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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

Axtra® PHY 20000 TPT2

(FAD-2015-0048; CRL/150029)

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-2015-0048 - CRL/150029**
Name of Product:	***Axtra® PHY 20000 TPT2***
Active Agent (s):	**6-phytase (EC 3.1.3.26)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **19/05/2016**
Report approved by: Date:	**Christoph von Holst** **23/05/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Axtra® PHY 20000 TPT2*, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for several animal species. The active agent of *Axtra® PHY 20000 TPT2* is *6-phytase* (EC 3.1.3.26), produced by fermentation of *Trichoderma reesei*. According to the Applicant, *Axtra® PHY 20000 TPT2* is a dry formulation with a guaranteed minimum enzyme activity of 20000 FTU/g. It is intended to be used in *premixtures* and/or complete *feedingstuffs* to obtain *6-phytase* activities of 250 FTU/kg *feedingstuffs*. The Applicant expresses the *phytase* enzymatic activity in FTU/g units, where "one phytase unit (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".

For the quantification of *phytase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted a single-laboratory validated and further verified colorimetric methods similar to the EN ISO 30024 standard method. Based on the experimental data available, the EURL recommends for official control these colorimetric methods for the quantification of *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

6-phytase, Axtra® PHY 20000 TPT2, zootechnical additives, digestibility enhancers, chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes, laying hens, minor poultry species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species

1. BACKGROUND

In the current application authorisation is sought for *Axtra® PHY 20000 TPT2*, under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes, laying hens, minor poultry species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species [1,2].

The active agent of *Axtra® PHY 20000 TPT2* is *6-phytase* (EC 3.1.3.26), produced by fermentation of a *Trichoderma reesei* [3]. The Applicant expresses the *phytase* enzymatic activity in FTU/g 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" [3]. This definition is in agreement with the *phytase* activity unit defined in the EN ISO 30024 [4].

According to the Applicant, *Axtra® PHY 20000 TPT2* is a dry formulation with a guaranteed minimum enzyme activity of 20000 FTU/g [2,3]. It is intended to be used in *premixtures* and/or complete *feedingstuffs* to obtain *6-phytase* activities of 250 FTU/kg *feedingstuffs* [2,5].

Note: The analytical methods for the determination of a different formulation of *Axtra® PHY* in relevant matrices of the target species were already evaluated by the EURL in the frame of the (FAD-2013-0049) dossier [6].

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 *Axtra® PHY 20000 TPT2* 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 (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [7].

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

For the quantification of the *phytase* activity in the *feed additive* [8], *premixtures* [9] and *feedingstuffs* [10], the Applicant submitted a single-laboratory validated and further verified methods already evaluated by the EURL [6] similar to the ring-trial validated ISO method (EN ISO 30024) [4].

Phytase is incubated with sodium phytate, resulting in the release of inorganic phosphate and forming a yellow complex with an acidic molybdate/vanadate reagent [4]. Based on the satisfactory experimental evidence presented (Table 1) [8] the EURL recommends for official control this single-laboratory validated and further verified method for the quantification of *phytase* activity in the *feed additive.*

For the quantification of *phytase* activity in *feedingstuffs* the Applicant applied the proposed method to feed samples containing *Axtra® PHY 20000 TPT2* [10]. Furthermore, the Applicant quantified the *phytase* activity in *premixture* samples containing *Axtra® PHY 20000 TPT2* [9] by first diluting the samples with heat treated whole grain wheat flour or maize and then analysing them as *feedingstuffs* samples.

The experimental data provided in the frame of the stability studies [8,9,10] were used by the EURL to calculated relative standard deviations (for *repeatability* and for *intermediate precision*) ranging from 0.5 to 14.4% [11,12,13]. The results obtained with the different matrices are summarized in Table 1 and demonstrate the applicability of the proposed method to the feed matrices containing the new formulation.

Additionally, in the frame of a previous dossier related to the liquid formulation of the same *feed additive* [6] the Applicant obtained similar performance characteristics for the in-house method and the EN ISO 30024 standard method when analysing mash feed fortified with their product [14], and therefore demonstrated the equivalence of the two methods.

Based on the satisfactory experimental evidences presented in Table 1 [9,10] the EURL recommends for official control the method submitted by the Applicant to quantify *phytase* in the *feed additive* and the EN ISO 30024 method to the determination of *phytase* in *premixtures* and *feedingstuffs* samples containing the *feed additive* under investigation.

Table 1: Performance characteristics of analytical methods for the determination of *phytase* in *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS)

Method	Matrix	Activity	RSD_r (%)	RSD_{ip} (%)	RSD_R (%)
Danisco	FA [8]	26640-30215 (FTU/g)	1.0-4.4	2.7-4.4	-
	PM [9]	136802-149287 (FTU/kg)	4.3-8.1	6.1-14.4	-
	FS (Mash)[10]	346-397 (FTU/kg)	0.5-11.3	9.1-11.3	-
	FS (Pellets) [10]	406-477 (FTU/kg)	7.5-10.8	7.5-17.6	-
EN ISO 30024	FS [4]	500-1500 (FTU/kg)	2.2-10.6	3.3-12.7	5.4-15.0

RSD_r, RSD_{ip}, and RSD_R : relative standard deviation for *repeatability,intermediate precision and reproducibility*.

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 EURL recommends for official control the single-laboratory validated and further verified colorimetric method for the quantification of *phytase* activity in the *feed additive* and the EN ISO 30024 colorimetric method for the quantification of phytase activity in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

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

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

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

- colorimetric method based on the enzymatic reaction of *phytase* on 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 *Axtra® PHY 20000 TPT2* 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 SANCO/G1: Forw. Appl. 1831/0005-2016

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

[3] *Technical dossier, Section II: II.1 Identity of the additive

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

[5] *Technical dossier, Section II: II.5 Conditions of use of the additive

[6] EURL Evaluation Report FAD 2013-0049
https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0049-axtraphy15000l.pdf

[7] 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

[8] *Technical dossier, Annex_II_22.18

[9] *Technical dossier, Annex_II_23.6

[10] *Technical dossier, Annex_II_24.3 & 25.3

[11] *Supplementary information, eurl_anova_axtraphy2000tpt2_fa.pdf

[12] *Supplementary information, eurl_anova_axtraphy2000tpt2_pm.pdf

[13] *Supplementary information, eurl_anova_axtraphy2000tpt2_fs.pdf

[14] *Supplementary information, Annex_II_29

*Refers to Dossier no: FAD-2015-0048

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union 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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)

- Laboratorio Arbitral Agroalimentario. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid (ES)
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