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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**

**Natuphos<sup>®</sup>E**  
(*FAD-2015-0040; CRL/150027*)



**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-2015-0040 - CRL/150027**

Name of Product : ***Natuphos<sup>®</sup>E***

Active Agent (s): **6-phytase**

Rapporteur Laboratory: **Austrian Agency for Health and Food  
Safety (AGES), Vienna, Austria**

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25/04/2016**

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Date: 25/04/2016**

## EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) of the Regulation (EC) No 1831/2003 for *Natuphos® E* under the category/functional groups 4 (a and c) "zootechnical additives"/"digestibility enhancers" and "substances which favourable affect the environment". Specifically, authorisation is sought for the use of the *feed additive* for all pigs and all avian species.

According to the Applicant, *6-phytase* is the active agent of *Natuphos® E*. 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".

The product is intended to be marketed as granulate formulations having a guaranteed minimum *phytase* activity of 5000 FTU/g (*Natuphos® E 5000G*) and of 10000 FTU/g (*Natuphos® E 10000G*). *Natuphos® E* is intended to be included into *feedingstuffs* directly and/or through *premixtures* to obtain a minimum activity ranging from 100 to 250 FTU/kg *feedingstuffs*, depending on the target species.

For the quantification of phytase activity in *feedingstuffs* the Applicant submitted the ring-trial validated colorimetric EN ISO 30024 standard method. Furthermore the Applicant applied (i) the ISO standard with minor experimental modifications to the analysis of the *feed additive* (*Natuphos® E*) and (ii) the ring-trial validated colorimetric method (VDLUF 27.1.3) for the quantification of the phytase activity in *premixtures* and obtained similar method performance characteristics. 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) is not considered necessary.

## KEYWORDS

*6-phytase, Natuphos® E, "zootechnical additives"/"digestibility enhancers" and "substances which favourably affect the environment", for all pigs, poultry and all avian species.*

## 1. BACKGROUND

In the current application authorisation is sought under article 4(1) of the Regulation (EC) No 1831/2003 for *Natuphos® E* 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 all pigs, and all avian species [1].

According to the Applicant, *6-phytase* is the active agent of *Natuphos® E* which is produced by fermentation of a genetically modified strain of *Aspergillus niger* (DSM 25770) [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].

The product is intended to be marketed as granulated (*Natuphos® E 5000G & 10000G*) formulations having a guaranteed minimum *phytase* activity of 5000 FTU/g and of 10000 FTU/g, respectively [3]. *Natuphos® E* is intended to be included into *feedingstuffs* directly and/or through *premixtures* to obtain a minimum activity of 100, 125, 200 or 250 FTU/kg *feedingstuffs*, depending on the target species [2][5].

## 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 *Natuphos® E* 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 [6].

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

For the quantification of *phytase* activity in *feedingstuffs* the Applicant submitted the ring-trial validated colorimetric standard method EN ISO 30024 [4] 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 nominal *phytase* activities ranging from 500 to 1500 FTU/kg [4]:

- a relative standard deviation for repeatability (RSD<sub>r</sub>) ranging from 2.2 to 10.6 %;
- a relative standard deviation for intermediate precision (RSD<sub>ip</sub>) ranging from 3.3 to 12.7 %;
- a relative standard deviation for reproducibility (RSD<sub>R</sub>) ranging from 5.4 to 15 %; and
- a limit of quantification (LOQ) of 60 FTU/kg feedingstuffs.

Furthermore, the Applicant determined recovery rates (R<sub>rec</sub>) ranging from 94 to 113% in *feedingstuffs* containing 1000 FTU/kg of *Natuphos®E 5000G & 1000G* [7].

For the quantification of the *phytase* activity in *feed additives* the Applicant applied the EN ISO 30024, adapting the sample extraction and dilution of the feed additive (*Natuphos®E 5000G & 1000G*) with buffer solution [8].

For the quantification of the *phytase* activity in *premixtures* the Applicant applied the VDLUFA 27.1.3 [9]. This method includes a solid dilution of the *premixtures* with blank feed and can be considered as the extension of the scope of EN ISO 30024 method [4].

The experimental data reported by the Applicant in the frame of the stability studies for the *feed additive* [8] and for *premixtures* [10] were used to calculate the repeatability and intermediate precision and led to values ranging from 0.3 % to 7.6 % [11][12]. These performance characteristics are in good agreement with those reported in the ring-trial validated colorimetric method (EN ISO 30024) confirming thus the applicability (extension of the scope) of the standard method to the analysis of the *feed additive* and *premixtures* samples.

Based on the performance characteristics available 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) 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

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 (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 *Natuphos® E* 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. 1831/0036-2015
- [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

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- [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] \*Technical dossier, Annex\_II\_63 & 64
- [8] \*Technical dossier, Annex\_II\_57 & 58
- [9] 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, 3<sup>rd</sup> Edition, 8<sup>th</sup> Supplementary volume, VDLUFA-Publishing house, Darmstadt
- [10] \*Technical dossier, Annex\_II\_72 and 73
- [11] \*Supplementary information, NatuphosE\_ANOVA\_FA.pdf
- [12] \*Supplementary information, NatuphosE\_ANOVA\_PM.pdf
- \*Refers to Dossier no: FAD-2015-0040

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Austrian Agency for Health and Food Safety (AGES), Vienna, 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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- Centro di referenza nazionale per la sorveglianza 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)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
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