

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Prostora Max (*Bifidobacterium animalis*) as a feed additive for dogs^{1, 2}

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)^{3, 4}

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ABSTRACT

Prostora Max is a feed additive based on a strain of *Bifidobacterium animalis* that has never been authorised in the EU. It is intended to be used in dogs at a proposed dose of 1.0×10^9 colony-forming units (CFUs)/day in the form of a supplemental treat. The species *B. animalis* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment, if its identity is confirmed and the absence of resistance to antibiotics of human and veterinary clinical significance demonstrated. However, the *B. animalis* strain is resistant to the antibiotic clindamycin. In the view of the FEEDAP Panel the antibiotic resistance qualification of the *B. animalis* strain has not been met and the QPS approach cannot therefore be applied. Although the applicant, on the basis of a bioinformatic analysis, could exclude the presence of many known antibiotic resistance genes, the genetic basis of the resistance to clindamycin was not established. In the absence of information on the genetic basis of this resistance, the extent of the risk of horizontal gene transfer to other bacteria in the food chain and in the environment cannot be established. All the excipients of the additive are authorised for food or feed use or feed materials. The product is in a tablet form so exposure to the mucous membranes and respiratory system of users is unlikely. Some skin exposure might occur, as the cocoa butter would melt at body temperature, but skin exposure would be limited. Treatment with Prostora Max at least 1×10^9 CFUs/day had an effect on gastrointestinal-related parameters in dogs. In two studies this took the form of an improved faecal score. However, in one study this improvement was marginal and of questionable biological/practical value. Therefore, taking into consideration the results of all three studies assessed, the FEEDAP Panel cannot conclude on the efficacy of Prostora Max for dogs.

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KEY WORDS

Zootechnical additive, Prostora Max, *Bifidobacterium animalis*, dogs, safety, efficacy

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² This scientific opinion has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety and efficacy of Prostora Max (*Bifidobacterium animalis* subsp. *animalis*) for dogs. This product has not been previously authorised in the European Union.

Prostora Max is the trade name for a feed additive based on a strain of *Bifidobacterium animalis* subsp. *animalis*. The additive is produced in the form of a tablet and is intended for dogs to supply a minimum of 1×10^9 CFUs of *B. animalis* per day.

The species *B. animalis* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment if its identity is confirmed and the absence of resistance to antibiotics of human and veterinary clinical significance is demonstrated. However, the *B. animalis* subsp. *animalis* strain is resistant to clindamycin, an antibiotic of human and veterinary importance. In the view of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), the antibiotic resistance qualification of the strain of *B. animalis* has not been met and the QPS approach cannot therefore be applied. Although the applicant, on the basis of a bioinformatic analysis, could exclude the presence of many known resistance genes, the reason for the resistance to clindamycin was not established. In the absence of information on the genetic basis of this resistance, the extent of the risk of horizontal gene transfer to other bacteria in the food chain and in the environment cannot be established.

All the excipients of the additive are authorised for food or feed use, or feed materials. The product is in a tablet form so exposure to the mucous membranes and respiratory system of users is unlikely. Some skin exposure might occur, as the cocoa butter would melt at body temperature, but skin exposure would be limited.

Treatment with Prostora Max at least 1×10^9 CFUs/day had an effect on gastrointestinal-related parameters in dogs. In two studies this took the form of an improved faecal score. However, in one study this improvement was marginal and of questionable biological/practical value. Therefore, taking into consideration the results of all three studies assessed, the FEEDAP Panel cannot conclude on the efficacy of Prostora Max for dogs.

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BACKGROUND

Regulation (EC) No 1831/2003⁵ establishes the rules governing the European Union 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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Procter & Gamble Eurocor⁶ for authorisation of the product Prostora Max, *Bifidobacterium animalis* ssp. *animalis* NCIMB 41617, to be used as a feed additive for dogs (category: zootechnical additive; functional group: gut flora stabiliser) under the conditions mentioned in Table 1.

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.⁷ According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 9 March 2010.

The additive Prostora Max is a preparation of *Bifidobacterium animalis* ssp. *animalis* NCIMB 41617. This product has not been previously authorised in the European Union.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 animal(s), user and the environment and the efficacy of the product Prostora Max (*Bifidobacterium animalis* ssp. *animalis* NCIMB 41617), when used under the conditions described in Table 1.

⁵ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

⁶ Procter & Gamble Eurocor, Temselaan 100, B-1853 Stombeek-Bever, Belgium.

⁷ EFSA Dossier reference: FAD-2009-0037.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	<i>Bifidobacterium animalis</i> AHC7
Registration number/EC No/No	none
Category(-ies) of additive	Zootechnical additive
Functional group(s) of additive	Gut flora stabiliser

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
Solid nutritional supplement	none	none	none

Trade name	Prostora Max
Name of the holder of authorisation	none

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/day		
Dogs	No limit	1 x 10 ⁹	none	none

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	none
Specific conditions or restrictions for handling	Store a cool dry place
Post-market monitoring	To be monitored by Procter & Gamble European Pet Care procedures
Specific conditions for use in complementary feedingstuffs	None

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
None	None	None	None

ASSESSMENT

1. Introduction

Prostora Max is the trade name for a feed additive based on a strain of *Bifidobacterium animalis* subsp. *animalis*. The applicant has requested an authorisation for use with dogs under the category zootechnical additive (functional group: gut flora stabiliser). This product has not been previously authorised in the European Union.

Bifidobacterium animalis is considered by EFSA to be a species suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007, 2011). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show resistance to antibiotics of human and veterinary importance.

2. Characterisation⁸

2.1. Characterisation of the active agent

The *B. animalis* subsp. *animalis* strain was isolated from canine intestinal tract and has not been subject to any genetic modification.⁹ It is deposited at the National Collection of Industrial, Marine and Food bacteria with accession number NCIMB 41617.¹⁰

The strain has been identified to the genus level with phenotypic tests (phosphofructotetolase test, Analytical Profile Index 50CHL) and to species level with 16S-23S rRNA gene spacer region sequencing.¹¹ Genetic stability of *B. animalis* NCIMB 41617 has been shown in a series of scale-up production batches (three batches, each about two months apart) using repetitive sequence-based polymerase chain reaction (PCR) (rep-PCR).¹²

2.1.1. Antibiotic resistance

Resistance to the antibiotics of clinical relevance recommended by EFSA (EFSA, 2012) was tested with the broth microdilution method using Isosensitest (90 %)—MRS (10 %) broth.¹³ The minimum inhibitory concentrations (MICs) of *B. animalis* NCIMB 41617 were below or equal to the cut-off values defined by EFSA for all antibiotics except clindamycin (4 mg/L vs 1 mg/L). D'Aimmo et al. (2007) studied the susceptibility of 21 *B. animalis* subsp. *lactis* strains with the broth microdilution technique and found that MICs for clindamycin were between 0.01 and 0.5 mg/L (MIC₅₀ 0.1 mg/L). In the study by Masco et al. (2006) the MICs of two *B. animalis* subsp. *animalis* and two *B. animalis* subsp. *lactis* strains were studied with broth microdilution. For *B. animalis* subsp. *animalis* clindamycin MICs were 0.5 and 1 mg/L and for *B. animalis* subsp. *lactis* ≤ 0.125 mg/L. In the more recent study of Xiao et al. (2010), MICs were determined with broth microdilution for 15 bifidobacterial isolates from foods, supplements and pharmaceuticals and for ATCC/other strains representing the same species (eight strains). For *B. animalis* subsp. *lactis* (five isolates from food and an ATCC strain) the MICs for clindamycin were ≤ 0.032 mg/L (except for one isolate 0.063 mg/L). For other *Bifidobacterium* species clindamycin MICs were typically ≤ 0.032 (12 strains); five strains had a MIC of 0.063–0.5 mg/L.

In the study of Mättö et al. (2007) bifidobacterial MICs for clindamycin were determined for 203 strains with the Etest. Nineteen *B. animalis* strains (17 *B. animalis* subsp. *animalis* and two *B. animalis* subsp. *lactis* strains) were studied. The vast majority of the strains had a MIC of ≤ 0.06 mg/L. Two *B. animalis* strains were considered to be potentially resistant; one with a MIC of 2–4 mg/L and

⁸ This section has been edited following the provision of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

⁹ Technical dossier/Section II/Annex_II_12.

¹⁰ Technical dossier/Section II/Annex_II_13.

¹¹ Technical dossier/Section II/Annexes_II_11 and 14.

¹² Technical dossier/Section II/Annex_II_15.

¹³ Technical dossier/Section II/Annex_II_16.

the other ≥ 256 mg/L. In the study of Ammor et al. (2008a) two *B. animalis* strains were studied among nine other *Bifidobacterium* spp. strains for MICs with the Etest. The MICs of sensitive strains to clindamycin were ≤ 0.25 mg/L (most commonly < 0.032 or 0.064 mg/L). A three-modal distribution of bifidobacterial MICs to clindamycin was seen: the sensitive strains; two strains with MICs of 1 and 2 mg/L; and eight strains with MICs of ≥ 256 mg/L. In the study of Mättö et al. (2006) the MICs of sensitive bifidobacterial strains to clindamycin were ≤ 0.12 mg/L when 42 strains altogether were studied (including six *B. animalis* subsp. *lactis* or *animalis* strains) using the Etest. Three strains were outside the uni-modal MIC distribution; the MIC values for these strains were 0.5–2 mg/L. In the study by Delgado et al. (2005) clindamycin MICs were determined for 76 bifidobacteria strains using both microdilution and agar dilution techniques (*B. animalis* strains were not included). Sixty-two strains had a MIC of ≤ 0.5 mg/L. Fourteen strains were separated from this group with MICs of 4–16 mg/L (eight strains), 64 mg/L (three strains) and ≥ 256 mg/L (three strains). Matteuzzi et al. (1983) studied 459 bifidobacterial strains (no *B. animalis* strains) for clindamycin MICs with broth dilution. No MIC distributions were given but the MIC₅₀ was between 0.05 and 0.38 mg/L and the MIC₉₀ between 0.09 and 0.89 mg/L.

The applicant also provided information about the clindamycin MICs of five additional *B. animalis* strains, besides the strain NCIMB 41617.¹⁴ Three of the strains were clearly susceptible to clindamycin (MIC < 0.032 mg/L), whereas the two others had MICs of 2 mg/L.

In conclusion, based on the scientific literature, clindamycin MICs for bifidobacteria, and also for *B. animalis* seem to have a three-modal distribution: clearly sensitive strains typically have MICs ≤ 0.12 mg/L; clearly resistant strains ≥ 256 mg/L; and potentially intermediately resistant strains 1–64 mg/L. As there are both clindamycin-susceptible and -resistant *B. animalis* strains, this species cannot be considered intrinsically resistant to clindamycin.

The whole genome of the *B. animalis* strain was sequenced, and similarities for antibiotic resistance genes were searched using the Antibiotic Resistance Genes Database. No genes conferring resistance to clindamycin were found.¹⁵ The *tet(W)* gene which confers tetracycline resistance, was identified in the genome. This gene is common in many bifidobacteria and can occasionally be found also in tetracycline-susceptible strains (Ammor et al., 2008b). Furthermore, eight multidrug transporter proteins or efflux pumps were identified in the genome, but their potential role in conferring resistance to clindamycin remains speculative.

The FEEDAP Panel concludes that, owing to the elevated MIC (4 mg/L) of *B. animalis* subsp. *animalis* NCIMB 41617, this strain has to be regarded as intermediately resistant for clindamycin. In the absence of information on the genetic nature of a demonstrated resistance, the FEEDAP Panel cannot conclude on the extent of the risk of horizontal gene transfer to other bacteria in the food chain and in the environment.

2.2. Production process

B. animalis NCIMB 41617 is grown in sterilised media typical of those used for bifidobacteria, concentrated and mixed with other ingredients. A detailed description of the manufacturing process of the additive is provided in the dossier.¹⁶

2.3. Characterisation of the additive

The final product is sold in the form of supplemental treat/tablet containing live *B. animalis* NCIMB 41617 cells at the minimum content of 1.0×10^9 CFU per supplemental treat.

¹⁴ Technical dossier/Supplementary information June 2012/Annex II 2 2 2 4.

¹⁵ Technical dossier/Supplementary information June 2012/Annexes II 2 2 2 3 and 5.

¹⁶ Technical dossier/Supplementary information June 2012/Annexes II 2 2 2 3 and 5.

For chemical impurities testing was conducted on three different batches of finished product.¹⁷ The parameters analysed were mycotoxins (aflatoxins B1, B2, G1, G2; deoxynivalenol; fumonisins B1, B2, B3; ochratoxin A; T-2 toxin; zearalenone); dioxins, polychlorinated biphenyls; insecticides (carbamates, chlorinated hydrocarbons, organophosphates), arsenic and heavy metals (cadmium, lead, mercury).¹⁸ In all samples the analytes were either not detected or below the acceptable ranges. Microbiological studies were performed on the same three samples. The numbers of enterobacteria, coliforms and *E. coli* were < 10 CFUs/g, and *Salmonella* spp. was not detected in the sample of 25 g of the final product.

The applicant states that no ongoing monitoring for contaminants is planned at this time because specifications of materials from suppliers require certification for contaminants.

2.4. Stability of the additive

Six independent batches of Prostora Max (with two dilutions of *B. animalis* NCIMB 41617), three batches with minimum 1×10^8 CFUs/treat and three batches with minimum 1×10^9 CFUs/treat) were stored under three different conditions (4 °C (relative humidity (RH) not monitored), 22 °C/33 % RH, and 38 °C/50 % RH).¹⁹ The *B. animalis* counts remained within one log value loss per treat at 4 °C and at 22 °C during the seven-month study period. *B. animalis* counts decreased rapidly at 38 °C, with losses of around five log₁₀ values per treat after four or five months' storage.

The applicant recommends storing Prostora Max treat in a cool and dry place (at or below 22 °C/33 % RH) in original, closed packaging, giving an interim shelf-life of at least seven months.

2.5. Conditions of use

The recommended application of Prostora Max is one treat per dog per day, supplying a minimum of 1×10^9 CFUs of *B. animalis* NCIMB 41617.

2.6. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the *B. animalis* in animal feed. The executive summary of the EURL report can be found in the appendix.

3. Safety

3.1. Safety for target species

The species *B. animalis* is considered by EFSA to be suitable for the QPS approach for the assessment of safety if its identity is confirmed and the absence of resistance to antibiotics of human and veterinary clinical significance is demonstrated. In the view of the FEEDAP Panel, the antibiotic resistance qualification of *B. animalis* (NCIMB 41617) has not been met and therefore the QPS approach cannot be applied.

As the QPS approach cannot be applied, a tolerance study would be required to demonstrate the safety for the target species. However, taking into consideration that:

- the resistance to clindamycin is not intrinsic in the *B. animalis* species
- the genetic basis of the antimicrobial resistance of *B. animalis* NCIMB 41617 has not been established
- a potential for horizontal gene transfer among bacteria has not been excluded,

¹⁷ Technical dossier/Section II/Annexes_II_6 to 9.

¹⁸ Technical dossier/Section II/Annex_II_10.

¹⁹ Technical dossier/Supplementary information Sep 11/Annex II_4.1.4.

the FEEDAP Panel concludes that *B. animalis* NCIMB 41617 poses a risk for the spread of genes coding for resistance to an important antibiotic of human and veterinary importance, namely clindamycin, in the environment and in the food chain. Therefore, in view of this concern, the FEEDAP Panel did not request a tolerance study for the target species. However, the Panel recognises the potential toxicity of chocolate for dogs and is concerned about the presence of cocoa butter in the additive.

3.2. Safety for user/owner

All the excipients of the additive are authorised for food or feed use or feed materials. The product is in a tablet form, so exposure to the mucous membranes and respiratory system of users is unlikely. Some skin exposure might occur, as the cocoa butter would melt at body temperature, but skin exposure would be limited.

4. Efficacy

Eight *in vivo* trials, all performed in the same extra-European country, have been provided. Out of these, five studies²⁰ are considered not acceptable owing to important flaws in the experimental design (e.g., absence of a negative control, inclusion of confounding factors) and thus have been disregarded.

Of the three remaining, the first trial involved 121 dogs (Labrador Retriever or Labrador–Golden Retriever cross; 13–15 months old) raised in private homes from approximately eight weeks of age and transferred from home to a training centre (referred to as kennel transfer, KT).²¹ The trial was divided into three periods:

- The first period lasted two weeks and the dogs were fed a basal diet to establish baseline values.
- In the second period, dogs were assigned to one of four treatments, control or Prostora Max at 1×10^7 , 1×10^8 or 1×10^9 CFUs/day. The animals received Prostora Max via a supplemental treat for five weeks; this period finished when the dogs were transferred to the kennel.
- The third period was the observation phase (with continuous Prostora Max supplementation) and lasted 20 days post KT.

Overall, the supplementation of Prostora Max lasted eight weeks. Blood samples were collected from all subjects once at baseline (five weeks before KT) and on days 3, 10 and 20 after KT to assess serum concentrations of a series of biological stress markers: C-reactive protein (CRP), α 1-acid glycoprotein (AGP) and cortisol. Faeces were monitored and samples from all subjects collected at baseline (five weeks prior to KT) and on days 3, 4, 9, 10, 19 and 20 after KT. Faecal consistency was monitored daily during the whole experiment and scored using a scale of 1 to 5 (whereby 5 denotes dry and hard, 4 denotes optimal, 3 denotes soft formed, 2 denotes soft unformed, and 1 denotes liquid diarrhoea). Total bifidobacteria and *B. animalis* NCIMB 41617 were also determined on days 3, 10 and 20 post-KT. The general health and behaviour of the dogs were monitored.

The number of bifidobacteria in faeces increased according to the dose. The increase in the acute-phase protein serum AGP was significantly lower 10 days after KT for all three groups and 20 days post KT for groups receiving *B. animalis* NCIMB 41617 at 1×10^7 or 1×10^9 but not 1×10^8 CFUs/day. However, CRP (another acute-phase protein) and cortisol, a marker of stress, did not differ significantly. Gruys et al. (2005) mentioned that AGP is not a major acute-phase protein in most animal species except the cat. AGP plasma levels are affected by pregnancy, certain drugs and certain diseases. Hayashi et al. (2001) observed a similar or lower number of dogs with AGP responses than CRP responses following inflammatory stimuli such as *S. aureus* infection, bone fracture and sterilisation. Therefore, the significance of the observed effect on AGP is not clear. A

²⁰ Technical dossier/Section IV and Supplementary information 11 September and 12 June/Annexes IV_3_1, IV_3_2, IV_4_3, IV_3_4, and IV_3_10.

²¹ Technical dossier/Section IV and Supplementary information 11 September/Annexes IV_3_5 and IV_4_1 or IV_3_7.

statistically significant but very small increase in faecal scores was observed with the supplementation of Prostora Max at all doses (Table 2). However, the biological and practical relevance of these small differences is questionable.

Table 2: Effect of Prostora Max supplementation on faecal scores of dogs subjected to stress

Treatment (<i>B. animalis</i> NCIMB 41617 CFU/day)	Week 1	Week 2	Week 3	Overall
0	3.8 ± 0.1	3.9 ± 0.1	3.7 ± 0.1 ^a	3.8 ± 0.0 ^a
1 × 10 ⁷	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1 ^b	3.9 ± 0.1 ^b
1 × 10 ⁸	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1 ^b	3.9 ± 0.1 ^b
1 × 10 ⁹	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1 ^b	3.9 ± 0.1 ^b
	<i>P</i> ≤ 0.29	<i>P</i> ≤ 0.69	<i>P</i> < 0.05	<i>P</i> < 0.03

In the second trial, 12 bitches (Labrador Retriever or Labrador–Golden Retriever crosses) were randomly assigned to one of two treatment groups (six animals per group) to determine the effects of the supplementation of Prostora Max on stool consistency during gestation and lactation.²² One group received a dose ≥ 1 × 10⁹ CFUs of *B. animalis* NCIMB 41617 per day in the form of an oral treat while animals in the control group received the same treat containing no additive from day 30 of gestation until weaning (42 days post-whelping). Daily stools were collected and scored (on a 5-point scale, as described in the study above). Unacceptable stools (rated ≤ 2) were recorded from day 30 of gestation until weaning, for a period of approximately 10 weeks. Furthermore, the faecal microbial populations were determined on day 30 (baseline) and day 56 of gestation and also on day 30 of lactation.

There was no significant effect (*P* < 0.18) of the additive on the number of unacceptable stools during gestation, but it significantly reduced the overall absolute number of unacceptable stools during lactation (Table 3), although the effect was not consistent over the whole period (i.e., it was significantly reduced in weeks 1, 2, 3 and 5, but not affected in weeks 4 and 6). The percentage of unacceptable stools was significantly improved only in weeks 3 and 5. Analysis of faecal material post supplementation demonstrated that Prostora Max increased faecal *B. animalis* NCIMB 41617 populations on day 30 of lactation (*P* < 0.05).

²² Technical dossier/Supplementary information 11 September/Annex Trial IV.3.8.

Table 3: Effects of Prostora Max supplementation on number and percentage of unacceptable stools (score ≤ 2) during the lactation period of bitches

Parameter Treatment (CFU <i>B. animalis</i> NCIMB 41617 /day)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Whole period
No of unacceptable stools							
0	14.3 \pm 1.4	1.8 \pm 0.7	1.3 \pm 0.4	1.0 \pm 0.5	0.7 \pm 0.2	0.0 \pm 0.0	19.2 \pm 2.9
$\geq 1 \times 10^9$	5.0 \pm 1.4	0.2 \pm 0.7	0.0 \pm 0.4	0.3 \pm 0.5	0.0 \pm 0.2	0.0 \pm 0.0	5.5 \pm 2.9
	$P \leq 0.01$	$P \leq 0.11$	$P \leq 0.06$	$P \leq 0.35$	$P \leq 0.08$	$P < 1.00$	$P < 0.01$
% Total Observations							
0	82.8 \pm 6.3	6.5 \pm 2.9	5.0 \pm 1.7	3.4 \pm 3.2	2.4 \pm 0.8	0.0 \pm 0.0	–
$\geq 1 \times 10^9$	91.1 \pm 6.3	2.8 \pm 2.9	0.0 \pm 1.7	6.1 \pm 3.2	0.0 \pm 0.8	0.0 \pm 0.0	–
	$P \leq 0.38$	$P \leq 0.40$	$P \leq 0.07$	$P \leq 0.56$	$P \leq 0.06$	$P \leq 1.00$	

The third trial was a double-blind study with 74 puppies from 12 litters. Animals received either a daily Prostora Max treat (37 puppies) or a placebo (37 puppies) from 35 days of age, approximately one week prior to weaning, until around 95 days of age, six weeks after relocation to a foster home.²³ The primary goal of the trial was to determine the effects of Prostora Max on digestive health, stool consistency and general welfare in growing puppies covering the weaning period, the introduction of dry food, and through the first six weeks in the new home. Faecal samples were collected from all puppies prior to treatment initiation at 35 days of age and on 52 ± 2 days of age. Faecal scores were reported on a 5-point scale (as described before). Blood was sampled from all puppies at 52 ± 2 days for determination of cortisol, AGP and CRP concentrations and immunoglobulin A (IgA) and IgM concentrations.

Supplementation with Prostora Max resulted in significantly higher faecal counts of *B. animalis* and significantly lower counts of *E. coli* in the treated group at day 52 compared with the control group. The supplementation did not elicit a significant effect on average weekly faecal scores. However, on a weekly basis, significantly fewer treated puppies experienced gastrointestinal events (faecal score ≤ 2) overall and in the first two weeks compared with puppies in the control group (81.1 ± 7.4 vs 56.8 ± 7.4 % $P < 0.10$). With respect to incidence, treated puppies had fewer gastrointestinal events overall (3.7 ± 1.2 vs 7.05 ± 0.2 , $P < 0.1$) and in the sixth week (0.3 ± 0.4 vs 1.2 ± 0.4 , $P < 0.10$). Furthermore, significantly ($P < 0.06$) fewer puppies in the treated group required elective (i.e., not routine) veterinary interventions owing to gastrointestinal-related problems in comparison with untreated puppies. Six pups in the placebo group and three animals in the supplemented group had gut-related veterinary interventions.

CONCLUSIONS

²³ Technical dossier/Supplementary information 11 September and 12 June/Annexes Trial IV.3.9 and Trial IV.3.9_updated.

The species *B. animalis* is considered by EFSA to be suitable for the QPS approach to safety assessment if its identity is confirmed and the absence of resistance to antibiotics of human and veterinary clinical significance is demonstrated. However, *B. animalis* subsp. *animalis* NCIMB 41617 is resistant to clindamycin. In the view of the FEEDAP Panel, the antibiotic resistance qualification of the strain of *B. animalis* has not been met. Therefore, the QPS approach cannot be applied. Furthermore, although the applicant, on the basis of a bioinformatic analysis, could exclude the presence of many known resistance genes, the genetic basis of the resistance to clindamycin was not established. In the absence of information on the genetic basis of this resistance, the extent of the risk of horizontal gene transfer to other bacteria in the food chain and in the environment cannot be established.

All the excipients of the additive are authorised for food or feed use or feed materials. The product is in a tablet form so exposure to the mucous membranes and respiratory system of users is unlikely. Some skin exposure might occur, as the cocoa butter would melt at body temperature, but skin exposure would be limited.

Treatment with *B. animalis* NCIMB 41617 $\geq 1 \times 10^9$ CFUs/day had an effect on gastrointestinal-related parameters in dogs. In two studies this took the form of an improved faecal score. However, in one study this improvement was marginal and of questionable biological/practical value. Therefore, taking into consideration the results of all three studies, the FEEDAP Panel cannot conclude on the efficacy of Prostora Max for dogs.

DOCUMENTATION PROVIDED TO EFSA

1. “Prostora Max” Supplement for dogs containing *Bifidobacterium animalis* AHC7 (NCIMB 41617) as a zootechnical additive and gut flora stabiliser. August 2009. Submitted by Procter & Gamble Eurocor.
2. “Prostora Max” Supplement for dogs containing *Bifidobacterium animalis* AHC7 (NCIMB 41617) as a zootechnical additive and gut flora stabiliser. Supplementary information. September 2011. Submitted by Procter & Gamble Eurocor.
3. “Prostora Max” Supplement for dogs containing *Bifidobacterium animalis* AHC7 (NCIMB 41617) as a zootechnical additive and gut flora stabiliser. Supplementary information. July 2012. Submitted by Procter & Gamble Eurocor.
4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the method(s) of analysis for Prostora Max.
5. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Prostora Max²⁴

In the current application authorisation is sought for the microbial feed additive *Bifidobacterium animalis* AHC7 under the category 'zootechnical additives', functional group 'gut flora stabiliser', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the feed additive placed on the market in the form of pills containing *Bifidobacterium animalis* AHC7. It is intended to be used to guarantee a minimum daily dosage of 1×10^9 CFUs *Bifidobacterium animalis* AHC7. The product is marketed in a sealed tray, containing 15 individually pills, for a total of one week's supplementation. There are no target levels of the feed additive in complete feedingstuffs.

For the enumeration of *Bifidobacterium animalis* AHC7 strain in *feed additives* the applicant proposed method EN 15785. The performance characteristics of the ring trial validated method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.13 to 0.15 \log_{10} CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.24 to 0.25 \log_{10} CFU/g, and
- a limit of detection (LOD) of 1×10^5 CFU/kg in *feedingstuffs*.

Molecular methods were used by the applicant for identification of the active agent. The EURL recommends for official control, pulsed-field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

Further testing or validation is not considered necessary.

²⁴ The full report is available on the EURL website: <http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2009-0037.pdf>