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JRC F.5/CvH/MGH/AS/Ares

**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**

Preparation of 6-phytase (EC 3.1.3.26)
(FAD-2019-0005; CRL/180073)



**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-2019-0005 - CRL/180073**

Name of Product: ***Preparation of 6-phytase***

Active Agent (s): **6-phytase (EC 3.1.3.26)**

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

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Date: **16/03/2020**

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Date: **16/03/2020**

EXECUTIVE SUMMARY

In the current application, authorisation of a *preparation of 6-phytase (EC 3.1.3.26)* is sought under Article 4(1) for avian species under the category/functional group 4 (a) "zootechnical additives"/"digestibility enhancers".

According to the Applicant, the active agent is *6-phytase*. The *phytase* activity is expressed in *phytase* units (FTU). One FTU unit, as described in EN ISO 30024, is defined as "the amount of enzyme that releases 1 µmol of inorganic phosphate from sodium phytate per minute under reaction conditions of pH 5.5 and 37 °C".

The product is intended to be marketed as solid (*Nutrase PD*, *Nutrase PG* and *Nutrase PTS*) and liquid (*Nutrase PL*) formulations with a guaranteed minimum *6-phytase* activity of 100000 FTU/g for the *Nutrase PD* and of 10000 FTU/g for the remaining formulations. It is intended to be included through *premixtures* or directly in *feedingstuffs* to obtain a minimum recommended activity of 250 FTU/kg *feedingstuffs*.

For the quantification of the *phytase* activity the Applicant submitted the ring-trial validated colorimetric standard method ISO 30024 (for the feed additive, *premixtures* and *feedingstuffs*). However as the scope of the ISO 30024 is limited to *feedingstuffs* the EURL requested to the Applicant to apply the ring-trial validated VDLUFA 27.1.4 and VDLUFA 27.1.3 methods to representative *feed additive* and *premixture* samples.

Based on the performance characteristics available the EURL recommends for official control the colorimetric ring-trial validated methods mentioned above for the quantification of the *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

Preparation of 6-phytase (EC 3.1.3.26), *Nutrase P*, "zootechnical additives"/"digestibility enhancers", chickens for fattening, other poultry for fattening, reared for laying and ornamental birds

1. BACKGROUND

In the current application, authorisation of a *preparation of 6-phytase (EC 3.1.3.26)* is sought under Article 4(1) (authorisation of a feed additive or a new use of a feed additive) for chickens for fattening, other poultry for fattening, reared for laying and ornamental birds [1,2,3] under the category/functional group 4 (a) "zootechnical additives"/"digestibility enhancers" according to Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, the active agent of the product is *6-phytase*, produced by fermentation of a genetically modified strain *Pichia Pastoris (CGMCC 7.19)* [2].

The activity of *6-phytase* is expressed in phytase units (FTU). One FTU unit, as described in EN ISO 30024, is defined as "the amount of enzyme that releases 1 µmol of inorganic phosphate from sodium phytate per minute under reaction conditions of pH 5.5 and 37 °C" [3].

The product is marketed as powder (*Nutrase PD*), granulate (*Nutrase PG*); thermostable (*Nutrase PTS*) and as liquid (*Nutrase PL*) formulations with a guaranteed minimum *6-phytase* (active agent) activity of 100000 FTU/g for the *Nutrase PD* and of 10000 FTU/g for the other formulations [1]. It is intended to be included through *premixtures* or directly in *feedingstuffs* to obtain a minimum recommended activity of 250 FTU/kg *feedingstuffs* [1,2,3,4].

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 *preparation of 6-phytase (EC 3.1.3.26)* 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 quantification of the *phytase* activity in *feedingstuffs (Nutrase P)* the Applicant submitted the ring-trial validated colorimetric standard method EN ISO 30024 [5] 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 [5]:

- 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 FTU/kg *feedingstuffs*.

The method VDLUFA 27.1.4 describes the preparation of phytases as *feed additives* for the determination of their *phytase* activity according to EN ISO 30024. This combination of methods has been ring-trial validated for *phytase* samples with activities between 6000 and 58000 FTU/g leading to the following performance characteristics [6]:

- a RSD_r ranging from 2.3 to 4.6 %; and
- a RSD_R ranging from 6.1 to 21 %.

Additionally the ring-trial validated VDLUFA 27.1.3 method [7], based on a solid dilution using maize meal, describes specifically the preparation of *premixtures* for quantification of the *phytase* activity according to EN ISO 30024. This combination of methods has been ring-trial validated for *premixtures* with *phytase* activities between 13000 to 228000 FTU/kg leading to the following performance characteristics [7]:

- a RSD_r ranging from 3.3 to 7.6 %; and
- a RSD_R ranging from 8.3 to 23 %.

Upon EURL request, the Applicant applied the methods VDLUFA 27.1.4 and VDLUFA 27.1.3 to representative *feed additive* [8] and *premixture* [9] samples. The EURL re-evaluated the experimental data reported by the Applicant in the frame of these verification studies and calculated an $RSD_r = 2.2$ % and a relative standard deviation for intermediate precision (RSD_{ip}) = 7.0 % for the *feed additive* [10] and an $RSD_r = RSD_{ip} = 3.5$ % for *premixtures* [11]. These performance characteristics are in good agreement with those reported in the VDLUFA 27.1.3 - VDLUFA 27.1.4 / EN ISO 30024 method combinations thus confirming the suitability for the analysis of these *feed additive* and *premixtures*.

Based on the performance characteristics available the EURL recommends for official control the colorimetric methods mentioned above for the quantification of the *phytase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

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)

The 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 phytate for the quantification of the *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 phytate - VDLUFA 27.1.4

For the quantification of phytase activity in *premixtures*:

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

For the quantification of phytase activity in *feedingstuffs*:

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

One *phytase* unit (FTU) is the amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute at pH 5.5 and 37 °C.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *preparation of 6-phytase (EC 3.1.3.26)* 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, Proposal for register entry, Annex A
- [2] *Application, Reference SANTE/E5: FORW. APPL. 1831-0009-2019 &
- [3] *Annex I Submission number 1548689133725-2348
- [4] *Technical dossier, Section II: 2.5 Conditions of use
- [5] EN ISO 30024:2009 - Animal feeding stuffs - Determination of phytase activity
- [6] Association of German Agricultural Analytic and Research Institute (VDLUFA): Method 27.1.3 Preparation of mineral feeds and mineral premixtures for the determination of the phytase activity
- [7] Association of German Agricultural Analytic and Research Institutes (VDLUFA): Method 27.1.4 Preparation of feed additives for the determination of the phytase activity
- [8] *Supplementary information, 1.a_Nutrased_PL10000_RSD.pdf
- [9] *Supplementary information, 1.b_Nutrased_PD100000_RSD.pdf
- [10] *ANOVA_EURL_ver_FA.pdf
- [11] *ANOVA_EURL_ver_PM.pdf

*Refers to Dossier no: FAD-2019-0005

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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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