SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Bonvital (*Enterococcus faecium)* as a feed additive for dogs[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The microbial feed additive Bonvital is a preparation of *Enterococcus faecium*. The applicant is seeking authorisation of Bonvital as a feed additive for dogs at the dose of 1 x 10^9 CFU/kg of complete feedingstuff. The safety for the user and the environment has been considered in the context of previous opinions. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has considered only the safety and the efficacy of Bonvital for dogs. A tolerance test was performed on adult dogs fed a diet to which Bonvital was added at 50 times the proposed dose. Although no adverse effects were observed on their immunological and haematological parameters, the inadequate experimental design did not allow the FEEDAP Panel to draw conclusions on the safety of Bonvital for dogs. Six experiments were provided examining the effects of the additive at use level in dogs. Two studies gave data on faecal consistency and four on immunological parameters. Microbiological parameters were also measured in one of these studies. The observation of reduced *Clostridium perfringens* counts and the results on faecal consistency do not support the functional group gut flora stabiliser. Data on immune response were inconsistent due in part to an inadequate experimental design and a lack of consistency in observations. Lymphocyte proliferation in response to a mitogen challenge was significantly greater in treated dogs in two studies, but it was not demonstrated that these animals will show an increased response to a specific antigen. Although there appeared to be a greater IgG response in vaccinated animals given Bonvital, this was seen only as an increase in total IgG in one study and as a specific IgG titer in one other study. Consequently the FEEDAP Panel was unable to identify any consistent immune response which might be considered beneficial to the animal.

KEY WORDS

Enterococcus faecium, zootechnical additive, dogs, safety, efficacy, immune response, IgG

[1] On request from the European Commission, Question No EFSA-Q-2006-318, adopted on 11 November 2009.

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[3] Acknowledgement: The European Food Safety Authority wishes to thank the members of the Working Group on on Micro-organisms including Eric Cox, Joaquim Brufau, Atte vonWright and Guido Rychen for the preparation of this opinion.

Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on of Bonvital as a feed additive for dogs. EFSA Journal 2009;7(11):1379. [11 pp.]. doi:10.2903/j.efsa.2009.1379. Available online: www.efsa.europa.eu

SUMMARY

The microbial feed additive Bonvital is a preparation of *Enterococcus faecium*. This product is authorised for use in piglets, pigs for fattening, and sows. The strain *Enterococcus faecium* in combination with *Lactobacillus rhamnosus* is also authorised for calves and piglets. The applicant is now seeking authorisation of Bonvital as a feed additive for dogs (category zootechnical additives, functional group gut flora stabiliser), at the dose of 1 x 10^9 CFU/kg of complete feedingstuff.

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 the product Bonvital (*Enterococcus faecium*) as a feed additive for dogs.

The safety for user and the environment has been considered in the context of previous opinions. The FEEDAP Panel is not aware of any new or additional information that would lead it to revise these conclusions. Consequently, the FEEDAP Panel has considered only the safety and the efficacy of Bonvital for dogs.

A tolerance test was performed on adult dogs fed a diet to which Bonvital was added at 50 times the proposed dose. Although no adverse effects were observed on immunological and haematological parameters of dogs, the inadequate experimental design did not allow the FEEDAP Panel to draw conclusions on the safety of Bonvital for dogs.

Six experiments were provided examining the effects of the additive at use level in dogs. Two studies gave data on faecal consistency and four on immunological parameters. Microbiological parameters were also measured in one of these studies. The observation of reduced *Clostridium perfringens* counts and the results on faecal consistency do not support the functional group gut flora stabiliser. No other parameters potentially related to the gut flora were measured. Data on immune response were inconsistent due in part to an inadequate experimental design and a lack of consistency in observations. Lymphocyte proliferation in response to a mitogen challenge was significantly greater in treated dogs in two studies, but this does not indicate that animals are able to show an increased response to a specific antigen. Although there appeared to be a greater IgG response in vaccinated animals given Bonvital, this was seen only as an increase in total IgG in one study and as a specific IgG titer in one other study. Consequently, the FEEDAP Panel was unable to identify any consistent immune response which might be considered beneficial to the animal.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lies down that any person seeking an 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 Lactosan Starterkulturen GmbH & Co[5] for authorisation of the product Bonvital (*Enterococcus faecium)* to be used as a feed additive for dogs (category: zootechnical additives; functional group: gut flora stabilisers) under 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.[6] 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 3 October 2006.

The additive Bonvital is a preparation of *Enterococcus faecium* (DSM 7134). This product is authorised for use in piglets and pigs for fattening,[7] and for sows.[8] The strain *Enterococcus faecium* (DSM 7134) in combination with *Lactobacillus rhamnosus* (DSM 7133) is also authorised for calves[9] and piglets.[10]

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety for pigs for fattening and calves, the consumer, user and environment of the product Bonvital containing *Enterococcus faecium* (DSM 7134) and *Lactobacillus rhamnosus* (DSM 7133) (EC, 1997, updated 2003). Another opinion on the safety for piglets, pigs for fattening and sows of the additive Provita E®, a preparation of *Enterococcus faecium* (DSM 7134), was issued by SCAN in 2003 (EC, 2003).

EFSA published one opinion on the safety of Bonvital (*Enterococcus faecium* DSM 7134) for chickens for fattening (EFSA, 2004), another one on the safety and efficacy of this product for the same target species (EFSA, 2009), one on the safety and efficacy for piglets and pigs for fattening (EFSA, 2007a) and another one on the safety and efficacy of Bonvital for sows (EFSA, 2007b).

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 efficacy and the safety for the target animal, user and the environment of the product Bonvital, which is a preparation of *Enterococcus faecium* DSM 7134, when used under the conditions described in Table 1.

[4] OJ L 268, 18.10.2003, p.29.
[5] Lactosan Starterkulturen GmbH & Co Industriestr. West 5 – 8605 Kapfenberg, Austria.
[6] EFSA dossier reference: FAD-2006-0006
[7] OJ L 128, 16.5.2007, p.16
[8] OJ L 335, 20.12.2007, p.24
[9] OJ L 243, 15.7.2004, p.10
[10] OJ L 370, 17.12.2004, p.24

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

Additive	*Enterococcus faecium* (DSM 7134)
Registration number/EC No/No	22
Categoryof additive	Zootechnical additives
Functional group of additive	Gut flora stabilisers

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
Preparation of *Enterococcus faecium* (DSM 7134) containing a minimum of : -Powder: 1 x 10^{10} CFU/g -Granules (micro-encapsulated): 1 x 10^{10} CFU/g		Impurities: Fungi: <100 CFU/g Clostridia: <10 CFU/g Enterobacteria: <10 CFU/g Salmonella: none detectable in 25 g	Quantification of lactic acid bacteria content (Code of the method: LAC-DO-EF1A)

Trade name	Bonvital
Name of the holder of authorisation	Lactosan Starterkulturen GmbH & Co

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
Dogs	-	1 x 10^9	1 x 10^9	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	
Specific conditions or restrictions for handling	The directions for use must indicate storage temperature, shelf life
Post-market monitoring	
Specific conditions for use in complementary feedingstuffs	-

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

The microbial feed additive Bonvital is a preparation of *Enterococcus faecium* (DSM 7134). This product is authorised for use in piglets, pigs for fattening, and sows. The strain *Enterococcus faecium* (DSM 7134) in combination with *Lactobacillus rhamnosus* (DSM 7133) is also authorised for use in calves and piglets (see Background).

The current dossier contains data supporting a request for authorisation under Regulation (EC) No 1831/2003 for the use of Bonvital as a feed additive for dogs.

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety for pigs for fattening and calves, the consumer, user and environment of the product Bonvital containing *Enterococcus faecium* (DSM 7134) and *Lactobacillus rhamnosus* (DSM 7133) (EC, 1997, updated 2003). Another opinion on the safety for piglets, pigs for fattening and sows of the additive Provita E®, a preparation of *Enterococcus faecium* (DSM 7134), was issued by SCAN in 2003 (EC, 2003). EFSA published one opinion on the safety of Bonvital (*Enterococcus faecium* DSM 7134) for chickens for fattening (EFSA, 2004), another one on the safety and efficacy of this product for the same target species (EFSA, 2009), one on the safety and efficacy for piglets and pigs for fattening (EFSA, 2007a) and another one on the safety and efficacy of Bonvital for sows (EFSA, 2007b).

The safety for the user and the environment has been considered in the context of the previous opinions. The FEEDAP Panel is not aware of any new or additional information that would lead it to revise these conclusions. Consequently, the FEEDAP Panel has considered only the safety and the efficacy of Bonvital for dogs.

2. Characterisation of the additive

The active ingredient of the additive Bonvital is the strain *Enterococcus faecium* DSM 7134.

This feed additive is available in two forms:

- Bonvital powder, with a guaranteed minimal concentration of viable bacterial cells in the additive of 1×10^{10} CFU/g in sweet whey powder.

- Bonvital granules, a microencapsulated form with a guaranteed minimal concentration of viable cells in the additive of 1×10^{10} CFU/g, containing saccharose (70%), maltodextrin (20%) and sodium citrate (1%).

The bacterial strain of Bonvital was deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen, with accession number DSM 7134.[11]

2.1. Conditions of use

The product is intended for use in feed for dogs at a dose of 1×10^{9} CFU/kg of complete feedingstuff.

2.2. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the *Enterococcus faecium* in animal feed. The Executive Summary of the CRL report can be found in Appendix A.

[11] Technical dossier/Section II_2

3. Safety for dogs

A tolerance test was conducted on ten Beagle adult dogs (four males and six females of approximately three years of age) fed with 50 times the recommended dose.[12] The experiment was divided in two phases of four weeks each: control and the tolerance period. The first week of both phases served as adaptation period to the diet, the remaining three weeks were used as experimental period. During phase 1, the control period, the animal received only the basal diet, while in phase 2, the tolerance period, the ten dogs received a dry diet supplemented with 4.7 x 10^{10} CFU/kg of feed of *E. faecium* DSM 7134. No experimental confirmation of the dose was provided.

Analysed parameters at the end of the three weeks experimental period were: health status of animals, faecal consistency, body score conditions, haematological parameters, concentration in serum of IgA, IgM, IgG and IgE, haptoglobin and C-reactive protein, nutrient digestibility and concentrations in faeces of IgA, ammonia and lactate. Microbiological analysis for the enumeration of *E. coli, Clostridium perfringens* and *Enterococcus spp.* in faeces was performed twice per phase.

Although, no adverse effects from a 50-fold overdose with the additive were observed on immunological (no decrease or increase in serum antibodies and faecal IgA) and haematological parameters of dogs, the inadequate experimental design (duration, lack of appropriate parameters) did not allow the Panel to draw conclusions on the safety of Bonvital for dogs.

4. Efficacy

A total of six studies made with dogs of various breeds and ages are described. With the exception of one trial, all the data originates from a single European country. Four studies aimed to demonstrate a positive effect on the immune system of dogs, while the remaining two, investigated faecal consistency of working dogs. In none of the experiments was the dose confirmed by analysis.

4.1. Immunological responses

The first study, performed with the same dogs as the tolerance trial, was divided into two phases: a control period of four weeks and a supplementation period of five weeks, when animals were given Bonvital at a dose of 1.8 x 10^9 CFU/kg feed.[13] The first week consisted of the adaptation period.
Analysed parameters at the end of the experimental periods were: health status, faecal consistency, defecation frequency, pH and dry matter (DM) of faeces, body mass, body score conditions, microbiological analysis of faeces (*Escherichia coli, Enterococcus* spp., *Clostridium perfringens),* differential blood counts, haematological examination. Analysed immunological parameters were: phenotyping of $CD4^+$, $CD8^+$, $CD21^+$ and $CD5^+$ lymphocytes, lymphocytes proliferation test after stimulation with Concanavallin A (ConA), Phytohaemoagglutinin (PHA) and Pokeweed Mitogen (PWM), concentration in serum of IgA, IgM, IgG and IgE, concentration in faeces of IgA. Ammonia and lactate concentration in faeces and nutrient digestibility were also determined.
Data were examined by analysis of variance (ANOVA) for significant differences between control and treated periods.

Microbiological analysis revealed an expected increase of *Enterococcus* spp. counts (7.5 Log *vs* 4.3 Log) and a statistically significant reduction (6.7 *vs* 7.6 Log) of *Clostridium perfringens* in the faeces of Bonvital treated animals.

The leukocyte analysis showed a significant increase in neutrophils (in five of ten dogs) and a significant decrease in eosinophils (in eight of ten dogs). Percentage of lymphocyte subsets showed a significant increase in $CD21^+$ B-lymphocytes (in nine of ten dogs) and a significant increase in $CD5^+$ lymphocytes (T-lymphocytes; in nine of ten dogs). However, the absolute numbers of $CD5^+$ lymphocytes were lower in the treated period.

[12] Technical dossier/Section IV and Supplementary information April 09
[13] Technical dossier/Section III/Trial 1

Lymphocyte proliferation showed a significant increase following Con A stimulation (in seven of ten dogs) but not for the other mitogens (PHA, PWM). However the stimulation index (SI) did not increase indicating that the background proliferation of the cells (proliferation without mitogen) was also increased (in six of ten dogs). The calculation of mean proliferation used was incorrect since the arithmetic mean was applied instead of the geometric mean.

The absence of a third period in which treatment is withdrawn makes uncertain the distinction between age-related and treatment-related effects.

Trials 2, 3 and 4, aimed to evaluate the effect of Bonvital supplementation on the immune response in vaccination trials, presented a similar experimental design. Animals were allocated to two groups: a control group receiving the basal diet supplemented with placebo and a treated group fed the basal diet supplemented with Bonvital at the recommended dose of 1 x 10^9 CFU/kg feed. Animals were vaccinated subcutaneously with a multivalent vaccine against distemper virus, hepatitis contagiosa canis, kennel cough, parvovirus, leptospirosis and rabies.

Analysed parameters in all three studies were: haematology, lymphocyte proliferation test with stimulation with three mitogens (Con A, PHA, PWM), lymphocyte phenotyping, vaccination titers of canine distemper virus and rabies. IgG and IgA concentration in serum were determined on collected samples in Trial 3 and 4 while only IgG in Trial 2. Statistical analysis was based on independent sample t-test and paired sample t-test. Normal distribution of the data was tested by Kolmogorov-Smirnov and Shapiro-Wilk test.

Trial 2 was performed on 19 adult dogs (Husky and Malamute breeds) randomly allocated to two groups, blocked for breed, age and gender.[14] Control and treated groups were, respectively composed by nine and ten animals. After four weeks of adaptation to diet, animals were subcutaneously immunised with the multivalent vaccine. Blood samples were collected at the first day of the study, on the day of vaccination, two weeks and four weeks after vaccination.

In this trial, Con A induced a significantly higher lymphocyte proliferation in the treatment group ($P<0.05$). However, only the SI was given and not the absolute data. No difference in antibody titers was seen between control and treatment group. The vaccine appeared to induce only a limited response. Normally antibody responses are only significant if there is a four-fold rise in the titer. However, it is unclear whether this observation is due to a limited response to vaccination or to inadequacies in methodology. Inflammation parameters were not determined.

Trial 3 was made with eight-week-old Beagle puppies from the same litter. The eight animals were allocated to two groups (two males and two females per group).[15] Animals were vaccinated subcutaneously with two doses of the multivalent vaccine at the beginning of the experiment and two weeks later. Blood samples were collected four days before the vaccination and two weeks after the second vaccination.

In this study lymphocyte proliferation was not significantly affected by mitogen induction ($P>0.05$). Total IgG was significantly higher two weeks after vaccination in the treatment group compared to the control group. However, this was only a weak effect since differences between treated and control groups was 1.94 mg IgG/mL before vaccination and 2.56 mg IgG/mL after vaccination and did not result in significantly higher canine distemper- and rabies-specific IgG response in the treatment group.

In trial 4, seven eight-week-old Siberian Huskies from the same litter were allocated either to control group (one male and two females) fed a basal diet, or to treated group (one male and three females).[16] Animals were vaccinated subcutaneously at the beginning of the experiment and two weeks later with

[14] Technical dossier/Supplementary information April 09/Trial 4
[15] Technical dossier/Supplementary information April 09/Trial 5
[16] Technical dossier/Supplementary information April 09/Trial 6

the multivalent vaccine. Blood samples were collected four days before the vaccination and two weeks after the second vaccination.

Lymphocyte proliferation was not significantly affected by mitogen stimulation. Lymphocyte phenotyping showed a decreased expression of Major Histocompatibility Complex class II (MHCII) in the treated group (p= 0.096). MHCII expression is needed for presentation of antigen towards $CD4^+$ T lymphocytes (helper T cells). Dogs vaccinated and fed the additive showed a significant higher canine distemper-specific IgG response. However, total IgG did not increase, nor did the rabies-specific IgG response.

4.2. Feacal consistency

Two studies with a common experimental design aimed to assess the effect of Bonvital supplementation on the faecal consistency of working dogs were provided. Cross-over studies were performed on 14 (trial 5)[17] and 21 (trial 6)[18] adult healthy dogs of different breeds. The studies were divided in two periods of four weeks, with a two weeks washout interval in between. In the treatment periods animals were given Bonvital at 1 x 10^9 CFU/kg feed. Animals in trial 5 were privately owned and received different diets. Consequently this study is confounded. A single basal diet was used in trial 6. The quality of faeces and the frequency of defecation were recorded and graded by the owners (trial 5) and attendants (trial 6). Single faecal samples were collected from each animal at the end of each period and faecal dry matter analysed.

No effect related to Bonvital supplementation was observed in trial 6 other than an increase in faecal DM (26.5 *vs* 24.7, p= 0.056).

5. Post-market monitoring

No risks associated with the use of the product are foreseen. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[19] and Good Manufacturing Practice.

CONCLUSIONS

Although, no adverse effects from a 50-fold overdose with the additive were observed on immunological and haematological parameters of dogs, the inadequate experimental design did not allow the FEEDAP Panel to draw conclusions on the safety of Bonvital for dogs.

The observation of reduced *Clostridium perfringens* counts and the results on faecal consistency do not support the functional group gut flora stabiliser. No other parameters potentially related to the gut flora were measured.

Data on immune response were inconsistent due in part to a poor experimental design and a lack of consistency in observations. Lymphocyte proliferation in response to mitogen challenge (Con A) was significantly greater in treated dogs in two studies, but this does not indicate that animals are able to show an increased response to a specific antigen. Although there appeared to be a greater IgG response in vaccinated animals given Bonvital, this was seen only as an increase in total IgG in one study and as a specific IgG titer against one antigen in one other study. Consequently, the FEEDAP Panel was unable to identify any consistent immune response which might be considered beneficial to the animal.

[17] Technical dossier/Supplementary information April 09/Trial 2
[18] Technical dossier/Supplementary information April 09/Trial 3
[19] OJ L 35, 8.2.2005, p.1

DOCUMENTATION PROVIDED TO EFSA

1. Request for authorisation of Bonvital for the animal category dogs. *Enterococcus Faecium* (DSM 7134). A zootechnical additive. May 2006. Submitted by Lactosan Starterkulturen GmbH & Co.
2. Supplementary information, April 09. Submitted by Lactosan Starterkulturen GmbH & Co.
3. Evaluation report of the Community Reference Laboratory feed additives authorisation on the methods(s) of analysis for Bonvital (*Enterococcus faecium* DSM 7134) for dogs.
4. Comments from Member States received through the ScienceNet.

REFERENCES

EC (European Commission), 1997, updated 2003. Opinion on the use of certain micro-organisms as additives in feedingstuffs. http://ec.europa.eu/food/fs/sc/scan/out93_en.pdf

EC (European Commission), 2003. Report of the Scientific Committee on Animal Nutrition on the safety of the Micro-organism product Provita E® for use as feed additive. http://ec.europa.eu/food/fs/sc/scan/out107_en.pdf

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the safety of product Bonvital (Provita E) for chickens for fattening. EFSA Journal (2004) 120, 1-4. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620783351.htm

EFSA (European Food Safety Authority), 2007a. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the safety and efficacy of product Bonvital, a preparation of *Enterococcus faecium*, as a feed additive for piglets and pigs for fattening in accordance with Regulation (EC) No 1831/2003. EFSA Journal (2007) 440, 1-9. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620781889.htm

EFSA (European Food Safety Authority), 2007b. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the safety and efficacy of product Bonvital, a preparation of *Enterococcus faecium*, as a feed additive for sows in accordance with Regulation (EC) No 1831/2003. EFSA Journal (2007) 521, 1-8. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178622724657.htm

EFSA (European Food Safety Authority), 2009. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the safety and efficacy of product Bonvital, a preparation of *Enterococcus faecium*, as a feed additive for chickens for fattening in accordance with Regulation (EC) No 1831/2003. EFSA Journal (2009) 990, 1-12 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902390754.htm

APPENDIX A

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Bonvital (*Enterococcus faecium* DSM 7134) for dogs

In the current application authorisation is sought for the microbial feed additive Bonvital under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. The active agent in the additive is *Enterococcus faecium* DSM 7134. The additive is available in two forms (powder or granules (micro-encapsulated)) both of which contain a minimum concentration of 1 x 10^{10} colony forming units (c.f.u.) per gram. Specifically, authorisation is sought to use Bonvital for dogs. The conditions of use are proposed with a recommended dosage of 1 x 10^{9} c.f.u./kg.

For the quantification of the active agent (*Enterococcus faecium* DSM 7134) of Bonvital in the *feed additive*, *premixtures* and *feedingstuffs*, an appropriate surface plate count method was proposed by the applicant. The method was in-house validated and shown to be transferable to four external laboratories. The method precision data resulting from the in-house and four laboratory trials were acceptable for the intended purpose.

For official controls regarding the quantitative determination of the active agent in the *feed additive*, *premixtures* and *feedingstuffs*, another plate count enumeration method is recommended which has been fully ring-trial validated (Leuschner R.G.K. et al. 2002. J. Appl. Microbiol. 93, 781-786). The method performance characteristics include a relative standard deviation for repeatability (RSD_r) ranging between 1.5 to 3.6 % and a relative standard deviation for reproducibility (RSD_R) ranging between 2.9 to 7.4 %. The limit of quantification (LOQ) for the method is around 2 to 3 x 10^{6} c.f.u./kg sample which is well below the minimum anticipated target level of application in feedingstuffs.

The identity of the bacterial strain, *Enterococcus faecium* DSM 7134, was analysed by a range of techniques including biochemistry, protein-fingerprinting and molecular methods such as polymerase chain reaction (PCR) and pulsed field gel electrophoresis (PFGE). PFGE is a generally recognised standard methodology for microbial identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.