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

FUMzyme[®]
(FAD-2017-0005; CRL/160042)

**Evaluation Report on the Analytical Methods submitted
in connection with the Application for Authorisation of a
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Dossier related to: **FAD-2017-0005 - CRL/160042**

Name of Product: ***FUMzyme*[®]**

Active Agent (s): **Fumonisin esterase EC 3.1.1.87**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
JRC Geel, Belgium**

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Date: **17/05/2017**

Report approved by: **Christoph von Holst**
Date: **17/05/2017**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *FUMzyme*[®] under the category/functional group 1(m) "technological additives"/"substances for reduction of the contamination of feed by mycotoxins", according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the *feed additive* for all pigs and all avian species. *FUMzyme*[®] is used for its ability to degrade fumonisin B₁. The enzyme detoxifies this mycotoxin by cleavage of the toxin's diester bonds and removal of the propane-1,2,3-tricarboxylic acid side chains. According to the Applicant, the active substance in *FUMzyme*[®] is *fumonisin esterase* (EC 3.1.1.87) which is added to a maltodextrin carrier to result in a minimum guaranteed enzyme activity of 3000 U/g. The Applicant defined the enzyme activity unit (U) as follows:

One unit (U) is the enzymatic activity that releases 1 µmol propane-1,2,3-tricarboxylic acid per minute from 100 µM fumonisin B₁ in 20 mM Tris-Cl buffer pH 8.0 with 0.1 mg/ml bovine serum albumin at 30 °C.

FUMzyme[®] is intended to be used in *premixtures* and *feedingstuffs*, with a proposed enzyme activity ranging from 15 to 300 U/kg *feedingstuffs*.

For the quantification of the *fumonisin esterase* activity in the *feed additive* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified method based on High Performance Liquid Chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). The method is based on the quantification of the propane-1,2,3-tricarboxylic acid released from the action of the enzyme on fumonisin B₁.

For the quantification of the enzyme activities in *premixtures*, the Applicant suggested to dilute *premixture* samples with animal feed (according to the recommended inclusion rate) and analyse them applying the method for *feedingstuffs* mentioned above.

Based on the experimental evidence available the EURL recommends for official control the HPLC-MS/MS method submitted by the Applicant for the quantification of the *fumonisin esterase* activity in the *feed additive*, *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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

FUMzyme[®], *fumonisin esterase*, technological additives, substances for reduction of the contamination of feed by mycotoxins, all pigs, all avian

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new use of an authorised feed additive) for *FUMzyme*[®] under the category/functional group 1(m) "technological additives" / "substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought for the use of the *feed additive* for all pigs and all avian species [1][2].

According to the Applicant, the active substance in *FUMzyme*[®] is *fumonisin esterase* (EC 3.1.1.87) which is added to a maltodextrin carrier to result in a minimum guaranteed enzyme activity of 3000 U/g. Another preparation of *fumonisin esterase* produced by a different strain is currently authorized as a technological additive for pigs by Commission Implementing Regulation (EU) No 1115/2014 [3].

Fumonisin esterase is used to degrade fumonisin B₁; it detoxifies this mycotoxin by cleavage of the toxin's diester bonds and removal of the propane-1,2,3-tricarboxylic acid (TCA) side chains [4].

The Applicant defined the enzyme activity unit (U) as follows:

One unit (U) is the enzymatic activity that releases 1 µmol propane-1,2,3-tricarboxylic acid per minute from 100 µM fumonisin B₁ in 20 mM Tris-Cl buffer pH 8.0 with 0.1 mg/ml bovine serum albumin at 30 °C.

FUMzyme[®] is intended to be used in *premixtures* and *feedingstuffs*, at a minimum recommended enzyme activity of 10 U/kg *feedingstuffs* [6].

Note: The EURL previously evaluated the analytical methods for the determination of *FUMzyme*[®] in the frame of FAD-2013-0002 [7].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *FUMzyme*® 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

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (arsenic, cadmium, lead, mercury and mycotoxins) are available from the respective European Union Reference Laboratories [8].

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

For the quantification of the *fumonisin esterase* activity in the *feed additive* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified method based on High Performance Liquid Chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). This method is based on the quantification of the propane-1,2,3-tricarboxylic acid (TCA) released from the fumonisin B₁ (FB1) [9].

Feed additive or *feedingstuffs* samples are mixed with a Tris-Cl buffer at pH 8.0 containing bovine serum albumin (FCE buffer) and added to a fumonisin B₁ solution. The mixture is then incubated at 30 °C. Aliquots of the reaction mixture are taken at five different incubation times (15, 30, 60, 120 and 240 min) and heat deactivated at 99 °C. TCA is then quantified in the five aliquots by HPLC-MS/MS (m/z 174.904 > 69.000) using external calibration and the enzyme activity is derived from the slope of the linear range of this curve [9][10].

Furthermore, the Applicant suggested to dilute the *premixture* samples with animal feed (according to the recommended inclusion rate) and to analyse them applying the above mentioned method for the quantification of *fumonisin esterase* activity in *feedingstuffs* [9].

The EURL recalculated the following performance characteristics [7] using the experimental data reported in the frame of the validation and verification studies [11] for *feed additive* and *feedingstuffs* samples containing 3000 U/g and 15 to 300 U/kg, respectively:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.6 to 7.8 %;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 3.4 to 8.2 %;
- and
- a recovery rate (R_{Rec}) ranging from 89 to 115 %.

Furthermore, the Applicant reported a limit of quantification (LOQ) of 2.1 U/kg *feedingstuffs*.

Based on the experimental evidence available the EURL recommends for official control the HPLC-MS/MS method submitted by the Applicant for the quantification of the *fumonisin esterase* activity in the *feed additive, 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the single-laboratory validated and further verified method based on High Performance Liquid Chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) for the quantification of *fumonisin esterase* in the *feed additive, premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

Quantification of *fumonisin esterase* activity in the *feed additive, premixtures* and *feedingstuffs*:

- High Performance Liquid Chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) method based on the quantification of the propane-1,2,3-tricarboxylic acid released from the action of the enzyme on fumonisin B₁ at pH 8.0 and 30 °C.

One unit (U) is the enzymatic activity that releases 1 µmol propane-1,2,3-tricarboxylic acid per minute from 100 µM fumonisin B₁ in 20 mM Tris-Cl buffer pH 8.0 with 0.1 mg/ml bovine serum albumin at 30 °C.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *FUMzyme*[®] have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

- [1] *Application, Reference SANTE/E5: FORW. APPL. 1831-0004-2017
 - [2] *Application, Proposal for Register Entry – Annex A
 - [3] Commission Implementing Regulation (EU) No 1115/2014 of 21 October 2014 concerning the authorisation of a preparation of fumonisin esterase produced by *Komagataella pastoris* (DMS 26643) as feed additive for pigs.
 - [4] *Technical dossier, Section II: II.2 Characterisation of the active substance/agent
 - [5] *Technical dossier, Section II: II.1 Identity of the additive
 - [6] *Technical dossier, Section II: II.5 Conditions of use of the additive
 - [7] EURL Evaluation Reports FAD-2013-0002
https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2013-0002-FUMzyme.doc_.pdf
 - [8] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
 - [9] *Technical dossier, Section II – Annex 73
 - [10] *Technical dossier, Section II – Annex 76
 - [11] *Technical dossier, Section II – Annex 74 & Annex 75
- *Refers to Dossier no: FAD-2017-0005

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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