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CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to: FAD-2009-0057
CRL/090039

Name of Additive: Fecinor/Fecinor Plus

Active Agent (s): *Enterococcus faecium* CECT 4515

Rapporteur Laboratory: Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium

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EXECUTIVE SUMMARY

In the current application authorisation is sought for the microbial feed additive *Enterococcus faecium* CECT 4515 under the category 'zootechnical additives', functional group 4(b), 'gut flora stabilisers' according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *Fecinor* and *Fecinor Plus* for chickens for fattening. The *feed additive* is intended to be used at a concentration of 1×10^9 CFU/kg of complete *feedingstuffs*.

For the enumeration of *Enterococcus faecium* CECT 4515 in *feed additives*, *premixtures* and *feedingstuffs* the CRL recommends CEN spread plate method using Bile Esculin Azide Agar (EN 15788), instead of the single laboratory validated method submitted by the applicant. The performance characteristics of the EN 15788 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 1×10^5 CFU/kg, well below the minimum dose proposed by the applicant (1×10^9 CFU/kg of *feedingstuffs*).

Molecular methods were used by the applicant for identification of the active agent. The CRL recommends 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.

KEYWORDS

Enterococcus faecium CECT 4515, zootechnical additives, gut flora stabilisers, chickens for fattening.

1. BACKGROUND

Enterococcus faecium CECT 4515 is a feed additive for which authorisation under Article 4(1) is sought under the category of 'zotechnical additives' functional group 4(b), 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003 [1]. The *feed additive* is already authorised under Regulation (EC) No 2036/2005 for the use for weaned piglets. The strain is deposited in 'Colección Española de Cultivos Tipo (CECT)' at the University of Valencia, Spain [2]. Specifically, authorisation is sought for the use of *Fecinor* and *Fecinor Plus* for chickens for fattening. *Fecinor* and *Fecinor Plus* are dry powders mixed with sepiolite with a final concentration of 1×10^9 CFU/g and 1×10^{10} CFU/g of *Enterococcus faecium* CECT 4515, respectively [2]. The *feed additive* is intended to be mixed at a concentration of 1×10^9 CFU/kg of complete *feedingstuffs* for chickens for fattening [3].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application or group of applications. For this dossier, the methods of analysis submitted in connection with *Fecinor/Fecinor Plus*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

For identification and characterisation of the strain *Enterococcus faecium* CECT 4515 the applicant used Polymerase Chain Reaction (PCR) [4], Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) [5] and Pulsed Field Gel Electrophoresis (PFGE) [6]. These methods are suitable for the purpose of analysis.

The CRL recommends for official control, Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification [7].

Qualitative and quantitative composition of any impurities in the additive

The applicant analysed the *feed additive* for microbial contaminants (such as *Salmonella*, *Clostridium perfringens*, coliform bacteria, *Escherichia coli*, *Staphylococcus aureus*, enterococci and yeasts and moulds) by using appropriate AOAC and FDA tests [8].

For undesirable substances (i.e. arsenic, cadmium, mercury, lead, fluor, aflatoxins) internationally recognised standard methods are available at the respective Community Reference Laboratories, in accordance with Commission Regulation (EC) No 776/2006.

Description of the analytical methods for the determination of the active substance in feed additive, premixtures, feedingstuffs and water

For enumeration of *Enterococcus faecium* CECT 4515 in *feed additive, premixtures and feedingstuffs*, the applicant proposes a single laboratory validated pour plate method using Slanetz and Bartley agar [9]. The sample is suspended and diluted in a saline solution; appropriate dilutions are then transferred into petri dishes and Slanetz and Bartley agar is added. When the agar is solidified, plates are incubated at 37°C for 48 hours before colony counting.

The CRL recommends instead the internationally recognised ring trial validated spread plate method developed by CEN for the enumeration of *Enterococcus* spp (EN 15788) [10]. The sample is suspended in phosphate buffered saline (PBS) and diluted in a peptone salt solution, the appropriate dilutions are then spread on Bile Esculin Azide Agar. The agar plates are incubated at 37°C for 24 hours before colony counting. 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 1×10^5 CFU/kg [11], well below the minimum dose proposed by the applicant (1×10^9 CFU/kg of *feedingstuffs*).

The CRL recommends, for official control, the CEN method EN 15788 for the enumeration of *Enterococcus faecium* CECT 4515 in *feed additives, premixtures and feedingstuffs*.

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

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends the CEN method - EN 15788 - for the enumeration of the active agent *Enterococcus faecium* CECT 4515 in *feed additive, premixtures* and *feedingstuffs*.

For the analysis of the identity of the bacterial strain, *Enterococcus faecium* CECT 4515 the CRL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories in accordance with article 10 of Commission Regulation (EC) No 378/2005 is not considered necessary.

Recommended text for the register entry (analytical method)

- Enumeration: Spread plate method using Bile Esculin Azide agar (EN 15788)
- Identification: Pulsed Field Gel Electrophoresis (PFGE)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Fecinor/Fecinor Plus* have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

- [1] *Application/Ref:SANCO/D/2:Forw.Appl.1831/0045-2009
- [2] *Technical dossier, Section II. Identity, characterisation and conditions of use of the additive
- [3] *Application, Proposal for Register Entry, Annex A
- [4] *Technical dossier, Section II. Annex II.13.
- [5] *Technical dossier, Section II. Annex II.14.
- [6] *Technical dossier, Section II. Annex II.15.
- [7] European Community Project SMT4-CT98-2235.'Methods for the Official Control of Probiotics Used as Feed Additives, Volume 1. 2002. Report 20873-1. Office for official Publications of the European Communities. ISBN 92-894-6250-7 (Vol. I)
- [8] *Technical dossier, Section II. Annex II.9.
- [9] *Technical dossier, Section II. Annex II.29.
- [10] EN 15788 'Animal feeding stuffs - Isolation and enumeration of *Enterococcus (E. faecium)* spp'
- [11] ISO 7218 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'

*Refers to Dossier no: FAD-2009-0057

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Community Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)