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Safety and efficacy of a feed additive consisting of *Bifidobacterium longum* CNCM I-5642 (PP102I) for cats and dogs (Nestlé Enterprises S.A.)

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, Mohan Raj, Montserrat Anguita, Rosella Brozzi, Jaume Galobart, Elisa Pettenati, Joana Revez, Jordi Tarrés-Call and Jordi Ortuño

Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of *Bifidobacterium longum* CNCM I-5642 (PP102I) when used as a feed additive for cats and dogs. The product under assessment consists of viable cells of a strain of *B. longum*, a species considered suitable for the qualified presumption of safety (QPS) approach to safety assessment. The strain was unambiguously identified as *B. longum* and was shown not to harbour antimicrobial resistance determinants for antibiotics of human and veterinary importance, thus meeting the QPS requirements. Following the QPS approach to safety assessment and since no concerns are expected from maltodextrin, the other component of the additive, PP102I was considered safe for the target species and the environment. Owing to the lack of data, no conclusions could be drawn on the skin/eye irritancy potential of PP102I. However, it should be considered a skin and respiratory sensitiser. The Panel was not in the position to conclude on the efficacy of PP102I for the target species.

Keywords: zootechnical additives, physiological condition stabilisers, *Bifidobacterium longum* CNCM I-5642, PP102I, cats, dogs, QPS

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Correspondence: feedap@efsa.europa.eu

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 Nestlé Enterprises S.A., (NESA), Nestlé Purina PetCare EMENA Division represented in the EU by Centres de Recherche et Développement Nestlé[2] for the authorisation of the additive consisting of *Bifidobacterium longum* CNCM I-5642 (PP102I) when used as a feed additive for cats and dogs (category: zootechnical additive; functional group: physiological condition 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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 28 May 2021.

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 and user and on the efficacy of the feed additive consisting of *Bifidobacterium longum* CNCM I-5642 (PP102I), when used under the proposed conditions of use (see Section 3.1.5).

1.2 Additional information

The feed additive consisting of *Bifidobacterium longum* CNCM I-5642 (PP102I) has not been previously authorised as a feed additive in the European Union (EU).

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 *Bifidobacterium longum* CNCM I-5642 (PP102I) as a feed additive.

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' 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 agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

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[5] 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 FEEDAP Panel, 2017a), Guidance on 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] 34–40, 34 Rue Guynemer 92,130 Issy-Le Moulineaux (France).
[3] FEED dossier reference: FAD-2021-0031.
[4] The full report is available on the EU Science Hub website: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0031_en
[5] 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.

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) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).

3 Assessment

The additive under assessment consists of *Bifidobacterium longum* CNCM I-5642 and is intended for use as a zootechnical additive (functional group: physiological condition stabilisers) in feed for cats and dogs. The additive will be referred to with its trade name PP102I.

3.1 Characterisation

3.1.1 Characterisation of the active agent

The *Bifidobacterium longum* strain was isolated from faeces of a healthy infant. It is deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) with the accession number CNCM I-5642.[6] It has not been genetically modified.

The taxonomic identification of the strain was confirmed using whole genome sequence (WGS)-based analyses. [redacted][7]

The antimicrobial susceptibility testing was performed [redacted] and included the antimicrobials recommended by the FEEDAP Panel Guidance (EFSA FEEDAP Panel, 2018b).[7] [redacted] the strain is considered susceptible to all relevant antibiotics.

The WGS data of the strain was interrogated for the presence of antimicrobial resistance (AMR) genes [redacted][7] No hits of concern were identified. The WGS data was also queried for the presence of virulence factors [redacted][7] [redacted] No hits of concern were identified.

3.1.2 Characterisation of the additive

[redacted][8]

PP102I consists of the active agent *Bifidobacterium longum* CNCM I-5642 with a minimum guaranteed content of 5×10^{10} colony forming units (CFU) per gram of additive. Analytical data of five batches of the additive confirmed the specifications, with an average of 7.72×10^{10} CFU/g (range $6.00–9.50 \times 10^{10}$ CFU/g).[9]

Three batches of the additive were analysed for chemical impurities.[10] Cadmium (Cd), lead (Pb) and mercury (Hg) contents showed the following average values: 0.061 (0.058–0.067) mg Cd/kg; 0.018 (0.017–0.019) mg Pb/kg; 0.016 (0.016–0.017) mg Hg/kg. For arsenic (As), two batches showed

[6] Technical dossier/Section II/Annex II_2_1_2 and Supplementary Information January 2022/ Annex_II_2_1_2.
[7] [redacted]
[8] [redacted]
[9] Technical dossier/Section II/Annex II_1_3.
[10] Technical Dossier/Section II/Annex II_1_4_1.

values below the limit of quantification (LOQ)[11] and one batch showed 0.017 mg As/kg. A series of other elements[12] were also determined with values below 1 mg/kg, except for manganese (range 1.9–2.7 mg/kg) and zinc (range 9.1–9.8 mg/kg; analysed in two batches only). The content of nickel ranged between 0.12 and 0.16 mg/kg.

In the same batches, the concentration of all mycotoxins analysed were below LOD.[13] The sum of polychlorinated dibenzodioxins and dibenzofurans (PCDDs and PCDFs) was 0.222 (0.099–0.463) ng WHO-PCDD/F-TEQ/kg and the sum of PCDDs and PCDFs and coplanar dioxin-like polychlorinated biphenyls (DL-PCBs) was 0.246 (0.112–0.505) ng WHO-PCDD/F-PCB-TEQ/kg; the sum of non-DL-PCBs ranged from 0.076 to 0.165 μg/kg additive.

The same batches of the additive were analysed for microbial contamination. The results showed values for Enterobacteriaceae (not detected in 10 g), *Salmonella* spp. (not detected in 25 g), yeasts and filamentous fungi (< 10 CFU/g), *Escherichia coli* (< 10 CFU/g), aerobic mesophiles (< 10 CFU/g), *Clostridium perfringens* (< 10 CFU/g), *Bacillus cereus* (< 10 CFU/g), *Cronobacter* spp. (not detected in 10 g), coliforms (< 10 CFU/g), coagulase positive staphylococci (< 10 CFU/g) and spores of mesophilic bacteria (< 10 CFU/g).

The detected amounts of the above described impurities do not raise safety concerns.

3.1.3 Physical properties of the additive

The additive appears as a dry powder with an average bulk density of 459 (439–493) kg/m^3 and a true density of 1,620 (1,570–1,680) kg/m^3.[14]

The dusting potential of three batches of the additive was determined using the Stauber–Heubach method and showed that the product is dust free (0 mg/m^3). The same batches were analysed for the particle size distribution by laser-diffraction method. The results showed that particles < 100, < 50, and < 10 μm were on average 30%, 8% and 1%, respectively.

3.1.4 Stability and homogeneity

The stability of the additive was studied for shelf-life in samples from three batches when stored in aluminium bags at 4°C for 8 months. Negligible losses (< 0.5 log of the initial value) were observed at the end of the storage period.[16]

[redacted]

The stability of the additive (one batch) in a complementary dry feed for cats and dogs was studied when supplemented at 1.6 × 10^{10} CFU/kg feed and stored in sealed aluminium sachets at 23°C or 32°C for 88 and 48 days, respectively. Negligible losses (< 0.5 log of the initial value) were observed under the above-mentioned conditions.

The homogeneous distribution of the additive was studied in 36 subsamples of a complementary dry pet feed supplemented with 1.6 × 10^{10} CFU/kg feed. The coefficient of variation was 15%.[17]

3.1.5 Conditions of use

The additive is intended for use in feed for dogs and cats via complementary feed at the minimum recommended daily dose of 1 × 10^9 CFU, which would be equivalent to 3.5 × 10^9 and 1.1 × 10^{10} CFU/kg complete feed, respectively.

[redacted]

[11] LOQs: 0.004 mg Cd/kg; 0.01 mg Hg/kg; 0.007 mg Pb/kg and 0.017 mg As/kg.
[12] Chromium (range 0.085–0.103 mg/kg), nickel (range 0.12–0.16 mg/kg), copper (range 0.45–0.57 mg/kg), molybdenum (range 0.054–0.065 mg/kg), selenium (two batches were below the limit of detection (LOD) – 0.01 mg/kg and one batch measured 0.013 mg/kg), antimony (three batches below LOQ – 0.005 mg/kg), aluminium (range 0.38–0.59 mg/kg; analysed in two batches only), cobalt (0.059 mg/kg; analysed only in one batch) and tin (three batches below LOD – 0.2 mg/kg).
[13] LODs: Aflatoxin B1, B2, G1 and G2–0.025 μg/kg; ochratoxin A – 0.25 μg/kg; zearalenone – 1 μg/kg; deoxynivalenol and fumonisins B1 and B2–25 μg/kg; nivalenol and HT-2 Toxin – 2.5 μg/kg; and T-2 toxin – 50 μg/kg.
[14] Technical Dossier/Section II/ /Annex_II_1_5a.
[15] Technical Dossier/Section II/ /Annex_II_1_5b.
[16] Technical dossier/Section II/Annex II_4.1.
[17] Technical dossier/Section II/Annex II_4.2.

3.2 Safety

The species *B. longum* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020). The strain was unambiguously identified as *B. longum* and was shown not to harbour any antimicrobial resistance genes to clinically relevant antimicrobials, thus meeting the QPS requirements. Consequently, the strain is presumed safe for the target animals and the environment. Since no concerns are expected from the other components of the additive (maltodextrin), PP102I can also be presumed safe for the target animals and the environment.

3.2.1 Safety for the user

Given the nickel content of the additive and the proteinaceous nature of the active agent, the additive should be considered a respiratory sensitiser. However, considering that the product is dust-free, exposure of users by inhalation is unlikely.[15]

No specific data on skin/eye irritation or skin sensitisation were provided for the additive under application. Therefore, no conclusions can be drawn on the skin/eye irritancy of PP102I. The additive is considered a skin sensitizer.

3.3 Efficacy

The additive is intended at reducing stress-related responses and, therefore, to contribute at improving animal welfare and resilience to stress factors when fed to dogs and cats at a daily minimum dose of 1×10^9 CFU per animal.

3.3.1 Efficacy for dogs

A total of three trials were submitted to support the efficacy of the additive in dogs. The details on the study design are provided in Table 1, whereas the results of the main physiological parameters measured in Table 2 and those of the behavioural evaluations in Tables A.1–A.3 (see Appendix A).

Table 1: Trial design and dosages of the efficacy trials performed in dogs

Trial	Design (no. of dogs) trial duration	Breed age body weight sex	Intended (CFU/dog per day)	Analysed (CFU/dog per day)	Calculated (CFU/kg feed 88% DM)
1[18]	Cross-over (24) 17 weeks	Labrador×Retriever 2–13 years 26–36 kg 50%♀♂	0 1×10^9	1.3×10^3 2.4×10^8	3.3×10^3 6.0×10^8
2[19]	Cross-over (20) 17 weeks	Beagle 2.5 years 8–15 kg 50%♀♂	0 1×10^9	9.0×10^3 9.5×10^8	5.4×10^4 5.7×10^9
3[20]	Cross-over (20) 25 weeks	Mixed breeds[21] 1–10 years 5–33 kg 60%♀/40%♂	0 1×10^9	< 100 9.8×10^8	< 100 2.9×10^9

CFU: colony forming unit.

The three trials followed a similar cross-over design, acting each dog as its own control. The studies lasted 17 (trials 1 and 2) or 25 (trial 3) weeks and were distributed in three phases: phase I – half of the dogs received a basal diet, whereas the other half received the same basal diet supplemented with the additive for 7 (trials 1 and 2) or 6 (trial 3) weeks; washout phase – all dogs

[18] Technical dossier/Section IV/Annex 3.1.
[19] Technical dossier/Section IV/Annex 3.2.
[20] Technical dossier/Section IV/Annex 3.3.
[21] Beagle (4), Brittany (2), Cairn Terrier (2), English Setter (1), Foxhound (1), German Shorthaired Pointer (2), Havanese (1), Miniature Pincher (1), Labrador Retriever (2), Miniature Schnauzer (2), Weimaraner (2).

receive the basal diet for 3 (trials 1 and 2) or 13 (trial 3)[22] weeks; phase II – the dogs previously receiving the basal diet were supplemented with the additive for 7 (trials 1 and 2) or 6 (trial 3) weeks and vice-versa.

In trials 1 and 3, performed in the same facilities, dogs were housed in groups of 2 animals and allocated to the two dietary treatments based on sex, age and/or body size. In trial 2, dogs were housed in groups of 5 animals with 2 pens per treatment and randomly allocated to two dietary treatments based on sex and baseline response to stress.[23] In all trials, dogs were fed a commercial extruded feed which was top-dressed with a complementary feed without (control) or with PP102I at an intended level of 1×10^9 CFU/dog per day (results of the analysis of the supplemented test item included in Table 1). Water was available *ad libitum* in all cases.

Feed intake and health status were recorded daily during the entire experimental period in all trials. Dogs were weighed biweekly (trials 1 and 3) or at the beginning and end of each phase (trial 2). In trials 1 and 3, the dog's behaviour[24] during each of the phases was checked on a weekly basis, twice per day (in the morning and afternoon), in the home kennel. At the end of each phase, a sample of fresh faeces was collected from the kennel and processed for the analysis of faecal moisture (trial 3) and bifidobacteria, lactobacilli and *Clostridium perfringens*,[25] and a formal reactivity test[26] (trial 1) or behavioural challenge[27] (trial 3) was performed to all dogs individually. The reactivity test and behavioural challenge measures included physiological condition[28] and behavioural changes.[29] In trial 2, at the end of each phase, an open-field test,[30] a thunderstorm testing[31] and a car ride assessment[32] were performed to all dogs individually. Two desensitisation sessions were performed after the open-field and thunderstorm testing in the same observational room.

In all trials, data were analysed with a generalised lineal model considering the treatment, phase and the interaction between both as the main effects, and the animal as random effect. Significance level was set as 0.05 in trial 1 and 0.10 in trials 2 and 3.

In trial 1, the dogs daily supplemented with the additive at 1×10^9 CFU/g feed for 7 weeks showed lower salivary cortisol and heart rate, and higher high frequency in comparison with control animals during the reactivity test. No effect was observed for pNN50, RMSSD and the ear/paw temperature. Out of the behavioural parameters measured during the reactivity test, higher lip-licking

[22] Note from the applicant: due to the impact of the COVID global pandemic, a decision was made to stop the study after phase I and to prolong the washout period until conditions allowed a more stable resumption of working conditions.

[23] A baseline pre-study (1 week) included two open field assessment to establish activity levels, thunderstorm testing to assess stress responses to the presentation of auditory stimuli, a desensitisation session to the testing room and a car ride session to evaluate stress during travel. Heart rate and serum cortisol parameters were evaluated during and after some of the tests, respectively, for the classification of the animals.

[24] Trial 1: frequency of barking, jumping, spinning, pacing; Trial 3: frequency of inside runs, outside runs, barking, jumping, spinning, pacing, upright, other vocals, lip licking, tail wagging.

[25] Gene copies of *Bifidobacteria*, *Lactobacillus* and *Clostridium perfringens* per gram of faeces based on quantitative PCR analysis.

[26] The reactivity test was designed to assess dogs' social and non-social stress response, separation-related stress behaviour responses and overall reactivity.

[27] The behavioural challenge was designed to assess generalised responses to situations that pet dogs might experience (visit to veterinary clinic, collection of blood samples, separation from owner/caregiver).

[28] Cardiac activity (heart rate (HR) and heart rate variability (HRV) – including high frequency (HF)), percent of heart beats where differences between the RR interval and the previous RR interval are greater than 50 ms (pNN50), the root mean square of successive differences between heartbeats (RMSSD), salivary/serum cortisol and body (eyes, ears, paws) temperature.

[29] Reactivity test assessment included frequency of sit, stand, lying down, tail-wagging, lip-licking, yawn, body shake, total activity and location in room. Behavioural challenge assessment included, among others, body position, eye contact, tail wagging, panting, lip-licking, posture and vocalisation; the overall assessment was expressed as an average score of positive/stress behaviour.

[30] The open-field test was performed in a special observational room and was designed to assess animal behaviour in the absence of noise stimuli. Activity measurements (distance travelled, inactivity duration, head movement frequency) were recorded and analysed.

[31] The thunderstorm testing was performed in a special observational room and was designed to assess animal behaviour in the presence of noise stimuli (thunder track for [redacted]). Activity measurements (distance travelled, inactivity duration, head movement frequency), global stress response (active – startle; bolting; vigilance; scanning; aimless pacing and circling; digging; scratching or climbing walls; actively retreating; and vocalisation – and reactive – panting; lip-licking; shaking; yawning; salivating; cowering; tail tucking; freezing near wall; vigilance; and general inactivity), heart rate and serum cortisol levels were recorded/sampled and analysed.

[32] The car ride assessment was designed to assess dogs' stress travel-induced response in a 10-min car-ride duration. Behaviour measurements during car ride (body position (duration of standing, sitting and lying down), frequency of lip licking, panting, yawning, escape attempts, vocalisation, vomiting, defecation, urination), heart rate and serum cortisol levels were recorded/sampled and analysed.

frequency and more time spent in central areas of the room were observed in the supplemented dogs. The scans performed daily to the dogs in their kennels revealed lower proportion of barking, jumping, spinning and pacing in the treated animals in comparison with the control ones. No effect of the dietary supplementation with the additive was observed in the overall body weight, feed intake and faecal bacteria.

In trial 2, the daily supplementation of dog feed with 1×10^9 CFU/g for 7 weeks showed no effect on any physiological parameter (heart rate and serum cortisol) during the car ride assessment or thunderstorm testing. Among the behavioural parameters measured by the different ethograms, lower lip-licking frequency score and shorter periods of standing were observed in supplemented animals during the car ride test.

In trial 3, during the behavioural challenge, the dogs receiving the additive for 6 weeks showed lower HR, faecal moisture content and pre/post-test difference in tympanic left/right temperature and higher HF, pNN50 and RMSSD in comparison with control dogs. No effect was observed in the salivary and serum cortisol or on the faecal bacteria. The average score of positive and stress-related behaviours in supplemented dogs was higher and lower, respectively, in comparison with the control ones. Among the behavioural parameters measured in the kennel, lower spinning, pacing, time upright and whines/growls frequencies were observed in the supplemented dogs in comparison with the control ones.

Table 2: Effects of the dietary supplementation with the additive (PP102I) on the physiological parameters measured during the different stress-induced tests in dogs

Trial	Treatments	Salivary cortisol(1)	Serum cortisol(2)	Heart rate(2)	HF	pNN50	RMSSD	Body Temp.
	CFU/dog per day	µg/dL	µg/dL(3)	bpm	ms²	%		°C
1	0	0.55[a]/0.54[a]	n/a	112[a]	821[b]	7.74	53.8	38.7/ 39.2(4)
	10^9	0.30[b]/0.32[b]	n/a	102[b]	1,465[a]	9.19	71.2	38.6/39.0
2	0	n/a	5.98/8.05	151/139	n/a	n/a	n/a	n/a
	10^9	n/a	6.13/7.61	159/140	n/a	n/a	n/a	n/a
3	0	0.31	1.80	115[a]	10,057[b]	53.2[b]	274[b]	0.5[a]/ 0.585[a](5)
	10^9	0.34	1.94	107[b]	20,725[a]	66.2[a]	327[a]	0[b]/0.023[b]

[a,b]: Mean values within a trial and within a column with a different superscript are significantly different $p < 0.10$.
n/a: not analysed.
(1): Play yard/Reactivity test.
(2): Post-thunder testing/Post- car ride test.
(3): Calculated from nmol/L values in the report (165/222–169/210); Post-thunder testing /Post-car ride test.
(4): Eye/Ear.
(5): Difference in tympanic left/right temperature before and after the behaviour test.

According to the definition provided in Regulation (EC) No 1831/2003, physiological condition stabilisers are 'substances or, when applicable, microorganisms, which, when fed to animals in good health, favourably affect their physiological condition, including their resilience to stress factors'. Therefore, the Panel would expect that the evaluation of the efficacy of this type of additives allows the demonstration of physiological effects on the animals regarding parameters commonly influenced by stress. The primary physiological stress-response system in mammals includes the activation of the hypothalamic–pituitary–adrenal axis, in which corticotropic releasing hormone is secreted by the hypothalamus, stimulating the pituitary to secrete adrenocorticotropic hormone which in turn stimulates the adrenal cortex to release glucocorticoids (Cockrem, 2013). This metabolic response leads to increase circulating cortisol, which might be considered the 'gold-standard' among the biomarkers used for assessing altered physiological states in response to stressful stimuli in most mammals, including dogs (Polgár et al., 2019). Changes in the cardiac response and body temperature are associated to stress stimuli in mammals through the activation of the sympathetic nervous system, and thus, have also been considered valid and effective parameters to evaluate these responses in dogs and cats (Travain et al., 2015; Wormald et al., 2017). Therefore, the joint evaluation of cortisol

and cardiac analysis aids the full evaluation of the complex stress response of mammals, which may be complemented by the temperature recording of the animals.

It is also regarded that the holistic evaluation of stress in pet animals might not exclusively rely on physiological measurements but also consider behavioural parameters (Kartashova et al., 2021). Indeed, behavioural responses to mild stress or day-to-day situations might not be related to the endocrine physiological response but could be considered an early adaptive response to a stimulus/ stress, and thus relevant in the context of the welfare evaluation of animals. However, there does not seem to be a generally accepted scale for visualising stress yet. Besides, behavioural responses reflecting anxiety and stress would be highly influenced by different aspects of the individual animal (sex, breed, physiological status, habituation to stimuli). Therefore, not all behavioural parameters can be regarded as fully valid and many can be context dependent.

Consequently, to support the efficacy of this type of additives when a claim to improve the animal welfare and the resilience to stress factors is done, positive changes in both relevant physiological and behavioural parameters are needed.

Out of the three trials submitted in dogs in the current application, trials 1 and 3 showed statistical differences in relevant physiological parameters (salivary cortisol, HR and HF in trial 1; HR, HF, pNN50, RMSSD and body temperature in trial 3) related to a likely lower activation of the metabolic stress responses. Therefore, this could be interpreted as a positive effect of the additive when the dogs are exposed to stressing situations. In both studies, the effect observed in the physiological responses was supported by changes in several behavioural parameters related to reduced stress and anxiety (see Appendix A). In contrast, no effect on any physiological parameter was observed in trial 2 and the behavioural changes observed were minor and limited to the car ride assessment.

With the current data, the FEEDAP Panel cannot conclude on the efficacy of the additive in dogs.

3.3.2 Efficacy for cats

The applicant submitted one trial in order to support the efficacy of the additive in cats. However, this study was not considered adequate to support the efficacy of the additive on welfare and stress resilience in the general population of healthy cats [redacted]. Therefore, in the absence of adequate data, the Panel cannot conclude on the efficacy of the additive in cats.

3.3.3 Conclusions on efficacy

Due to the lack of sufficient data, the FEEDAP Panel cannot conclude on the efficacy of the additive in cats and 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[33] and Good Manufacturing Practice.

4 Conclusions

The additive consisting of viable cells of *Bifidobacterium longum* CNCM I-5642 (PP102I) is considered safe for the target species and the environment.

The additive should be considered a skin and respiratory sensitiser, but inhalation exposure of users is considered unlikely. No conclusions can be drawn on the skin/eye irritancy potential of the additive.

In the absence of sufficient evidence, the FEEDAP Panel cannot conclude on the efficacy of PP102I for the target species.

[33] 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.

5 Documentation provided to EFSA/Chronology

Date	Event
19/03/2021	Dossier received by EFSA. PP102I (*Bifidobacterium longum* CNCM I-5642) for cats and dogs. Submitted by Nestlé Enterprises S.A., (NESA), Nestlé Purina PetCare EMENA Division represented in the EU by Centres de Recherche et Dévelopement Nestlé
09/04/2021	Reception mandate from the European Commission
28/05/2021	Application validated by EFSA – Start of the scientific assessment
24/08/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation*
30/08/2021	Comments received from Member States
20/09/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
23/09/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
13/10/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation / efficacy*
31/01/2022	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

ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CFU	colony forming unit
CV	coefficient of variation
DL-PCB	dioxin-like polychlorinated biphenyl
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
HACCP	hazard analysis and critical control points
LOD	limit of detection
LOQ	limit of quantification
OECD	Organisation for Economic Co-operation and Development
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
RH	relative humidity
WHO	World Health Organization

Appendix A – Results of the evaluation of the behavioural and parameters on the trials submitted to support the efficacy of the additive in dogs

Table A.1: Results of the effect of the additive on the weekly scan of day-to-day behavioural parameters in trials 1 and 3

Parameter[(1)]	Control	Treatment
Trial 1		
Barking	**56**	**42**
Jumping	**28**	**13**
Spinning	**10**	**5**
Pacing	**6**	**0**
Trial 3		
Barking	10	14
Jumping	6	4
Spinning	**1**	**0**
Pacing	**11**	**8**
Inside run at front	67	69
Inside run at back	14	14
Outside run	26	31
Upright on kennel	**29**	**20**
Other vocals	**7**	**3**
Lip licking	12	9
Tail wagging	51	54

Values in bold letter indicate statistical difference ($p < 0.10$) between treatments.
(1): All values are provided as the proportion of the total number of scans where the behaviour was observed.

Table A.2: Results of the effect of the additive on the behavioural parameters measured during the reactivity test (trial 1) and behavioural challenge (trial 3)

Parameter	Control	Treatment
Trial 1		
Sit	1.04	1.21
Stand	1.83	1.92
Lying down	0.33	0.33
Tail wagging (s)	102	121
Lip-licking	**8.21**	**13.4**
Yawn	0.50	0.08
Body shake	0.58	0.46
Trial 3[(1)]		
Positive behaviour	**3.09**	**7.79**
Stress behaviour	**18.6**	**7.94**

Values in bold letter indicate statistical difference ($p < 0.10$) between treatments.
(1): Average scores were calculated as the average of the assessment at four time points (1) initial saliva/tympanic temperature, (2) fitting with HR monitor, (3) blood draw, (4) final saliva/tympanic temperature by multiplying the frequency (1-none to 6-most of the time) by intensity (1-none to 6-severe).

Table A.3: Results of the effect of the additive on the behavioural parameters measured during thunder and car ride tests performed in trial 2

Parameter[(1)]	Control	Treatment
Thunder test		
Inactivity frequency	6.5	6.0
Inactivity duration	157	154

Parameter[(1)]	Control	Treatment
Head movement frequency	45.7	48.9
Head movement duration	9.0	9.3
Global	4.2	4.2
Intensity	2.8	2.8
Scanning	2.3	2.2
Active	0.7	0.6
Car ride test		
Lip licking frequency score	**4.4**	**3.9**
Vocalisation frequency	3.2	1.1
Standing	**163**	**127**
Panting	1.6	1.8
Yawning	1.6	1.8
Escape attempt	0.4	0.2
Salivating	0.7	0.8
Laying down	130	121

Values in bold letter indicate statistical difference ($p < 0.10$) between treatments.
(1): All values are provided as percentage of animals showing the behaviour.

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for *Bifidobacterium longum* (CNCM I-5642)

In the current application an authorisation is sought under Article 4(1) (new feed additive) for *Bifidobacterium longum* (CNCM I-5642) under the category/functional group 4(e) 'zootechnical additives'/'physiological condition stabilisers', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for cats and dogs.

According to the Applicant, the *feed additive* contains viable cells of the non-genetically modified strain *Bifidobacterium longum* (CNCM I-5642) as the active substance. The *feed additive* is to be marketed as a preparation containing a minimum content of the active substance of 5×10^{10} Colony Forming Unit (CFU)/g. The *feed additive* is intended to be used in *feedingstuffs* at minimum doses of 1.1×10^{10} and 3.5×10^{9} CFU/kg complete *feedingstuffs* for cats and dogs, respectively.

For the identification of *Bifidobacterium longum* (CNCM I-5642), the EURL recommends for the official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for the genetic identification of bacterial strains.

For the enumeration of *Bifidobacterium longum* (CNCM I-5642) in the *feed additive* and *feedingstuffs*, the Applicant submitted a single-laboratory validated and further verified pour plate count method using a reinforced clostridial agar medium.

The following performance characteristics were obtained in the frame of the validation and verification studies:

i) for the average measured content of the active substance in the *feed additive* of 1.3×10^{11} CFU/g: a standard deviation for *repeatability* (Sr) and *intermediate precision* (Sip) of 0.06 $\log_{10}$ CFU/g;
ii) for the average measured content of the active substance in *feedingstuffs* ranging from 9.2×10^{8} to 8.0×10^{9} CFU/g: Sr ranging from 0.04 to 0.13 $\log_{10}$ CFU/g and Sip ranging from 0.04 to 0.17 $\log_{10}$ CFU/g.

Furthermore, a limit of quantification (LOQ) of 100 CFU/g *feedingstuffs* can be derived following the recommendations of ISO 7218 standard.

Based on the performance characteristics and the experimental data available, the EURL recommends the pour plate count method using a reinforced clostridial agar medium for the official control for the enumeration of *Bifidobacterium longum* (CNCM I-5642) in the *feed additive* and *feedingstuffs*.

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