European Food Safety Authority

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SCIENTIFIC OPINION

Safety and efficacy of Probiotic LACTINA® (*Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermophilus, Enterococcus faecium*) for chickens for fattening, piglets and pigs[1]

Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed

(Question N° EFSA-Q-2006-135)

Adopted on 9 December 2008

PANEL MEMBERS

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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 Probiotic LACTINA® for chickens for fattening, piglets and pigs.

The additive Probiotic LACTINA® is proposed be used as a feed additive for chickens for fattening and piglets (category: zootechnical additives; functional group: gut flora stabilisers). It has not been previously authorised in the Community. Probiotic LACTINA® is a preparation of *Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermophilus* and *Enterococcus faecium*. The total content of lactic acid bacteria is 5 x 10^9 CFU g^{-1} product, but no information is given about the relative amounts of different bacterial strains.

The product is intended for chickens for fattening (5 x 10^8 – 9 x 10^9 CFU kg^{-1} complete feedingstuff), for piglets up to 9 – 12 kg (5 x 10^9 – 1 x 10^{10} CFU kg^{-1} complete feedingstuff) and for pigs up to 35 kg (9 x 10^8 – 5 x 10^9 CFU kg^{-1} complete feedingstuff).

[1] For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of Probiotic LACTINA® for chickens for fattening, piglets and pigs. *The EFSA Journal* (2009) 912, 1-13

Due to the lack of data presented in the dossier and in the supplementary information, the FEEDAP Panel is not able to describe the composition, stability and homogeneity in feed of the product.

Serious deficiencies in the study design, conduct and reporting of the studies do not allow conclusions to be drawn on the efficacy of Probiotic LACTINA® in chickens for fattening. Acceptable evidence of efficacy has been provided only in one study made with weaned piglets and one study with suckling piglets. Therefore, the FEEDAP Panel cannot conclude on the efficacy of Probiotic LACTINA® for piglets. The applicant did not provide any study on pigs for fattening and therefore no conclusion can be drawn on the efficacy of the product for this target species.

In the absence of tolerance studies, the FEEDAP Panel cannot conclude on the safety of Probiotic LACTINA® for the target species. The lack of data on the antibiotic resistance of the six Probiotic LACTINA® strains and on the presence of known virulence factors in *E. faecium* NBIMCC 8270 prevents drawing conclusions on the safety of the product for the consumer. No experimental data on the user safety was provided. Because of its proteinaceous nature, the possibility for the product to act as a respiratory sensitiser cannot be excluded.

The use of this product as a feed additive would not pose a risk for the environment.

Key words: zootechnical additive, gut flora stabiliser, Probiotic LACTINA®, micro-organisms, lactic acid bacteria, *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Streptococcus thermophilus*, *Enterococcus faecium*, chickens for fattening, piglets, efficacy, safety

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BACKGROUND

Regulation (EC) No 1831/2003[2] 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 Lactina SK s.r.o.[3] for authorisation of the product Probiotic LACTINA® to be used as a feed additive for chickens for fattening and piglets (category: zootechnical additives; functional group: gut flora stabilisers) under the conditions described 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.[4] 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 26 October 2007.

The additive Probiotic LACTINA® is a preparation of *Lactobacillus acidophilus* (NBIMCC 8242), *Lactobacillus helveticus* (NBIMCC 8269), *Lactobacillus bulgaricus* (NBIMCC 8244), *Lactobacillus lactis* (NBIMCC 8250), *Streptococcus thermophilus* (NBIMCC 8253) and *Enterococcus faecium* (NBIMCC 8270). This product has not been authorised in the Community.

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. Therefore, EFSA shall deliver an opinion on the efficacy and the safety for the target animals, the consumer, user and the environment of the product Probiotic LACTINA®, a preparation of *Lactobacillus acidophilus* (NBIMCC 8242), *Lactobacillus helveticus* (NBIMCC 8269), *Lactobacillus bulgaricus* (NBIMCC 8244), *Lactobacillus lactis* (NBIMCC 8250), *Streptococcus thermophilus* (NBIMCC 8253) and *Enterococcus faecium* (NBIMCC 8270), when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on micro-organisms for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29.
[3] Lactina SK Ratislavova 2 149 01 Nitra Slovak Republic
[4] Dossier reference: FAD-2006-0029

Table 1. **Register entry as proposed by the applicant**

Additive	Probiotic LACTINA®
Registration number/EC No/No (if appropriate)	Pending
Category of additive	Zootechnical
Functional group of additive	Gut flora stabilisers

Description			
Composition, description	**Chemical formula**	**Purity criteria** (if appropriate)	**Method of analysis** (if appropriate)
$5X10^9$ CFU (LAB) *Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermophilus, Enterococcus faecium*	Not applicable	Complies with EU law on microbial quality, heavy metals, toxins, undesirable substances	IDF146: 1991, IDF 149: 1997 And IDF microbial methods for purity control

Trade name (if appropriate)	Probiotic LACTINA®
Name of the holder of authorisation (if appropriate)	Lactina Ltd, Bulgaria

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period** (if appropriate)
		CFU kg^{-1} of complete feedingstuffs		
Chickens for fattening	Slaughter age	5×10^8	9×10^9	Nil MRLs Not applicable
Piglets	Up to 9-12 kg	5×10^9	1×10^{10}	Nil MRLs Not applicable
Pigs	Up to 35 kg	9×10^8	5×10^9	Nil MRLs Not applicable

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	None
Specific conditions or restrictions for handling (if appropriate)	None
Post-market monitoring (if appropriate)	EU distributor by making use of HACCP (Hazard Analysis and Critical Control Points) and Traceability principles, including routine postmarketing sampling and

	analysis, and monitoring of customer feedback, involving documentation and resolution of products and service complaints
Specific conditions for use in complementary feedingstuffs (if appropriate)	Dosage used should supply at least 1 x 10^9 CFU/kg final complete feedingstuff.

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
Not applicable (N/a)	Not applicable	Not applicable	Not applicable

ASSESSMENT

1. Introduction

The product Probiotic LACTINA® is a mixture of six strains of lactic acid bacteria, *Lactobacillus acidophilus* LAT180 (NBIMCC 8242), *Lactobacillus delbrueckii* ssp. *bulgaricus* LAT187 (NBIMCC 8244), *Lactobacillus helveticus* LAT179 (NBIMCC 8269), *Lactobacillus delbrueckii* ssp. *lactis* LAT182 (NBIMCC 8250), *Streptococcus thermophilus* LAT205 (NBIMCC 8253), and *Enterococcus faecium* LAT E-253 (NBIMCC 8270). Although five out of the six strains have QPS status (EFSA, 2007), the presence of *E. faecium* makes a complete safety assessment necessary.

2. Characterisation of the product

2.2.1 Description of the product

The product is a mixture of six strains of lactic acid bacteria with a total content of 5 x 10^9 CFU g^{-1}, but no information is given about the relative amounts of the different bacterial species. The carrier materials are stated to be carbohydrates (dextrose) and calcium carbonate but their relative amounts are not indicated. The product is a powder with a particle size distribution (determined by a sieve method) showing a fraction of particles with a diameter ≤ 90 µm equal to 15.5 %. The water content is 4–6 % and the percentage of lactic acid 0.6–2 %.

The product is monitored for arsenic, cadmium, mercury and lead, using 'Arsenic limit test' and 'Heavy metal limit test', according to Japan Pharmacopoeia, and for aflatoxin (M_1 only), using an HPLC method. The levels are stated to be below the limits set by EU-legislation (no information on the detection limits of the methods is given).

The presence of contaminating Enterobacteriaceae, yeasts and molds, *Salmonella* and coagulase positive staphylococci is checked using the standard microbiological techniques and action limits set.

The manufacturing process is schematically presented. The growth medium of the strains consists of reconstituted skim milk and whey powder. Each strain is cultivated separately. The bacteria are freeze-dried, then the concentrates are mixed together and with the carrier materials.

2.2.2 The active agents

The bacterial strains used in the product have been isolated from home-made yoghurt and cheese and subsequently deposited in the National Bank of Industrial Microorganisms and Cell Cultures (NBIMCC, Sofia, Bulgaria) with designations NBIMCC 8242 (*L. acidophilus*), NBIMCC 8250 (*L. delbrueckii* ssp. *lactis*), NBICCM 8244 (*L. delbrueckii* ssp. *bulgaricus*), NBIMCC 8269 (*L. helveticus*), NBIMCC 8253 (*S. thermophilus*) and NBIMCC 8270 (*E. faecium*). The strains are not genetically modified. The species identification is based on morphological and biochemical criteria, and 16S rDNA analysis. No information is provided on the characterisation of *E. faecium*. The lactobacilli and *S. thermophilus* are reported to produce bacteriocins as indicated by inhibition of *E. coli, Listeria* and *Staphylococcus aureus*. The chemical nature of these antimicrobial compounds has not been described. The lactobacilli are further reported to have inhibitory activity against *Proteus mirabilis.*

The strains are stated to be both phenotypically and genetically stable, but the methods to asses the genetic stability have not been given.

The absence of virulence factors has been studied with animal experiments performed on mice. The whole product and each individual strain were tested, but for the scope of this assessment only information on *E. feacium* NBIMCC 8270 and the whole product were considered. The animals were intraperitoneously injected with either bacterial cultures or culture supernatants and were followed for appearance and clinical symptoms for three or four days. No positive pathogenic control strains were included. Although no adverse effects were observed, due to the absence of information on the dose used, the low number of animals (three mice per sample) and the lack of reference confirming the validity of the test, the FEEDAP Panel cannot conclude on the safety of *E. faecium* and Probiotic LACTINA®. Moreover, no specific experimental evidence on the absence of known virulence factors of *E. faecium* is reported.

2.2.3 Antibiotic resistance

The strains are reported to be moderately resistant against penicillins, but no data is given on the minimum inhibitory concentration (MIC) values against the antibiotics recommended in the Opinion on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human and veterinary importance (EFSA, 2005).

2.2.4 Stability and homogeneity

The stability of the product in closed packages has been tested at several temperatures ranging from -18 °C to 43 °C. The product is stated to be stable (based on CFU determination) at temperatures below 20 °C. However, 12-month stability tests are only reported for temperatures of -18 °C and 5 °C; consequently, the actual use by dates cannot be concluded for higher temperatures. The relative stabilities of the product strains have not been reported.

The product is stated to be stable for up to six months in premixes and feedingstuffs at temperatures ≤ 20 °C and at relative humidity of 60 %. However, again, no experimental evidence is presented.

The product is stated to mix homogenously in premixtures and feedingstuffs but no supporting data are presented.

2.2.5 Proposed conditions of use

The product is intended for chickens for fattening (minimum and maximum concentrations: 5 x 10^8 and 9 x 10^9 CFU kg^{-1} complete feedingstuff, respectively), for piglets up to 9 – 12 kg (minimum and maximum concentrations: 5 x 10^9 and 1 x 10^{10} CFU kg^{-1} complete feedingstuff, respectively) and for pigs up to 35 kg (minimum and maximum concentrations: 9 x 10^8 and 5 x 10^9 CFU kg^{-1} complete feedingstuff, respectively).

2.2.6 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 active substance in animal feeds. The Executive Summary of the CRL report can be found in the Appendix.

3. Efficacy

2.2.7 Efficacy in chickens for fattening

The applicant provided the results of six trials (including two field trials) in chickens for fattening. The experimental design of all six trials was based on a control group and one or two groups supplemented with Probiotic LACTINA®. In three trials, diets were supplemented with two doses (higher dose from day 1 to 28, lower dose from day 29). However, serious deficiencies in the study design, conduct and reporting (e.g. no confirmation of doses in feed, number of replicates unknown, details of statistical evaluation missing) of the studies do not allow to conclude on the efficacy of Probiotic LACTINA® in chickens for fattening.

3.1. Efficacy in piglets and pigs

The applicant provided ten trials with piglets and pigs to demonstrate the efficacy of Probiotic LACTINA® in improving the animal performance. However, only three trials included statistics. No analytical data on the concentration of the active agents in feed was provided.

Trial 1

The control group consisted of 45 piglets (mean weight at birth: 1.51 kg) and the treatment group of 38 piglets (mean weight at birth: 1.72 kg).[5] The dose of Probiotic LACTINA® was 4.0 x 10^9 CFU kg^{-1} feed for suckling piglets and 2.5 x 10^9 CFU kg^{-1} feed for growing pigs. The suckling period was 35 days, after which the average weights of the pigs in the control and test groups were 7.6 and 8.9 kg, respectively. There was a statistically significant ($P < 0.05$) difference between the average daily weight gain in the test group (204 ± 6.9 g) and in the controls (174 ± 8.5 g). No information of the growth performance during the growing period was provided. The confounding effects of unequal weights at the start of the experiment raise doubts about the significance of the differences of weights at the end of the experiment.

Trial 2

The control group consisted of 29 and the treatment group of 27 growing pigs, with initial average weights of 7.1 and 6.8 kg, respectively. The experiment lasted 61 days.[6] The dose of Probiotic LACTINA® was 2.5 x 10^9 CFU kg^{-1} feed. The final weights were 17.7 kg (control) and 18.9 kg (treatment). There was a statistically significant difference ($P < 0.05$) between the average daily weight gain between the control animals (173 ± 7.7 g) and the treated group (198 ± 7.6 g).

Trial 3

The study[7] was carried out with 24 pregnant sows and their offspring divided into four groups according to Probiotic LACTINA®-supplementation:

- group I: no Probiotic LACTINA®-supplementation for either sows or piglets,
- group II: both sows and piglets supplemented,
- group III: only sows supplemented,
- group IV: only piglets supplemented.

The Probiotic LACTINA® treatment started on the 85th day of pregnancy and lasted until the end of lactation. The piglets receiving Probiotic LACTINA® were given the product by

[5] Technical dossier/Section III.2.3.2.6.1
[6] Technical dossier/Section III.2.3.2.6.1
[7] Technical dossier/Section III.2.3.2.7

stomach intubation from day 1 onwards until day 14 and additionally in feed from day 5 onwards until day 35. A significant ($P < 0.001$) increase of the live weight at weaning was observed in piglets supplemented with Probiotic LACTINA® (via stomach intubation for the first 14 days of life and then from creep feed). The administration of Probiotic LACTINA® to sows did not have any effect on weight gain for piglets.

3.2. Conclusions on efficacy in piglets and pigs

Acceptable evidence of efficacy has been provided only in one study made with weaned piglets and one study with suckling piglets. Therefore, the FEEDAP Panel cannot conclude on the efficacy of Probiotic LACTINA® for piglets. The applicant did not provide any study on pigs for fattening and therefore no conclusion can be drawn on the efficacy of the product for this target species.

3.3. Compatibility with coccidiostats

The compatibility of the product has been tested *in vitro* against the coccidiostats diclazuril, maduramycin ammonium and robenidine hydrochloride at feed concentration, following the total CFU counts and the acidification of the skimmed milk-based growth medium. With all the antimicrobials there was an initial drop of more than three orders of magnitude during the first hour of exposure. With diclazuril, the CFU counts reached the levels of the control on the third day of the experiment, while with the other compounds the CFU counts remained considerably reduced. No incompatibility data on the individual product strains were given.

The poor experimental design does not allow drawing any conclusion on the compatibility of Probiotic LACTINA® with the tested coccidiostats.

4. Safety for the target species

4.1. Tolerance

In the absence of tolerance studies, the FEEDAP Panel cannot conclude on the safety for the target species.

4.2. Effects on the intestinal microbiota

The applicant has provided a study on the effect of Probiotic LACTINA® on the intestinal microbiota.[8] Despite the dose-dependent increase of cultivable micro-organisms in treated animals, the poor experimental design does not allow a reliable identification of the representative bacterial groups from caecal samples.

4.3. Safety for the consumer

The applicant has not provided data on the antibiotic resistance of the six Probiotic LACTINA® strains or on the presence of known virulence factors in *E. faecium* NBIMCC 8270. Therefore, the FEEDAP Panel is unable to conclude on the safety of the product for the consumer.

4.4. Safety for the user

The company states that the product is not irritant to skin and eyes, does not have allergenic potential and it is not a respiratory sensitiser. However, no experimental data is presented.

[8] Technical dossier/Section IV.1.2

Because of its proteinaceous nature, the possibility for the product to act as a respiratory sensitiser cannot be excluded.

4.5. Safety for the environment

The bacteria present in the product are common species in foods and/or in the intestinal tract of animals, and their use in the product is not likely to increase their presence in the wider environment. Consequently, no risks for the environment are foreseen from the use of this product.

5. Post-market monitoring

The FEEDAP Panel is unable to conclude on the safety of this product and therefore on the need for post-market monitoring.

CONCLUSIONS

Due to the lack of data presented in the dossier and in the supplementary material, the FEEDAP Panel is not able to describe the composition, stability and homogeneity in feed of the product.

Serious deficiencies in the study design, conduct and reporting of the studies do not allow conclusions to be drawn on the efficacy of Probiotic LACTINA® in chickens for fattening. Acceptable evidence of efficacy has been provided only in one study made with weaned piglets and one study with suckling piglets. Therefore, the FEEDAP Panel cannot conclude on the efficacy of Probiotic LACTINA® for piglets. The applicant did not provide any study on pigs for fattening and therefore no conclusion can be drawn on the efficacy of the product for this target species.

In the absence of tolerance studies, the FEEDAP Panel cannot conclude on the safety of Probiotic LACTINA® for the target species. The lack of data on the antibiotic resistance of the six Probiotic LACTINA® strains and on the presence of known virulence factors in *E. faecium* NBIMCC 8270 prevents drawing conclusions on the safety of the product for the consumer. No experimental data on the user safety was provided. Because of its proteinaceous nature, the possibility for the product to act as a respiratory sensitiser cannot be excluded.

No risk for the environment is foreseen from the use of this product as a feed additive.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Lactina® *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus bulgaricus* NBIMCC 8244, *Lactobacillus lactis* NBIMCC 8250, *Streptococcus thermophilus* NBIMCC 8253, *Enterococcus faecium* NBIMCC 8270 for chickens for fattening, pigs and piglets. March 2006. Submitted by Lactina Ltd.
2. Evaluation report of the Community Reference Laboratory feed additives authorisation on the methods(s) of analysis for Lactina® *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus bulgaricus* NBIMCC 8244, *Lactobacillus lactis* NBIMCC 8250, *Streptococcus thermophilus* NBIMCC 8253, *Enterococcus faecium* NBIMCC 8270 for chickens for fattening, pigs and piglets.
3. Supplementary information. September 2008. Submitted by Lactina Ltd.
4. Comments from the Member States submitted through the ScienceNet.

REFERENCES

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of safety (QPS) approach of selected microorganisms referred to EFSA. <http://www.efsa.eu.int/cs/BlobServer/Scientific_Opinion/sc_op_ej587_qps_en.pdf?ssbinary=true>

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human and veterinary importance. <http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/feedap_op_ej223_antibiotics_en_obs,3.pdf?ssbinary=true>

APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory Feed Additives Authorisation on the Method(s) of Analysis for Probiotic LACTINA®, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus bulgaricus* NBIMCC 8244, *Lactobacillus lactis* NBIMCC 8250, *Streptococcus thermophilus* NBIMCC 8253, *Enterococcus faecium* NBIMCC 8270, for chickens for fattening, pigs and piglets

In the current application authorisation is sought for the microbial feed additive Probiotic LACTINA® under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Probiotic LACTINA® for chickens for fattening, piglets and pigs is requested. Probiotic LACTINA® consists of a minimum of 5 x 109 of viable cells (colony-forming units, c.f.u.) of lactic acid bacteria (LAB) per gram which comprise six strains as active agents, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus bulgaricus* NBIMCC 8244, *Lactobacillus lactis* NBIMCC 8250, *Streptococcus thermophilus* NBIMCC 8253, *Enterococcus faecium* NBIMCC 8270. The feed additive is intended to be mixed into complete feedingstuffs at final concentrations of 5 x 108 to 9 x 109 c.f.u./kg for chickens for fattening, of 5 x 109 to 1 x 1010 c.f.u./kg for piglets and of 9 x 108 to 5 x 109 c.f.u./kg for pigs.

For the determination of the active agents (LAB), in the *feed additive*, identification and control methods for lactic acid bacteria monocultures in accordance to International Dairy Federation Standard Methods IDF 146:1991 and IDF 149A:1997 are used by the applicant. For enumeration of the active agents de Man, Rogosa, Sharp (MRS) agar is used whereby for *Streptococcus thermohilus* M17 agar is suggested. The incubation temperature used is 37 ºC. These methods are considered appropriate. ISO 4833 is used for the enumeration of the active agents in *premixtures and feedingstuffs*.

For official controls of the active agents (LAB) in the *feed additive, premixtures and feedingstuffs* a spread plate method using MRS agar is suggested by the CRL-FA. The enumeration method was validated in a collaborative study [Food Microbiol., (2003), 20, 57-66]. The method's performance characteristics of the enumeration method using MRS, acidified MRS or MRS supplemented with triphenyl tetrazolium chloride (TTC) agar and an incubation temperature of 37 ºC revealed standard deviations for repeatability (sr) and reproducibility (sR) of around 0.10 – 0.26 log10 and 0.18 – 0.39 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively.

The limit of quantification (LOQ) of this method is 100 colony forming units (c.f.u) per gram (g) feed additive or premixture and 10000 c.f.u./g feedingstuff. These performance characteristics are considered acceptable.

For identification of the active agents, methods suitable for the purpose of analysis were used by the applicant. For official controls pulsed-field gel electrophoresis (PFGE) is recommended in principle, however - as the concentrations for individual strains in the product are not provided - it may not be applicable.

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