EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

D08/FSQ/CVH/SY/D(2008)27454

CRL Evaluation Report on the Analytical Methods submitted in connection with Section II, 2.5 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2008-0003 EFSA-Q-2008-272
Product name:	Phyzyme XP 10000 TPT and Phyzyme XP 10000 L
Active Substance(s):	6-Phytase (EC 3.1.3.26)
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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Date:	22/10/2008
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Date:	23/10/2008

EXECUTIVE SUMMARY

In the current application authorisation is sought for Phyzyme XP 10000 TPT and Phyzyme XP 10000 L under the category zootechnical additives, groups 4.a and 4.c, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Phyzyme XP 10000 L and Phyzyme XP 10000 TPT as a digestibility enhancer and as a substance that favourably affects the environment for chickens for fattening, laying hens, ducks for fattening, turkeys for fattening, piglets, pigs for fattening and sows. The product is intended to be marketed as liquid (Phyzyme XP 10000 L) and as dry (Phyzyme XP 10000 TPT) form.

The active substance of Phyzyme XP 10000 TPT and Phyzyme XP 10000 L is 6-Phytase (EC 3.1.3.26) produced by S*chizosaccharomyces pombe* (ATCC 5233). The enzymatic activity is expressed in FTU, where 1 FTU is the amount of enzyme which liberates one µmol of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C. The liquid and the solid form of the product have an minimum enzymatic activity of 10000 FTU/g and the product is intended to be mixed into compound feedingstuffs to obtain a phytase activity range of 150 – 1000 FTU/kg depending on the target species or category of animal.

For the determination of the phytase activity in the *feed additive* a spectrophotometric method is proposed. The method is based on the principle that phytase releases inorganic phosphate from a sodium phytate substrate, which in the presence of a molybdate/vanadate reagent forms a yellow complex. The yellow complex is measured with a spectrophotometer and the inorganic phosphate is quantified against a phosphate standard curve. The applicant method was in-house validated, obtaining 7.7% for the percentage relative standard deviation for repeatability (RSD_r). The CRL considers this method suitable for the intended purpose.

For the determination of the phytase activity in *premixtures* the applicant proposes a method which is based on the dilution of the premixture sample into blank feed matrix and applying the corresponding method for the determination of the phytase activity in *feedingstuffs*. The CRL confirms the principle validity of such an approach. However, since a precise protocol of this method and corresponding validation data are missing, the suitability of the method for official control cannot be evaluated.

For the determination of the phytase in *feedingstuffs* a harmonised method is available, which is based on the same principle as the applicant's method for the determination of the phytase activity in the feed additive. The harmonised method is currently under evaluation to become a standard of the European Committee for Standardisation (CEN) and has been

validated in an interlaboratory study which was performed on *feedingstuffs* fortified with different phytase products including a feed additive that contained the specific enzyme of the present application (Gizzi et al., J. of AOAC International, 91, 259-267, (2008)). The obtained values of the precision for the various products could be pooled, obtaining 10% for the RSD_r and 12% for the relative standard deviation for reproducibility (RSD_R). Based on the acceptable method performance profile the CRL recommends this method for official control of 6-phytase in the *feedingstuffs*.

No further testing or validation is required.

KEYWORDS

Phyzyme XP 10000 TPT and Phyzyme XP 10000 L, phytase, enzyme activity, *Schizosaccharomyces pombe (ATCC 5233)*

1. BACKGROUND

Phyzyme XP 10000 TPT and Phyzyme XP 10000 L is a product containing 6-phytase (EC 3.1.3.26), which is produced by *Schizosaccharomyces pombe (ATCC 5233*), as active substance. The applicant proposes to classify the additive belonging to the category 'zootechnical additives, group 4.a and 4.c'.

The intended use (*cf.* EFSA-Q-2008-272) of the current application is to enhance the digestibility of chickens for fattening, laying hens, ducks for fattening, turkeys for fattening, piglets, pigs for fattening and sows and that favourably affects the environment by mixing the product into compound feedingstuffs at various concentrations varying from 150 to 1000 FTU/kg, depending on the target species or category of animal.

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis, submitted in connection with

Phyzyme XP 10000 L and Phyzyme XP 10000 TPT, (EFSA-Q-2008-272), and their suitability to be used for official controls in the frame of authorisation, were evaluated.

3. EVALUATION

Identification/Characterisation of the feed additive

Quantitative and qualitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of impurities in the *additive* (e.g. arsenic and heavy metals - cadmium, mercury and lead) are available at the respective Community Reference Laboratories [1].

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

For the determination of the enzyme activity of the active substance (6-phytase) in the *feed additive* a spectrophotometric (colorimetric) method is proposed which is based on the release of inorganic phosphate during the hydrolysis of sodium phytate at pH 5.5 and 37°C by the enzyme phytase. The sample (10 g dry formulation or 1 mL liquid formulation) is extracted with acetate buffer and subjected to the enzymatic reaction under defined conditions and after addition of phytate as substrate. The reaction is stopped by addition of acid molybdate/vanadate reagent which also produces a coloured complex with the inorganic phosphate. The colour of the yellow complex is measured at 415 nm and the amount of phosphate is quantified through a phosphate standard curve [2]. The method has been in-house validated, providing a relative standard deviation for repeatability (RSD_r) of 7.7 % [3], which is considered acceptable. Based on these acceptable performance characteristics, the applicant method is found suitable for the intended purpose.

For the determination of the phytase activity in *premixtures* the applicant proposes a method which is based of the dilution of the premixture sample into blank feed matrix and applying the corresponding method for the determination of the phytase activity in *feedingstuffs*. The CRL confirms the principle validity of such an approach. However, since a precise protocol of this method and corresponding validation data are missing, the suitability of the method for official control cannot be evaluated.

For the determination of the phytase in *feedingstuffs* a harmonised method is available, which is based on the same principle as the applicant's method for the determination of the phytase activity in the feed additive. The method is an *absolute* method, since it quantifies the phytase activity against a phosphate standard. Therefore, this method does not require the use

of a specific phytase product for calibration [4]. According to the protocol two portions of pellets or mash, of about 50 g each, are weighed into 500 ml Erlenmeyer flasks and extracted with a mixture of 500 ml distilled water and 0.5 ml of 10 % Tween 20. After incubation at the defined conditions, the phosphate concentration is determined as specified in the method description for the analysis of the feed additive. The harmonised method has been validated in an interlaboratory study which was performed on *feedingstuffs* fortified with different phytase products including a feed additive that contained the specific enzyme of the present application [5]. The obtained values of the precision for the various products could be pooled, obtaining 10% for the RSD_r and 12% for the RSD_R and a limit of quantification of 60 FTU/kg. Based on the acceptable method performance profile this method which is currently under evaluation to become a standard of the European Committee for Standardisation (CEN), is recommended for official control of 6-phytase in the *feedingstuffs*.

4. CONCLUSIONS AND RECOMMENDATIONS

For official control of the phytase activity in *feedingstuffs* at or around the proposed minimum and maximum content (150 to 1000 FTU / kg complete feedingstuffs) the CRL recommends the Draft CEN method "Animal feeding stuffs – Determination of phytase activity".

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

Colorimetric method based on reaction of vanadomolybdate on inorganic phosphate produced by action of 6-phytase on a phytate-containing substrate (sodium phytate) at pH 5.5 and 37°C, quantified against a standard curve from inorganic phosphate.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of Phyzyme XP 10000 L & Phyzyme XP 10000 TPT have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] 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 Community reference laboratories, Official Journal of the European Union L 136.

[2] *Ref B93 Phytase in product.pdf

[3] *Technical Annex II.pdf – chapter 2.5.1 (Analytical methods for routine assay of Phytase in product) page 109

[4] CEN-method draft: Animal feeding stuffs – Determination of phytase activity. CEN TC 327 WG 3 (version 19 June 2008).

[5] Gizzi, G. Thyregod P., von Holst C., Bertin G., Vogel K., Faurschou-Isaksen M., Betz R., Murphy R., Brandt Andersen B.:" Determination of Phytase Activity in Feed: Interlaboratory Study" J AOAC International (2008) Vol. 91, No. 2, 259-267.

* Refers to Dossier No: FAD-2008-0003

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was Community Reference Laboratory for Feed Additives, IRMM, Geel, Belgium.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Laboratoire de Rennes, direction générale de la concurrence, de la consommation et de la répression des frauds (DGCCRF), Rennes, France
- Plantedirektoratets Laboratorium, Lyngby, Lyngby, Denmark
- Sächsische Landesanstalt für Landwirtschaft. Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig, Germany
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin, Poland
- Państwowy Instytut Weterynaryjny, Puławy, Poland