European Food Safety Authority

EFSA Journal 2013;11(2):3098

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Cylactin® (*Enterococcus faecium*) as a feed additive for cats and dogs[1, 2]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[3,4]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Cylactin® is the trade name for a preparation of *Enterococcus faecium.* It is currently authorised for use in chickens for fattening, pigs for fattening, piglets, sows, calves, turkeys for fattening, cats and dogs in various formulation. The product is intended for use with dogs at a minimum dose of 4.5×10^6 and a maximum dose of 2.0×10^9 CFU/kg feed and with cats at a minimum dose of 5.0×10^6 and a maximum dose of 8.0×10^9 CFU/kg feed. The Cylactin® strain does not contain marker genes typical of hospital-associated isolates responsible for clinical infections and is susceptible to clinically relevant antibiotics, except for kanamycin, which is considered of no concern. Cylactin® is safe for dogs and cats at the recommended dose range. The additive is not a skin/mucosal irritant or a skin sensitiser. As this formulation has a large particle size and the dusting potential is low, the potential for exposure via the respiratory route is considered minimal. Three studies carried out in dogs demonstrated that the additive has the potential to produce a beneficial effect in dogs, when added to feedstuffs at a dose of 2.5×10^9 CFU kg, by increasing the intestinal or serum concentration of IgA. Cylactin® showed inconsistent effects on faecal quality in three studies in which cats were fed the additive at the dose of 7×10^9 CFU/kg of feed.

KEY WORDS

Zootechnical additive, Cylactin®, *Enterococcus faecium*, gut flora stabiliser, dogs, cats, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2012-00420, adopted on 29 January 2013.

[2] This scientific opinion has been edited following the adoption of the Commission decision regarding certain confidentiality claims submitted by the applicant in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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[4] Acknowledgement: The Panel wishes to thank the members of the Working Group on Micro-organisms, including Andrew Chesson, Pier Sandro Cocconcelli, Eric Cox and Ingrid Halle, for the preparatory work on this scientific opinion.

Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of Cylactin® (*Enterococcus faecium*) as a feed additive for cats and dogs. EFSA Journal 2013;11(2):3098. [15 pp.] doi:10.2903/j.efsa.2013.3098. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

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 Cylactin® LBC ME5 PET for dogs and cats. Cylactin® is the trade name for a preparation of a strain of *Enterococcus faecium* which is currently authorised for use in chickens for fattening, pigs for fattening, piglets, sows, calves, turkeys for fattening, cats and dogs in various formulations.

The product is intended for use in dogs at a minimum dose of 4.5×10^6 and a maximum dose of 2.0×10^9 CFU/kg feed and in cats at a minimum dose of 5.0×10^6 and a maximum dose of 8.0×10^9 CFU/kg feed.

Many elements of the assessment of safety of this additive were considered in previous opinions. Consequently, the focus of the present opinion is on safety and efficacy when used with cats and dogs. Additional data were also provided on virulence determinants and on the susceptibility to antibiotics.

Enterococcus faecium NCIMB 10415 does not contain marker genes typical of hospital-associated isolates responsible for clinical infections and is susceptible to clinically relevant antibiotics, except for kanamycin, which is considered of no concern.

No adverse effects were seen when Cylactin® LBC ME5 PET was administered to cats and dogs at up to at least a 70-fold overdose. Consequently, it is concluded that the product is safe for cats and dogs at the recommended dose.

The additive is not a skin/mucosal irritant or a skin sensitiser. As this formulation has a large particle size and the dusting potential is low, the potential for exposure via the respiratory route is considered minimal.

Three studies carried out in dogs demonstrated that Cylactin® has the potential to produce a beneficial effect in dogs when added to feedstuffs at a dose of 2.5×10^9 CFU kg, by increasing the intestinal or serum concentration of IgA. Cylactin® showed inconsistent effects on faecal quality in three studies in which cats were fed the additive at the dose of 7×10^9 CFU/kg of feed. No significant effects on IgA were observed in the two studies in which this parameter was measured.

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BACKGROUND

Regulation (EC) No 1831/2003[5] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from DSM Nutritional Products Ltd[6] for re-evaluation of the product Cylactin®,[7] (*Enterococcus faecium* NCIMB 10415), when used as a feed additive for cats and 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 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[8] 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 30 April 2012.

Cylactin® has been authorised for chickens for fattening,[9] pigs for fattening,[10] piglets,[11] sows,[12] calves,[13] and cats and dogs.[14] The same strain is also authorised under a different trade name for calves,[15] piglets,[16] chickens for fattening,[17] turkeys for fattening and dogs.[18]

[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] DSM Nutritional Products Ltd, Switzerland represented by DSM Nutritional Products Spz.o.o., UI Tarcynska 113, PL 96-320, Mszczonow, Poland.
[7] The Applicant intends to market the product also under the tradename Cernivet.
[8] EFSA Dossier reference: FAD-2010-0294.
[9] Commission Implementing Regulation (EU) No 361/2011 of 13 April 2011 concerning the authorisation of *Enterococcus faecium* NCIMB 10415 as a feed additive for chickens for fattening (holder of authorisation DSM Nutritional products Ltd represented by DSM Nutritional Products Sp. z o.o) and amending Regulation (EC) No 943/2005. OJ L 100, 14.4.2011, p. 22.
[10] Commission Regulation (EC) No 943/2005 of 21 June 2005 concerning the permanent authorisation of additives in feedingstuffs. OJ L 159, 22.6.2005, p. 6.
[11] Commission Regulation (EC) No 252/2006 of 14 February 2006 concerning the permanent authorisations of certain additives in feedingstuffs and the provisional authorisations of new uses of certain additives already authorised in feedingstuffs . OJ L 44, 15.2.2006, p. 3.
[12] Commission Regulation (EC) No 1200/2005 of 26 July 2005 concerning the permanent authorisation of certain additives in feedingstuffs and the provisional authorisation of a new use of an additive already authorised in feedingstuffs. OJ L 195, 27.7.2005, p. 6.
[13] Commission Regulation (EC) No 1288/2004 of 14 July 2004 concerning the permanent authorisation of certain additives and the provisional authorisation of a new use of an additive already authorised in feedingstuffs. OJ L 243, 15.7.2004, p. 10.
[14] Commission Regulation (EC) No 102/2009 of 3 February 2009 concerning the permanent authorisation of an additive in feedingstuffs. OJ L 34, 4.2.2009, p. 8.
[15] Commission Regulation (EC) No 255/2005 of 15 February 2005 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 45, 16.2.2005, p. 3.
[16] Commission Regulation (EC) No 1200/2005 of 26 July 2005 concerning the permanent authorisation of certain additives in feedingstuffs and the provisional authorisation of a new use of an additive already authorised in feedingstuffs. OJ L 195, 27.7.2005, p. 6.
[17] Commission Regulation (EC) No 1259/2004 of 8 July 2004 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 239, 9.7.2004, p. 8.
[18] Commission Regulation (EC) No 1520/2007 of 19 December 2007 concerning the permanent authorisations of certain additives in feedingstuffs. OJ L 355, 20.12.2007, p. 17.

The Scientific Committee on Animal Nutrition issued on the safety for the target animals, consumers, users and environment for this product when used as a feed additive for chickens for fattening, piglets, pigs for fattening and calves (EC, 1997, updated 2003). EFSA has issued one opinion on the safety of Cylactin® (*Enterococcus faecium* NCIMB 10415) for dogs and cats (EFSA, 2004a) and another one on the safety and efficacy of Cylactin® (*Enterococcus faecium* NCIMB 10415) for chickens for fattening (EFSA, 2010a). The Scientific Committee on Animal Nutrition (SCAN) issued two opinions on the safety of Oralin (*Enterococcus faecium* NCIMB 10415/DSM 10663) for turkeys (EC, 2002), and for pigs for fattening, calves and chickens for fattening (EC, 2003). EFSA issued an opinion on the safety of Oralin for dogs (EFSA, 2004b).

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 animals, the user and the efficacy of the product Cylactin® (*Enterococcus faecium* NCIMB 10415), when used under the conditions described in Table 1.

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

Additive	*Enterococcus faecium* NCIMB 10415
Registration number/EC No/No	E 1705
Category(-ies) of additive	Zootechnical additive
Functional group(s) of additive	Gut flora stabiliser

Description			
Composition, description	Chemical formula	Purity criteria	Method of analysis
E. faecium NCIMB 10415 Microencapsulated form: Minimum 5 x 10^9 CFU/g	-	-	Enumeration: Spread plate method using bile esculin azid agar Identification: Pulse-filed gel elctrophoresis

Trade name	Cylactin®, Cernivet®
Name of the holder of authorisation	DSM Nutritional Products Ltd

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
Dogs Cats	Not relevant	4.5 x 10^6 5.0 x 10^6	-	none

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	
Specific conditions or restrictions for handling	*E. faecium* NCIMB is a class 1 biological agent. No specific labelling required.
Post-market monitoring	No additional requirements further to the need for traceability and recall procedures established by Regulation No 178/2002
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
Not relevant	Not relevant	Not relevant	Not relevant

ASSESSMENT

1. Introduction

The microbial feed additive Cylactin® is a product consisting of dehydrated cells of *Enterococcus faecium* NCIMB 10415.

Cylactin® in various formulations is currently authorised for use in chickens for fattening, pigs for fattening, piglets, sows, calves, turkeys for fattening, cats and dogs. However, a number of these authorisations arise from assessments made under Directive 70/524/EEC and under the transitional arrangements laid down in Regulation (EC) No 1831/2003. Such applications are required to undergo a re-evaluation for the authorisation to remain in force. EFSA has already assessed the safety and efficacy of Cylactin® for chickens for fattening as a result of this re-evaluation process (EFSA, 2010b). The applicant has now applied for the re-evaluation of Cylactin® when used in diets for dogs and cats.

The product and its technical characteristics have been fully described in the context of the previous application. However, since the previous application of 2010, new guidance on the safety assessment of *E. faecium* (EFSA, 2012a) and a revision of the guidance on the susceptibility to antimicrobials (EFSA, 2012b) have been published. The applicant has provided new data relevant to the changes introduced by these guidance documents, which are assessed below. Since the product is intended for use in pet animals, the present assessment considers only the safety for the target animals and user/owner and the efficacy of the additive.

2. Characterisation[19]

2.1. Characterisation of the active agent

The active agent is a strain of *E. faecium*, isolated from faeces of a healthy newborn baby and deposited in the UK National Collection of Industrial, Marine and Food Bacteria (NCIMB) with the accession number NCIMB 10415.[20] The strain was identified by phenotypic tests and molecular taxonomy and characterised by polymerase chain reaction fingerprinting methods.[21]

The susceptibility of *E. faecium* NCIMB 10415 to the list of antibiotics recommended by the FEEDAP Panel in its Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA, 2012b) was tested using broth serial two-fold microdilution procedure.[22] The minimum inhibitory concentrations (MICs) of the strain were lower than the defined EFSA cut-off values for all antibiotics tested with the exception of kanamycin. This fact was previously assessed by the Panel in 2010, who concluded that "the genetic determinants conferring kanamycin resistance to *E. faecium* NCIMB 10415 are intrinsic to *E. faecium* and that consequently; the risk of horizontal transfer of these genes is minimal" (EFSA, 2010a, b).

The minimum inhibitory concentration (MIC) of ampicillin for *E. faecium* NCIMB 10415 was 1 mg/L and the analysis of complete genome sequence demonstrated the absence of the genetic determinants IS*16*, *hylEfm* and *esp*, typical of hospital-associated strains.[23] Therefore, the FEEDAP Panel concludes that *E. faecium* NCIMB 10415 does not contain marker genes typical of hospital-associated isolates responsible for clinical infections.

[19] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[20] Technical dossier/Section II/ Annex 2_13.
[21] Technical dossier/Section II/ Annex 2_14-15.
[22] Technical dossier/Section II and Supplementary information July 2012/Annexes 2_36 and 3_2.
[23] Technical dossier/Section II and Supplementary information July 2012/Annexes 2_21 to 32 and 3_3 to 7.

2.2. The nature of the additive

Cylactin® LBC ME5 PET is a free-flowing granulated product consisting of dehydrated cells of *Enterococcus faecium* NCIMB 10415 (5×10^9 CFU/g additive), with saccharose as carrier and a cellulose derivative and resin as encapsulating agents. The safety of the coating agent has been previously assessed (EFSA, 2004a).[24]

The product contains no particles below 100 µm in diameter; 0.2 % of particles are between 100 and 400 µm in diameter and 99.8 % between 400 and 2000 µm, as determined by laser diffraction on three batches.[25] Analysis of dusting potential, based on three batches, shows that the additive can be considered practically dust free.[26]

2.3. Stability and homogeneity

The additive is added as a surface coat to extruded pet feed after cooling. Losses associated with this coating process are low and in the region of 10 %.[27] Storage of the extruded feed in closed containers at 25 °C showed essentially no loss of viability over 11 months.[28] In open containers at 25 °C and 65 % relative humidity, losses were estimated to be in the region of 1.5 log per annum. Both sets of data were based on three batches of the additive.

No evidence of stability was provided for pelleting or the inclusion of the additive in wet food.

To determine the homogeneous distribution of the additive in feed, samples of dry pet food were taken from the production line at 10-minute intervals to determine consistency of application.[29] The coefficient of variation between counts was 7 %.

2.4. Conditions of use

Cylactin® LBC ME5 PET is intended for use in feeds for dogs at a minimum dose of 4.5×10^6 and a maximum dose of 2.0×10^9 CFU/kg feed and for cats at a minimum dose of 5.0×10^6 and a maximum dose of 8.0×10^9 CFU/kg feed.

2.5. 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 active agent in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

3.1. Safety for the target species

Two tolerance studies were provided, one in dogs and one in cats.

A tolerance test lasting 34 days was performed in 20 adult male and female large-breed (Labrador retriever) and small-breed dogs (Manchester terrier).[30] Dogs ranged in age from 1.6 to 9 years. Age, sex and breed were balanced across both trial groups. The control group received an extruded dog food with no additive whilst the test group was given Cylactin® at 5×10^{10} CFU/dog per day mixed with a small amount of food. This corresponded to approximately 100 times the maximum recommended dose expressed in terms of kilograms complete feed. Dogs were housed in pairs in

[24] The coating agent is currently under evaluation by the ANS Panel as part of the food additives re-evaluation programme specified under Regulation (EU) No 257/2010. This opinion is expected to be delivered by the end of 2018.
[25] Technical dossier/Section II/ Annex 2_11.
[26] Technical dossier/Section II/ Annex 2_9.
[27] Technical dossier/Section II/ Annex 2_61.
[28] Technical dossier/Section II/Annex 2_63.
[29] Technical dossier/Section II/Annex 2_64.
[30] Technical dossier/Section III/ Annex 3_7.

indoor/outdoor kennel runs. Samples for blood chemistry (albumin, cholesterol, creatinine, globulin, glucose, total bilirubin, total protein, triglycerides, urea nitrogen, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase, serum gamma-glutamyl transferase (GGT), electrolytes) and haematology (white blood cells, red blood cells, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils) and urine analysis (pH and specific gravity) were obtained at the start and at the end of the study. Health status, food intake and faecal consistency were recorded daily. Faecal consistency was scored on a four-point subjective scale.

Animals remained in good health through the study and maintained a constant weight. No significant differences in any of the clinical chemistry or haematological parameters were seen between treatments. Faecal consistency was not affected.

A tolerance test lasting 31 days was performed in 19 adult male and female cats housed in individual pens.[31] Animals ranged in age from 2 to 10 years. Age, sex and breed were balanced across both trial groups. The control group received an extruded cat food with no additive whilst the test group was given Cylactin® at 3.6×10^{10} CFU/cat/day mixed with a small amount of food. This corresponded to approximately 70 times the maximum recommended dose expressed in terms of kilograms complete feed. Samples for blood chemistry (albumin, cholesterol, creatinine, globulin, glucose, total bilirubin, total protein, triglycerides, urea nitrogen, alkaline phosphatase, ALT, AST, creatine kinase, GGT, electrolytes) and haematology (white blood cells, red blood cells, haematocrit, MCV, MCH, MCHC, platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils) and urine analysis (pH and specific gravity) were obtained at the start and at the end of the study. Health status, food intake and faecal consistency were recorded daily. Faecal quality was scored on a four-point subjective scale.

Animals remained in good health through the study. No significant differences in any of the clinical chemistry or haematological parameters were seen between treatments. Faecal consistency was not affected.

3.2. Safety for the user/owner

Cylactin® LBC ME20, a more concentrated formulation of Cylactin® (2×10^{10} CFU/g additive) containing the same excipients, was shown not to be a skin/eye irritant or a skin sensitiser in tests performed following OECD Guidelines 404, 405 and 429.[32] The FEEDAP Panel does not expect the formulation under assessment to behave differently. As this formulation has a large particle size and the dusting potential is low, the potential for exposure via respiratory routes is considered minimal.

4. Efficacy

Secretory IgA (SIgA) is the major antibody isotype present in mucosal secretions and has many functional attributes, both direct and indirect, serving to prevent infective agents such as bacteria and viruses from breaching the mucosal barrier. IgA is also present in serum in humans and rodents, where it functions as an inflammatory antibody through interactions with FcαR on immune effector cells, enabling systemic clearance of pathogens. In dogs, 31 % of the total plasma IgA originates from local intestinal synthesis, reaching the blood via mesenteric lymph. The majority of serum IgA in the dog originates from the plasma cells in the intestinal lamina propria since the daily output of IgA from mesenteric lymph into the circulation is greater than the quantity present in the serum and is sufficient to maintain the circulating pool of IgA (Vaerman et al., 1970; Kaartinen et al., 1978). SIgA exerts its function in mucosal secretions as the first line of defence by limiting invasion of pathogens. However, serum IgA may function as a second line of defence by eliminating pathogens that have breached the mucosal surface (Otten and van Egmond, 2004) and could have inflammatory properties in humans and rats.

[31] Technical dossier/Section III/ Annex 3_8.
[32] Technical dossier/Section III/ Annexes 3_21 and 3_22.

4.1. Efficacy for dogs

To support the efficacy of the additive in dogs, the applicant has provided four studies evaluating the effects of Cylactin® LBC ME5 PET supplementation on faecal quality and on some immune parameters such as IgA.

In the first study, 16 puppies of four different breeds were assigned from eight weeks of age (weaning) until 52 weeks of age (44 weeks in total) to two groups (eight in the control and eight in the test group) and were given a food either supplemented (test group) or non-supplemented (control group) with Cylactin® at 5×10^8 CFU/animal per day, which equates to approximately 2×10^9 CFU/kg complete feed.[33] The additive was top dressed on a dry food. Faecal quality was scored daily using a four-point scale (1 denoting ideal stools and 4 watery faeces) and expressed as average percentage of acceptable stools (scores 1 and 2). Immune parameters were assessed at five time points (weeks 0, 10, 18, 31 and 44 of the trial). Puppies were allotted to treatment according to the litter, with a puppy from the litter assigned to the control group and a littermate to the treatment group. Puppies were vaccinated at nine weeks of age and boosted at 12 weeks of age with a vaccine containing canine distemper virus (CDV), adenovirus type 2, parainfluenza virus and parvovirus. Blood samples and faeces were collected for the determination by enzyme-linked immunosorbent assay (ELISA) of total IgA concentration, total serum IgG and total serum IgA concentrations, CDV-specific serum IgG and serum IgA. Blood was also used to determine the percentage of CD4+ and CD8+ T-lymphocytes and B-lymphocytes (Wilkerson et al., 2005) within the lymphocyte population, and to calculate the CD4/CD8 T-cell ratio at 18, 31 and 44 weeks. Lymphocyte proliferation was measured following stimulation with different mitogens that induce proliferation of T-lymphocytes (concanavalin A (conA), phytohaemagglutinin (PHA)), B-lymphocytes (pokeweed mitogen (PWM)) and all lymphocytes (phorbol myristate acetate/ionomycin (PMA/iono)) at all the time points. Proliferation was expressed as mean of the stimulation indices (SI = ratio of stimulated and non-stimulated cells) (Benyacoub et al., 2003).

The supplementation with Cylactin® resulted in a significant increase, of approximately 50 %, in the total amount of faecal IgA after 44 weeks of treatment. Significant increases (approximately 60 %) in total serum IgA but not serum IgG were seen at weeks 18, 31 and 44 and significant increases (> 60 %) in CDV-specific serum IgA (approximately 30 %) and IgG (> 60 %) at 31 and 44 weeks. The mature B-lymphocyte population remained at a significantly higher level in the treatment group than in the control group at 31 and 44 weeks. There were no differences in mitogen-induced proliferation.

The average percentage of acceptable stools was 92.8 % in the test group and 87.1 % in the control group. In addition, the overall average faecal score was significantly better ($P < 0.01$) in the test (1.36) group than in the control group (1.51). However, the significance of such a difference can be questioned.

In the second study, a total of 30 male and female dogs (Labrador retriever, schnauzer and Siberian husky) were fed a control food supplemented with placebo or the same food supplemented with two different levels of Cylactin® for 11 weeks.[34] Dogs were housed in pairs and were given the same basal food. Three groups of 10 dogs received the diet without Cylactin® (control), a second group was given 5×10^7 CFU/day and the third group received 1×10^8 CFU/day. These doses of Cylactin® equate to 2×10^8 and 4×10^8 CFU/kg complete feed, respectively. The placebo and bacteria were administered in capsules and given daily. Food intake was measured daily and body weight weekly. Total serum IgA was measured by ELISA at the start and end of the study. The significance of differences between the initial and final concentrations was determined using a one-sided *t*-test, whereas differences between the test groups and the control group were measured using a two-sided *t*-test.

Dogs maintained body weight during the study. At the beginning of the study, serum IgA was similar in the three groups of dogs. In animals receiving the high dose of Cylactin®, serum IgA concentration

[33] Technical dossier/Section IV/ Annex 4_6.
[34] Technical dossier/Section IV/Annex 4_8.

at the end of the treatment (2 615 ng/mL) was significantly higher than both the initial concentration (1 804 ng/mL; $P < 0.04$) and the end concentration in the control group (1 340 ng/mL; $P < 0.025$).

The third study involved 16 healthy elderly male and female Beagle dogs (average age 13.2 years, range 10–15 years) fed a control diet ($n = 8$) or the same diet supplemented with Cylactin® at a dose of 5×10^8 CFU/day, which equates to 2.5×10^9 CFU/kg complete feed ($n = 8$), for six months.[35] The additive was mixed with a small amount of food. Total faecal IgA was measured at the start and end of the study by ELISA. The Student's *t*-test was used to evaluate significant differences between the control and test groups. A paired *t*-test was used to evaluate differences between initial and final values.

Faecal IgA in dogs given Cylactin® was significantly higher than in control dogs at the end of the trial ($P < 0.001$) and higher than the initial value.

A fourth study was provided in which faecal consistency and some microbiological parameters were measured.[36] Although a small but significant effect on faecal consistency was recorded, this is not considered of any relevance. The microbiological analyses did not identify any consequences of Cylactin® addition.

4.1.1. Conclusions of efficacy for dogs

Cylactin®, when added to diets for dogs, was shown to significantly increase the concentration of IgA in serum and faeces, which may improve the animals' defence against pathogens. This was demonstrated at a minimum dose of 5×10^8 CFU/animal per day, which equates to a dose of 2.5×10^9 CFU/kg complete feed for a medium-sized dog.

4.2. Efficacy for cats

The applicant has provided three studies with a similar experimental design to support the efficacy of the additive in cats. Studies were aimed at investigating the influence of Cylactin® LBC ME5 PET supplementation on faecal quality and, in two studies, on immune parameters.

In the first study, 11 post-weaning kittens were divided in two groups, a control unsupplemented group and a group which received Cylactin® at an average dose of 5×10^8 CFU/cat per day, equating to approximately 6.9×10^9 CFU/kg feed ($n = 6$).[37] The additive or a placebo containing only carriers was administered daily in a small amount of canned food. Food (extruded) was fed from weaning (8–13 weeks of age) until one year of age. Serum IgA was measured at the beginning of the trial and at 19, 26, 40 and 52 weeks of age. Faecal IgA was measured at 52 weeks of age. Faecal quality (daily scoring using a four-point scale), food intake (daily) and body weight (weekly) were assessed throughout the trial. The statistical significance of differences between groups was determined using the Student's *t*-test.

Kittens fed the additive had a better average faecal quality score than those in the control group during the first month of the study (2.2 vs. 1.92 for treated kittens; $P < 0.025$) and overall (2.4 vs. 1.9 for treated kittens; $P < 0.025$). Kittens fed the additive had significantly fewer daily defecation events than the control animals (1.6 vs. 2.4; $P < 0.025$). Faecal IgA did not differ significantly between groups at 52 weeks post supplementation. Serum IgA was not affected by Cylactin® supplementation during the study.

The second study involved 15 adult cats selected on the basis of the production of poor-quality faeces and assigned to one of two groups, a control group (seven cats) and a treatment group (eight cats).[38] Faecal quality was assessed using a four-point subjective scale for three days before the beginning of

[35] Technical dossier/Section IV/Annex 4_9.
[36] Technical dossier/Section IV/Annex 4_5.
[37] Technical dossier/Section IV/ Annex 4_10.
[38] Technical dossier/Section IV/ Annex 4_11.

the study and daily thereafter. Animals from the treated group received Cylactin® at 5×10^8 CFU/cat per day, equating to approximately 6.6×10^9 CFU/kg feed, for 25 days. As above, animals received either a placebo or the additive mixed with a small amount of canned food. Analysis of variance (ANOVA) was used to test for differences between the control and treated periods.

There was no significant difference between the mean faecal score in the control group and the treatment group at the end of the trial, although a small but significant difference was seen between initial and final values in the treatment group (3.6 vs. 3.3; $P < 0.0005$). However, there was an indication of increased number of firmer faeces (from 42 % to 62 % scoring 2 or 3) and a significant reduction in faeces scoring 4 (from 58 % vs. 38 %; $P < 0.02$) in the treated animals. Over the same period there was no significant improvement in faecal quality in the control group.

In the third study, groups of five post-weaning kittens were fed either a control feed or the same food supplemented with Cylactin® at 5×10^7, 1×10^8 or 5×10^8 CFU/kitten per day. These values equate to 7×10^8, 1.4×10^9 or 7×10^9 CFU/kg feed.[39] As above, the additive was added daily to a small amount of canned food while control animals received a placebo consisting of carriers mixed with the same canned food. Food was provided from weaning (8–14 weeks of age) until one year of age. Faecal quality (four-point scale) and food intake were measured daily and body weight weekly for a period of four months. Total serum IgA was measured at the beginning of the trial and at 19, 26, 40 and 52 weeks of age. Total faecal IgA was measured at the end of the trial. The Student's *t*-test was used to determine differences between animals of the control and test groups and between initial values and final values. Faecal scores were analysed by ANOVA.

All kittens remained healthy throughout the study and grew as expected. Although faecal quality score was significantly ($P < 0.01$) better after four months in kittens consuming Cylactin® at all doses (2.6 vs. 2.1–2.3 for the treatment groups), effects were not consistent in the monthly measurements and differences were small and of questionable relevance.

There were no significant differences in the total faecal IgA between the test groups and the control group at the end of the trial. Similarly, there were no significant differences in total serum IgA between the treatment groups and the control group at any of the times of measurement.

4.2.1. Conclusions of efficacy for cats

Cylactin® showed inconsistent effects on faecal quality in three studies in which cats were fed the additive at the dose of 7×10^9 CFU/kg of feed. No significant effects on IgA were observed in the two studies in which this parameter was measured.

5. 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[40] and Good Manufacturing Practice.

6. Conclusions

Enterococcus faecium NCIMB 10415 does not contain marker genes typical of hospital-associated isolates responsible for clinical infections and is susceptible to clinically relevant antibiotics, except for kanamycin, which is considered of no concern.

Cylactin® LBC ME5 PET is safe for dogs and cats at the recommended dose range.

[39] Technical dossier/Section IV/Annex 4_12.

[40] 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.

The additive is not a skin/eye irritant or a skin sensitiser. As this formulation has a large particle size and the dusting potential is low, the potential for exposure via respiratory routes is considered minimal.

Cylactin® has the potential to produce a beneficial effect in dogs when feed is supplemented with a dose of 2.5×10^9 CFU/kg, by increasing the intestinal or serum concentration of IgA. Cylactin® showed no consistent effects on faecal quality when fed to cats at the dose of 7×10^9 CFU/kg feed.

DOCUMENTATION PROVIDED TO EFSA

1. *E. faecium* NCIMB 10415 as a gut flora stabiliser, zootechnical additive for dogs and cats. November 2010. Submitted by DSM Nutritional Products.

2. *E. faecium* NCIMB 10415 as a gut flora stabiliser, zootechnical additive for dogs and cats. Supplementary information July 2012. Submitted by DSM Nutritional Products.

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Cylactin®.

4. 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 Cylactin[41]

In the current application authorisation is sought under Article 10(2) for *Enterococcus faecium* NCIMB 10415, under the category/functional group 4(b), "zootechnical additives/gut flora stabilisers", according to the classification system of Annex I of Regulation (EC) No 1831/2003. The *feed additive* will be marketed as micro-encapsulated or granulated form with concentrations of *Enterococcus faecium* NCIMB 10415: ranging from $5x10^9$ to $3.5x10^{10}$ CFU/g. Specifically, authorisation is sought for the use of the *feed additive* for pigs for fattening, piglets (suckling and weaned), sows, cats and dogs. The *feed additive* is intended to be used either via *premixtures* or incorporated directly into the *feedingstuffs*. The Applicant proposed the following content of *Enterococcus faecium* NCIMB 10415 in complete *feedingstuffs*: - for dogs and cats a minimum content of (4.5 and 5) $x10^6$ CFU/kg; - for pigs and piglets a content range of (3.5 – 10) $x10^8$ CFU/kg; and - for sows a content range of (0.7 – 1.25) $x10^9$ CFU/kg.

For the enumeration of *Enterococcus faecium* NCIMB 10415 in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed two single laboratory validated pour plate/spread plate methods. The EURL identified instead the internationally recognised ring-trial validated spread plate CEN method (EN 15788), using Bile Esculin Azide Agar, for the enumeration of Enterococcus spp. The performance characteristics of the CEN method reported after logarithmic transformation (CFU) are:

- a repeatability standard deviation (s_r) ranging from 0.12 to 0.2 $\log_{10}$ CFU/g,
- a reproducibility standard deviation (s_R) ranging from 0.23 to 0.41 $\log_{10}$ CFU/g; and
- a limit of detection (LOD) of $1x10^5$ CFU/kg *feedingstuffs*.

Based on the performance characteristics presented, the EURL recommends for official control, the CEN method (EN 15788) for the enumeration of *Enterococcus faecium* NCIMB 10415 in *feed additive* and *feedingstuffs*.

Molecular methods were used by the Applicant to identify the active agent in the *feed additive*. The EURL recommends instead for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

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) is not considered necessary.

[41] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0269+0294.pdf