
Natuphos®E
(FAD-2014-0044; CRL/140034)

Dossier related to: FAD-2014-0044 - CRL/140034
Name of Product: Natuphos® E
Active Agent (s): 6-phytase
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: Piotr Robouch (EURL-FA)
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Report approved by: Christoph von Holst
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EXECUTIVE SUMMARY

In the current application authorisation is sought under article 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". 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 powder and liquid formulations having a guaranteed minimum phytase activity ranging from 5000 FTU/g to 25000 FTU/g. Natuphos® E is intended to be included through premixtures to obtain a minimum activity of 100 125, 200 or 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 (VDLUFA 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", all pigs 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]. Specifically, authorisation is sought for the use of the feed additive for all pigs, and all avian species[1][2].

According to the Applicant, 6-phytase is the active agent of Natuphos® E [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 powder (Natuphos® E 5000) and liquid (Natuphos® E 5000 L, Natuphos® E 10000 L and Natuphos® E 25000 L) formulations having a guaranteed minimum phytase activity of 5000 FTU/g, 10000 FTU/g and 25000 FTU/g respectively [3]. Natuphos® E is intended to be included 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 (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 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 and mycotoxins) are available from the respective European Union Reference Laboratories [6].

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 a nominal phytase activities ranging from 500 to 1500 FTU/kg:

- a relative standard deviation for repeatability (RSD_r) ranging from 2.2 to 10.6 %;
- a relative standard deviation for intermediate precision (RSD_ip) ranging from 3.3 to 12.7 %
- 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 also determined recovery rates (R_{rec}) ranging from 91 to 99% in feedingstuffs containing 500 and 1000 FTU/kg Natuphos® E [7].

For the characterisation of the feed additive the Applicant implemented the EN ISO 30024 standard method, adapting the sample preparation of the feed additive (Natuphos® E) – by including a sample dilution step in the buffer solution.

For the quantification of the enzyme activity in premixtures the Applicant applied the VDLUFA 27.1.3 [8] in the frame of the stability study. This method includes a solid dilution of the premixtures with a blank feed and can be considered as the extension of the scope of the EN ISO 30024 method [9].

The EURL used the experimental data reported by the Applicant in the frame of the validation study for the feed additive [7] and the stability study of premixtures [9] to calculate the following precision (repeatability and intermediate precision) values: 2.3 % for the feed additive [9] and ranging from 8.0% to 12% for the premixtures [11]. These performance characteristics are in good agreement with those reported in the ring-trial validated
colorimetric method (EN ISO 30024) thus confirming the applicability (extension of the scope) of the standard method to the analysis of 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 and premixtures:
- colorimetric method based on the enzymatic reaction of phytase on the phytate

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

[3] *Technical dossier, Section II: II.1 Identity of the additive*
[5] *Technical dossier, Section II: II.5 Conditions of use of the additive*
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)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)