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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

Preparation of 3-phytase (FSF10000)

(FAD-2017-0043; CRL/170004)

European Union Reference Laboratory
Feed Additives

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

Dossier related to:	**FAD-2017-0043 - CRL/170004**
Name of Feed Additive:	***Preparation of 3-phytase (FSF10000)***
Active Agent (s):	**3-phytase (EC 3.1.3.8)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Piotr Robouch & Christoph von Holst** **06/07/2018**
Report approved by: Date:	**Christoph von Holst** **10/07/2018**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) of the Regulation (EC) No 1831/2003 for a *Preparation of 3-phytase (FSF10000)* under the category/functional groups 4 (a and c) "zootechnical additives"/"digestibility enhancers" and "substances which favourably affect the environment". Specifically, authorisation is sought for the use of the *feed additive* for different poultry and porcine species. The liquid formulation of this *feed additive* is currently authorised by Commission Implementing Regulation (EU) 2017/895 for chickens for fattening and laying hens.

According to the Applicant, *FSF10000* is a solid formulation containing *3-phytase* (active agent), sodium citrate, water and wheat bran. The *3-phytase* enzymatic activity is expressed in FTU units, where "one FTU is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C".

The product is intended to be marketed as a solid formulation having a guaranteed minimum *phytase* activity of 10000 FTU/g. *FSF10000* is intended to be used directly in *feedingstuffs* or through *premixtures* to obtain a minimum activity of 500 or 1000 FTU/kg *feedingstuffs* depending of the target species and a maximum activity of 1000 FTU/kg *feedingstuffs*.

For the quantification of the *phytase* activity in *feedingstuffs* the Applicant applied the colorimetric EN ISO 30024 method. Furthermore the Applicant applied this method with slight modifications to analyse of the *feed additive* (*FSF10000*) and *premixtures*, and obtained similar performance characteristics. Based on the performance characteristics obtained, the EURL recommends for official control these colorimetric methods for the quantification of the *phytase* 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) is not considered necessary.

KEYWORDS

3-phytase, *Preparation of 3-phytase (FSF10000),* "zootechnical additives"/"digestibility enhancers" and "substances which favourably affect the environment", chickens and minor poultry for fattening and reared for laying, laying hens, pigs for fattening and minor porcine species

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new use) of the Regulation (EC) No 1831/2003 for a *Preparation of 3-phytase (FSF10000)* under the category/functional groups 4 (a and c) "zootechnical additives"/"digestibility enhancers" and "substances which favourably affect the environment" [1][2]. Specifically, authorisation is sought for the use of the *feed additive* for chickens and minor poultry for fattening and reared for laying, laying hens, pigs for fattening and minor porcine species [1][2]. The *feed additive* in its liquid formulation is currently authorised by Commission Implementing Regulation (EU) 2017/895 for chickens for fattening and laying hens [3].

FSF10000 is a *3-phytase* formulation produced by *Komagataella pastoris* (CECT 13094) [4]. According to the Applicant, *FSF10000* is a solid formulation containing *3-phytase* (active agent), sodium citrate, water and wheat bran [5]. The phytase enzymatic activity is expressed in FTU units, where "one FTU is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C" [5][6].

The product is intended to be marketed with a guaranteed minimum *phytase* activity of 10000 FTU/g [2][5]. *FSF10000* is intended to be used directly in *feedingstuffs* or through *premixtures* to obtain a minimum activity of 500 or 1000 FTU/kg *feedingstuffs* depending of the target species and a maximum activity of 1000 FTU/kg *feedingstuffs* [2][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 *Preparation of 3-phytase (FSF10000)* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the quantification of the *phytase* activity in *feedingstuffs* the Applicant submitted the ring-trial validated colorimetric method (EN ISO 30024) [6] based on the enzymatic reaction of *phytase* on phytate.

Samples containing *phytase* are incubated with sodium phytate, triggering the release of inorganic phosphate and forming a yellow complex with an acidic molybdate/vanadate reagent. The optical density of the yellow complex is measured at 415 nm and the inorganic phosphate released is quantified against a phosphate standard calibration curve. The following performance characteristics were reported for *feedingstuffs* at a nominal *phytase* activities ranging from 500 to 1500 FTU/kg [6]:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.2 to 10.6 %;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.4 to 15 %; and
- a limit of quantification (LOQ) of 60 FTU/kg *feedingstuffs*.

The Applicant applied few experimental modifications to the abovementioned ISO method to analyse the *feed additive* and *premixtures* [9][10]. The EURL re-evaluated the experimental data reported by the Applicant in the frame of the validation study for the *feed additive, premixtures* and *feedingstuffs* to calculate a precision (repeatability and intermediate precision) ranging from 2.5 and 12.5 % [11][12][13]. These performance characteristics are in good agreement with those reported in the ring-trial validated colorimetric method (EN ISO 30024) thus confirming the applicability of the proposed method to the analysis of *feed additive (FSF10000)* and *premixture* samples.

Upon request of one of the NRLs the EURL requested to the Applicant to apply the respective ring-trial validated VDLUFA methods [14][15] for the product (VDLUFA 27.1.4) and premixtures (VDLUFA 27.1.3). The Applicant provided experimental evidences of the equivalence of both analytical approaches when applied to the *feed additive* and to the *premixture* samples by applying the experimental design proposed by the EURL [16].

Based on the performance characteristics available the EURL recommends for official control the colorimetric methods mentioned above for the quantification of the *phytase* activity in the *feed additive, premixtures* and *feedingstuffs.*

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

Evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

Evaluation of corresponding methods of analysis is not considered necessary by the EURL.

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 colorimetric method based on the enzymatic reaction of *phytase* on the phytate for the quantification of the *phytase* activity in the *feed additive*, *premixtures* and *feedingstuffs.*

Recommended text for the register entry (analytical method)

For the quantification of the *phytase* activity in the *feed additive and premixtures:*

- colorimetric method based on the enzymatic reaction of *phytase* on the phytate

For the quantification of the *phytase* activity in *feedingstuffs*:

- colorimetric method based on the enzymatic reaction of *phytase* on the phytate – EN ISO 30024

One phytase unit (FTU) is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at 37°C and pH 5.5.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Preparation of 3-phytase (FSF10000)* 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, Application Form – Annex 1 – Subm. No. 1501176074256-2110

[2] *Application, Proposal for Register Entry – Annex A

[3] Commission Implemeting Regulation (EU) 2017/895 of 24 May 2017 concerning the authorisation of a preparation of 3-phytase produced by Komagataella pastoris (CECT 13094) as a feed additive for chickens for fattening and laying hens (holder of authorisation Fertinagro Nutrientes S.L.)

[4] *Technical dossier, Annex_II_7_Microbial indentification

[5] *Technical dossier, Section II: 2.1 Identity of the additive

[6] EN ISO 30024:2009 - Animal feeding stuffs -- Determination of phytase activity

[7] *Technical dossier, Section II: 2.5 Conditions of use of the additive

[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: 2.6.1 Methods of analysis of the active substance

[10] *Technical dossier, Annex_II_19

[11] *Supplementary information, ANOVA_FSF10000_FA.pdf

[12] *Supplementary information, ANOVA_FSF10000_PM.pdf

[13] *Supplementary information, ANOVA_FSF10000_FSs.pdf

[14] Association of German Agricultural Analytic and Research Institute (VDLUFA) (Ed.) 2012: Method 27.1.3 Preparation of mineral feeds and mineral premixtures for the determination of the phytase activity. Methods Book III. The Chemical analysis of feedingstuffs, 3nd Edition, 8th Supplementary volume, VDLUFA-Publishing house, Darmstadt

[15] Association of German Agricultural Analytic and Research Institute (VDLUFA): Method 27.1.4 Preparation of feed additives for the determination of the phytase activity.

[16] f*Supplementary information, equivalence analytical methods.pdf

*Refers to Dossier no: FAD-2017-0043

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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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
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- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)