



JRC.DG.D.6/CvH/DM/ag/ARES(2010)793478

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

Dossier related to: FAD-2009-0022
CRL/ 090009

Name of additive: Lactiferm®

Active Agent (s): *Enterococcus faecium* M74® NCIMB 11181

Rapporteur Laboratory: Central Control and Testing Inst. of Agriculture Laboratory of Analysis of Feedingstuffs
Bratislava, Slovakia

Report prepared by: Katarina Sikhartova (UKSUP)

Report checked by: DM, PR (CRL-FA)
Date: 09/11/2010

Report approved by: Christoph von Holst (CRL-FA)
Date: 10/11/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought under Articles 4(1) (new use) and 10 (2) (re-evaluation of an authorised additive) for feed additive *Lactiferm*® under the category 'zootechnical additives', functional group 4(b), 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. The active component of *Lactiferm*® is a pure culture of strain *Enterococcus faecium* M74® NCIMB 11181. The *feed additive* is intended to be marketed in three forms with different concentration of *Enterococcus faecium* M74® NCIMB 11181: - powder form *Lactiferm*® Basic 50 (5×10^{10} CFU/g), - coated form *Lactiferm*® Caps 50 (5×10^{10} CFU/g), and - water soluble form *Lactiferm*® WS 200 (2×10^{11} CFU/g).

It is intended to be mixed in powder and coated form at a dose ranging from 5×10^8 to 2×10^{10} CFU/kg of complete *feedingstuffs* for calves and piglets and from 2.5×10^8 to 1.5×10^{10} CFU/kg for chickens for fattening. In *water* the Applicant proposed a non classical value, but a minimum content of *Lactiferm*® per 100 piglets and 1000 birds in drinking water is recommended in the registry entry.

For the enumeration of *Enterococcus faecium* M74® NCIMB 11181 in *feed additives*, *premixtures*, *feedingstuffs* and *water* the Applicant proposes ring trial validated spread plate method developed by CEN for the enumeration of *Enterococcus* spp (EN 15788). The CRL recommends the CEN spread plate method using Bile Esculin Azide Agar. 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 (2.5×10^8 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 M74® NCIMB 11181, zootechnical additives, gut flora stabilisers, calves, piglets, chickens for fattening.

1. BACKGROUND

In the current application authorisation is sought under Articles 4(1) (new use) and 10 (2) (re-evaluation of an authorised additive) for feed additive *Lactiferm*® under the category 'zootechnical 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 Commission Regulation (EC) No 1333/2004. The active component of *Lactiferm*® is a pure culture of strain *Enterococcus faecium* M74® NCIMB 11181. The strain is deposited at the 'National Collection of Industrial and Marine Bacteria (NCIMB)' in Aberdeen, Scotland [2]. The *feed additive* is intended to be marketed in three forms with different concentration of *Enterococcus faecium* M74® NCIMB 11181:

- powder form *Lactiferm*® Basic 50 (5×10^{10} CFU/g),
- coated form *Lactiferm*® Caps 50 (5×10^{10} CFU/g), and
- water soluble form *Lactiferm*® WS 200 (2×10^{11} CFU/g)[3].

It is intended to be mixed in powder and coated form at a dose ranging from:

- 5×10^8 to 2×10^{10} CFU/kg of complete *feedingstuffs* for calves and piglets, and
- 2.5×10^8 to 1.5×10^{10} CFU/kg for chickens for fattening [3].

In *water* the Applicant proposed a minimum content of *Lactiferm*® per 100 piglets and 1000 birds in drinking water as recommended in the registry entry [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 *Lactiferm*®, 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* M74® NCIMB 11181 the Applicant used modern microbial techniques such as SDS-PAGE (1 D-protein gel-electrophoresis) and cluster analysis [4]. These methods are suitable for the purpose of analysis. The CRL recommends instead for official control, Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification .

Qualitative and quantitative composition of any impurities in the additive

The Applicant analysed the *feed additive* for microbial contaminants (such as coliforms, *Escherichia coli*, *Salmonella*, moulds and yeasts) by using appropriate ISO tests [5].

For undesirable substances (i.e. arsenic, cadmium, mercury, lead, selenium, copper, zinc, chrome, 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* M74® NCIMB 11181 in *feed additive, premixtures, feedingstuffs* and *water* the Applicant proposes the ring trial validated spread plate method developed by CEN for the enumeration of *Enterococcus* spp (EN 15788) [6]. 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 [7], well below the minimum dose proposed by the applicant (2.5×10^8 CFU/kg of *feedingstuffs*).

Based on these performance characteristics the CRL recommends for official control the above mentioned CEN method EN 15788 for the enumeration of *Enterococcus faecium* M74® NCIMB 11181 in *feed additives, premixtures, feedingstuffs* and *water*.

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* M74® NCIMB 11181 in *feed additive, premixtures, feedingstuffs* and *water*.

For the identification of the bacterial strain *Enterococcus faecium* M74® NCIMB 11181 the CRL recommends Pulsed Field Gel Electrophoresis (PFGE) for official control.

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 *Lactiferm*® 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.1831/017-2009
- [2] *Technical dossier, Section II. Annex II 2-1-2a
- [3] *Application, Proposal for Register Entry, Annex A
- [4] *Technical dossier, Section II. Annex II 2-1-2b
- [5] *Technical dossier, Section II. BS_EN_ISO
- [6] EN 15788 'Animal feeding stuffs - Isolation and enumeration of *Enterococcus (E. faecium)* spp'
- [7] ISO 7218:2007 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'

*Refers to Dossier no: FAD-2009-0022

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Central Control and Testing Inst. of Agriculture Laboratory of Analysis of Feedingstuffs (UKSUP), Bratislava, Slovakia. 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

The following National Reference Laboratories contributed to this report:

- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Państwowy Instytut Weterynaryjny, Puławy (PL)