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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

APSA PHYTAFEED®

(FAD-2018-0031; CRL/160026)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2018-0031 - CRL/160026**
Name of Product:	***APSA PHYTAFEED® GR and L***
Active Agent (s):	**6-Phytase**
Rapporteur Laboratory:	**Austrian Agency for Health and Food Safety (AGES), Austria**
Report prepared by:	**Irmengard Strnad**
Report checked by: Date:	**María José González de la Huebra** **11/01/2019**
Report approved by: Date:	**Christoph von Holst** **16/01/2019**

EXECUTIVE SUMMARY

In the current application authorisation in is sought under article 4(1) of Regulation (EC) No 1831/2003 for APSA PHYTAFEED® under the category/functional group 4(a) "zootechnical additives"/"digestibility enhancers". Specifically, authorisation is sought for chickens for fattening, chickens reared for laying and minor poultry species.

According to the Applicant, *6-phytase* is the active substance of APSA PHYTAFEED® *produced by Komagataella pastoris appa T75* (CGMCC 12056). The Applicant expresses the phytase enzymatic activity in units (U), where "one U is the amount of enzyme which releases one micromole of inorganic phosphate from phytate per minute at pH 5.5 and 37°C".

The product is intended to be marketed as a granulated and a liquid formulation having a guaranteed minimum *phytase* activity of 20000 U/g (APSA PHYTAFEED® 20000GR) and of 20000 U/ml (APSA PHYTAFEED® 20000 L). APSA PHYTAFEED® is intended to be included into feedingstuffs directly and/or through premixtures to obtain a minimum activity of 250 U/kg feedingstuffs.

For the determination of *phytase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant applied a modified protocol of the EN ISO 30024 standard method. Upon request of the EURL the Applicant applied (i) the ring-trial validated colorimetric EN ISO 30024 standard method for the quantification of the *phytase* activity in *feedingstuffs*, (ii) the ring-trial validated colorimetric method (VDLUFA 27.1.3) for the quantification of the *phytase* activity in *premixtures* and (iii) the ring-trial validated colorimetric method (VDLUFA 27.1.4) for the quantification of the *phytase* activity in the *feed additives*. Comparable results and method performance characteristics were obtained and demonstrate the applicability of these methods to the determination of phytase activity in *feed additive*, *premixtures* and *feedingstuffs* of the product under investigation.

Based on the performance characteristics provided the EURL recommends for official control the colorimetric methods mentioned above 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

6-phytase, APSA PHYTAFEED®, "zootechnical additives"/"digestibility enhancers", chickens for fattening, chickens reared for laying and minor poultry species

1. BACKGROUND

In the current application authorisation is sought under article 4(1) of the Regulation (EC) No 1831/2003 for APSA PHYTAFEED® under the category/functional groups 4 (a) "zootechnical additives"/"digestibility enhancers" [1][2]. Specifically, authorisation is sought for the use of the feed additive for chickens for fattening, chickens reared for laying and minor poultry species [1].

According to the Applicant, *6-phytase* is the active agent of APSA PHYTAFEED® which is produced by fermentation of a genetically modified yeast *Komagataella pastoris appa T75* (CGMCC 12056) [3]. The Applicant expresses the *phytase* enzymatic activity in units (U), where "one U is the amount of enzyme which releases one micromole of inorganic phosphate from 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].

The product is intended to be marketed as a granulated and a liquid formulation (APSA PHYTAFEED® 20000 GR & 20000 L) having a guaranteed minimum *phytase* activity of 20000 U/g and of 20000 U/ml, respectively [3]. APSA PHYTAFEED® is intended to be included into *feedingstuffs* directly and/or through *premixtures* to obtain a minimum activity of 250 U/kg *feedingstuffs* [2][3].

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 *APSA PHYTAFEED®* 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 determination of *phytase* activity in the *feed additive*, premixtures and *feedingstuffs*, the Applicant applied a modified protocol of the EN ISO 30024 standard method. Upon request of the EURL the Applicant applied (i) the ring-trial validated colorimetric EN ISO 30024 standard method for the quantification of the *phytase* activity in *feedingstuffs*, (ii) the

ring-trial validated colorimetric method (VDLUFA 27.1.3) for the quantification of the *phytase* activity in *premixtures* and (iii) the ring-trial validated colorimetric method (VDLUFA 27.1.4) for the quantification of the *phytase* activity in the *feed additive* [7, 8]. The Applicant provided experimental evidences, summarised in Table 1, of the equivalence of the Applicant method (APSARD) and the ones described in the mentioned standards when applied to the *feed additive*, *premixtures* and *feedingstuffs* samples by applying the experimental design proposed by the EURL [9][10][11]. No significant differences in the activity were detected. Recovery rate was calculated on the basis of target activity from the amount of supplemented feed additive, specified by the applicant's method.

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 nominal phytase activities ranging from 500 to 1500 U/kg [4]:

- a relative standard deviation for repeatability (RSD_r) ranging from 2.2 to 11 %;
- a relative standard deviation for reproducibility (RSD_R) ranging from 5.4 to 15 %; and
- a limit of quantification (LOQ) of 60 U/kg *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.

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

Matrix	Activity (U/kg)	Method	RSD_r (%)*	RSD_{ip} (%)*	R_{rec} (%)
FA[9]	23,353,000	APSARD	2.7	3.0	102
		VDLUFA 27.1.4	3.9	4.1	101
PM [10]	250,000	APSARD	5.2	6.3	98
		VDLUFA 27.1.3	3.3	4.0	98
FS [11]	1000	APSARD	5.5	5.7	100
		EN ISO 30024	2.3	2.3	94

RSD_r: relative standard deviation for repeatability; RSD_{ip}: relative standard deviation for intermediate precision; R_{rec}: recovery rate; (*) Recalculated by the EURL

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)

An 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 *phytase* activity in the *feed additive, 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 - VDLUFA 27.1.4

For the quantification of phytase activity in premixtures:

- colorimetric method based on the enzymatic reaction of phytase on the phytate - VDLUFA 27.1.3

For the quantification of phytase activity in 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 *APSA PHYTAFEED®* 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. 1831-0037-2018
[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] 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
[7] 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, VDLUFA-Publishing house, Darmstadt
[8] Association of German Agricultural Analytic and Research Institute (VDLUFA) (Ed.) 2012: Method 27.1.4 Preparation of feed additives for the determination of the phytase activity. Methods Book III. The Chemical analysis of feedingstuffs, VDLUFA-Publishing house, Darmstadt
[9] *Supplementary information, APSA PHYTAFEED, Annex II 6-5
[10] *Supplementary information, APSA PHYTAFEED, Annex II 6-6
[11] *Supplementary information, APSA PHYTAFEED, Annex II 6-7

*Refers to Dossier no: FAD-2018-0031

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the Österreichische Agentur für Gesundheit und Ernährungssicherheit - Austrian Agency for Health and Food Safety (AGES), Wien Austria.. 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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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural**.** Generalitat de Catalunya, Cabrils (ES)

- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)