with an analysis of [redacted] In trials 2 and 3, the data were analysed with [redacted]

Statistical significance was set at 0.10.

Table 2: Effects of the additive on faecal consistency and moisture at the end of the experiment (day 28)

Trial	Group	Faecal consistency* (score)	Moisture (%)
1	Control	4.3	68.7
	K-9 Heritage Probiotic Blend®	3.9	68.1

[32] Annex_IV_6_IPD19003_final_study_report_combined.
[33] Annex_IV_7_9_NWBP_final_study_report_combined.
[34] Annex_IV_8_KFI Final Study Report.
[35] Trial 1: scale from 1 (very hard and dry faeces) to 7 (watery faeces); Trial 2: scale from 0 (absent) to 4 (poorly formed with viscous consistency); Trial 3: scale from 0 to 7 (scoring system not specified).
[36] Annex_IV_5_Report_quantification_of_microbiota_composition_and_SCFAs_QO.

Trial	Group	Faecal consistency* (score)	Moisture (%)
2	Control	2.6	61.8
	K-9 Heritage Probiotic Blend®	2.6	62.7
3	Control	3.5	71.6
	K-9 Heritage Probiotic Blend®	3.7	74.1

*: Trial 1: 1 (very hard and dry faeces) to 7 (watery faeces); Trial 2: 0 (absent) to 4 (poorly formed with viscous consistency); Trial 3: 0 to 7 (scoring system not specified).

The supplementation of the dogs' diets with the additive at the recommended levels had no significant effect on the faecal consistency and/or moisture content in any of the trials. The dogs' body weight and feed intake were not affected by the additive in any trial.

3.3.1. Conclusions on efficacy

The Panel is not in the position to conclude on the efficacy of the additive K-9 Heritage Probiotic Blend® for the target animals at the proposed conditions of use.

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[37] and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel is not in the position to conclude on the identification of the active agents present in the additive. The use of *E. faecium* IDAC 181218-03 represents a safety concern because it harbours an acquired antimicrobial resistance gene. The hazard related to the presence of additional antimicrobial resistance genes in the active agents cannot be excluded.

The FEEDAP Panel is not in the position to conclude on the safety for the target animals.

Regarding user safety, no conclusions can be drawn on the potential of the additive to cause skin/eyes irritation or skin sensitisation. Owing to its proteinaceous nature, it is considered a respiratory sensitiser.

The use of K-9 Heritage Probiotic Blend® in animal nutrition represents a safety concern for the environment due to the carryover of at least an antimicrobial resistance gene.

The FEEDAP Panel is not in the position to conclude on the efficacy of K-9 Heritage Probiotic Blend® for the target species.

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), 2021. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. EFSA Journal 2021;19(7):6506, 14 pp. https://doi.org/10.2903/j.efsa.2021.6506

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Álvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Prieto Maradona M, Querol A, Sijtsma L, Suarez JE, Sundh I, Vlak JM, Barizzone F, Hempen M, Correia S and Herman L, 2023. Scientific Opinion on the "Update of the list of Qualified Presumption of Safety (QPS) recommended microorganisms intentionally added to food or feed as notified to EFSA". EFSA Journal 2023;21(1):7747, 27 pp. https://doi.org/10.2903/j.efsa.2023.7747

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

[37] 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.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017a. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017b. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018a. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16 (5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018b. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, 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, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

Flórez AB, Ammor MS and Mayo B, 2008. Identification of tet(M) in two Lactococcus lactis strains isolated from a Spanish traditional starter-free cheese made of raw milk and conjugative transfer of tetracycline resistance to lactococci and enterococci. International Journal of Food Microbiology, 121, 189–194. https://doi.org/10.1016/j.ijfoodmicro.2007.11.029

Jones CH, Tuckman M, Murphy E and Bradford PA, 2006. Identification and sequence of a tet(M) tetracycline resistance determinant homologue in clinical isolates of *Escherichia coli*. Journal of Bacteriology, 188, 7151–7164. https://doi.org/10.1128/JB.00705-06

Lengliz S, Cheriet S, Raddaoui A, Klibi N, Chehida NB, Najar T and Abbassi MS, 2022. Species distribution and genes encoding antimicrobial resistance in *enterococcus* spp. isolates from rabbits residing in diverse ecosystems: a new reservoir of linezolid and vancomycin resistance. Journal of Applied Microbiology, 132, 2760–2772. https://doi.org/10.1111/jam.15461

León-Sampedro R, Novais C, Peixe L, Baquero F and Coque TM, 2016. Diversity and evolution of the Tn5801-tet (M)-like integrative and conjugative elements among *Enterococcus*, *Streptococcus*, and *Staphylococcus*. Journal of Antimicrobial Agents and Chemotherapy, 60, 1736–1746. https://doi.org/10.1128/AAC.01864-15

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CFU	colony forming unit
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction
WGS	whole genome sequence