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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 15000L

(FAD-2013-0049 CRL/120034)



**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-2013-0049 - CRL/120034**

Name of Product: **Axtra® Phy 15000L**

Active Agent (s): **6-phytase (E.C. 3.1.3.26)**

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

Report prepared by: **Johanna Keltti**

Report checked by: **Piotr Robouch (EURL-FA)**
Date: **19/06/2014**

Report approved by: **Christoph von Holst**
Date: **20/06/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for Axta[®] Phy 15000L, 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 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 avian species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species. The active agent of Axta[®] Phy 15000L is 6-*phytase* (EC 3.1.3.26), produced by fermentation of *Trichoderma reesei*. According to the Applicant, Axta[®] Phy 15000L is a liquid preparation with a guaranteed minimum enzyme activity of 15000 U/g. It is intended to be used in *premixtures* and/or complete *feedingstuffs* to obtain 6-*phytase* activities ranging from 250 to 2000 U/kg *feedingstuffs*. The Applicant uses the international phytase unit defined in the EN ISO 30024, where "one phytase unit (U) 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 determination of *phytase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted colorimetric methods derived from the EN ISO 30024 standard method. Phytase is incubated with sodium phytate, resulting in the release of inorganic phosphate and forming a yellow complex with an acidic molybdate/vanadate reagent. Based on the experimental data available, the EURL recommends for official control the in-house validated and further verified method for the quantification of *phytase* activity in the *feed additive* and the EN ISO 30024 standard method for the quantification of *phytase* activity in *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*, Axta[®] Phy 15000L, zootechnical additives, digestibility enhancers, chickens and turkeys for fattening, chickens reared for laying, turkeys reared for breeding, turkeys for breeding purposes, laying hens, minor avian species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species

1. BACKGROUND

In the current application authorisation is sought under article 4(1) for Axta[®] Phy 15000L, 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 avian species, weaned piglets, pigs for fattening, sows for reproduction, and minor porcine species [1,2].

The active agent of Axta[®] Phy 15000L is *6-phytase* (EC 3.1.3.26), produced by fermentation of a *Trichoderma reesei* [3].

According to the Applicant, Axta[®] Phy 15000L is a liquid preparation with a guaranteed minimum enzyme activity of 15000 U/g, [2,3]. It is intended to be used in *premixtures* and/or complete *feedingstuffs* to obtain *6-phytase* activities ranging from 250 to 2000 U/kg *feedingstuffs* [2].

The Applicant uses the internationally agreed enzyme activity unit defined in the EN ISO 30024 [4], where "*one phytase unit (U) is the amount of enzyme which releases one micromole of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C*".

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 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 Axta[®] Phy 15000L 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 [5].

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*, the Applicant submitted in-house validated and further verified method [6] derived from ring-trial validated CEN method (EN ISO 30024) [4].

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 performance characteristics obtained in the frame of the validation and verification studies are presented in the Table 1.

Based on the satisfactory experimental evidence presented the EURL recommends for official control this in-house 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 EN ISO 30024 standard method to feed samples containing *Axtra Phy 15000L* [7] and reported a limit of quantification (LOQ) of 61 U/kg *feedingstuffs* [8]. Furthermore, the Applicant quantified the *phytase* activity in *premixture* samples containing *Axtra Phy 15000L* by first diluting the samples with heat treated whole grain wheat flour or maize and then analysing them as *feedingstuffs* samples [8,9]. The satisfactory results presented [7,8] demonstrate the applicability of the CEN method to the determination of *phytase* in *premixture* 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)

Matrix	Activity	RSD _r (%)	RSD _{ip} (%)	RSD _R (%)	R _{rec} (%)	Reference
FA	24000 U/g	4.6	9.2	-	100.3	Val. [10]
FA	7060 U/g	3.7	5.2	-	102.1	Ver. [11]
PM	200 U/g	7.8	10.4	-	-	Val. [8]
FS	500-1500 U/kg	6-8.3	10.4-12.7	8-15	-	EN ISO, [4]

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

Based on the experimental evidence available, the EURL recommends for official control the ring-trial validated colorimetric method EN ISO 30024 for the quantification of the *phytase* activity in *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 EURL recommends for official control the in-house 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 the phytate

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

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

One phytase unit (U) 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 Axta[®] Phy 15000L 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. 1831/0049-2013
 - [2] *Application, Proposal for Register Entry
 - [3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; methods of analysis
 - [4] EN ISO 30024:2009 - *Animal feeding stuffs -- Determination of phytase activity*
 - [5] 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
 - [6] *Technical dossier, Section II, Annex II.24 Method Analysis Product
 - [7] *Technical dossier, Section II, Annex II.29 Verification feed and premixtures
 - [8] *Technical dossier, Section II, Annex II.27 Validation feed
 - [9] *Technical dossier, Section II, Annex II.25 Method Analysis feed and premixtures
 - [10] *Technical dossier, Section II, Annex II.26 Validation product
 - [11] *Technical dossier, Section II, Annex II.28 Verification product
- *Refers to Dossier no: FAD-2013-0049

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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)