
Endo-1,4-beta-xylanase produced by Trichoderma citrinoviride Bisset (IM SD135) (4a1617)  
(FAD-2017-0010; CRL/170000)

Dossier related to: FAD-2017-0010 - CRL/170000

Name of Feed Additive: Endo-1,4-beta-xylanase produced by Trichoderma citrinoviride Bisset (IM SD135) (4a1617)

Active Agent (s): Endo-1,4-β-xylanase (E.C. 3.2.1.8)

Rapporteur Laboratory: European Union Reference Laboratory for Feed Additives (EURL-FA)
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Date: 04/07/2017
EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Endo-1,4-beta-xylanase produced by Trichoderma citrinoviride Bisset (IM SD135) (4a1617)*, under the category/functional group 4 (a) "zootechnical additive"/"digestibility enhancers", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for carp. *Endo-1,4-beta-xylanase (EC 3.2.1.8)* has been already authorised as feed additive under Commission Implementing Regulation (EU) 2015/1043.

The activity of *endo-1,4-beta-xylanase* is expressed as Endo-Pentosanase Units (EPU). According to the Applicant, one EPU is the amount of enzyme which releases 0.0083 μmol of reducing sugars (xylose equivalent) per minute from oat spelt xylan at pH 4.7 and 50 °C.

The *feed additive* is intended to be marketed in solid or liquid formulations (Hostazym® X) with a guaranteed minimum activity of endo-1,4-β-xylanase ranging from 6000 to 30000 EPU/g. The formulated *feed additive* is proposed to be incorporated via *premixtures* or directly into *feedingstuffs* with a minimum recommended *endo-1,4-beta-xylanase* activity of 1050 EPU/kg complete feed.

For the quantification of the *endo-1,4-beta-xylanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted spectrophotometric methods based on the quantification of water soluble dyed fragments produced at pH 4.7 and 50 °C by the action of *endo-1,4-beta-xylanase* on commercially available azurine cross-linked wheat arabinoxylan substrates from Megazyme. The analytical methods presented were already evaluated in the frame of the authorised dossier FAD-2010-0001. Furthermore the Applicant submitted a verification study demonstrating the fitness for purpose of the analytical method for the quantification of *endo-1,4-beta-xylanase* in fish feed. The following performance characteristics were derived from the data presented: a relative standard deviation for *repeatability* (RSD_r) and for *intermediate precision* (RSD_ip) ranging from 1.9 to 16 %; and a recovery rate (RRec) ranging from 93 to 116 %. Based on the satisfactory performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified colorimetric methods submitted by the Applicant for the quantification of *endo-1,4-beta-xylanase* 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

*Endo-1,4-beta-xylanase produced by Trichoderma citrinoviride Bisset (IM SD135) (4a1617)*, Hostazym® X, zootechnical additive, carp
1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new feed additive) for endo-1,4-β-xylanase, under the category/functional group 4 (a) "zootechnical additive"/"digestibility enhancers", according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the feed additive for carps [1,2].

Endo-1,4-β-xylanase (EC 3.2.1.8) is produced by fermentation of Trichoderma citrinoviride Bisset (IM SD135) (formerly Trichoderma longibrachiatum) [3]. The production strain has been deposited at the International Mycological Institute (IMI) in Surrey, UK [4].

The activity of endo-1,4-β-xylanase is expressed as Endo-Pentosanase Units (EPU). According to the Applicant, one EPU is the amount of enzyme which releases 0.0083 μmol of reducing sugars (xylose equivalent) per minute from oat spelt xylan at pH 4.7 and 50 °C [5].

The feed additive is intended to be marketed in solid or liquid formulations (Hostazym® X) with a guaranteed minimum activity of endo-1,4-β-xylanase ranging from 6000 to 30000 EPU/g [2,6]. The formulated feed additive is proposed to be incorporated via premixtures or directly into feedingstuffs with a minimum recommended endo-1,4-β-xylanase activity of 1050 EPU/kg complete feed [2,6,7].

Endo-1,4-β-xylanase (EC 3.2.1.8) has been already authorised under Commission Implementing Regulation (EU) 2015/1043 [8]. The authorisation as feed additive is for turkeys for fattening, minor poultry species for fattening, chickens for fattening, laying hens, minor poultry species for laying, weaned piglets and pigs for fattening. The corresponding analytical methods were evaluated by the EURL in the frame of dossier FAD-2010-0001 [9].

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 endo-1,4-β-xylanase (4a1617) 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 [10].

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

For the quantification of the endo-1,4-β-xylanase activity in the feed additive, premixtures and feedingstuffs, the Applicant submitted spectrophotometric methods based on the quantification of water soluble dyed fragments produced at pH 4.7 and 50 °C by the action of endo-1,4-β-xylanase on commercially available azurine cross-linked wheat arabinoxylan substrates from Megazyme [11]. These validated and verified methods were previously evaluated by the EURL in the frame of the authorisation dossier FAD-2010-0001 [9]. Furthermore, the Applicant submitted a verification study demonstrating the fitness for purpose of the analytical method for the quantification of the endo-1,4-β-xylanase activity in fish feed [12].

For the quantification of the endo-1,4-β-xylanase activity in the feed additive and premixtures, 1.0 g of sample is extracted in 100 ml of acetate buffer. After centrifugation the sample solution is diluted with buffer to an enzyme concentration ranging from 400 to 800 EPU/ml. After a 30 min incubation with the above mentioned substrate at pH 4.7 and 50 °C, the reaction is stopped with tris(hydroxymethyl)aminomethane (TRIS) solution. The rate of dye released is measured with a spectrophotometer at 590 nm and quantified against a reference enzyme standard available from the Applicant upon request. This reference standard has a certified activity determined by the Applicant at the conditions specified in the definition of the EPU unit [5,9].

For the quantification of the endo-1,4-β-xylanase activity in feedingstuffs, 10 g of sample is extracted in 200 ml acetate buffer (containing spiked solution). After centrifugation, the solution is incubated with the above mentioned substrate at 50 °C and pH 4.7 for 150 min. The reaction is stopped by addition of TRIS solution. The rate of dye released is measured with a spectrophotometer at 590 nm and the quantification is carried out via the standard addition technique [9,13,14].

The performance characteristics (Table 1) derived from the validation [5,14] and verification studies [9,12,15] were recalculated by the EURL [16]. In addition, a Limit of Quantification (LOQ) of 360 EPU/kg of feedingstuffs was recalculated by the EURL based on the validation data provided by the Applicant [9,16].
Table 1: Method performance characteristics for the determination of endo-1,4-β-xylanase in feed additive (FA), premixtures (PM), feedingstuffs (FS) and fish feed (FF) [16].

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<th>RSD, (%)</th>
<th>RSDint, (%)</th>
<th>RRec, (%)</th>
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RSD, RSDint Relative Standard Deviation for repeatability and intermediate precision, RRec recovery rate (calculated as activity measured/activity expected)

Based on the satisfactory performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified colorimetric methods submitted by the Applicant for the quantification of the endo-1,4-beta-xylanase 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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the single-laboratory validated and further verified colorimetric method submitted by the Applicant for the quantification of the endo-1,4-beta-xylanase activity in the feed additive, premixtures and feedingstuffs.

**Recommended text for the register entry (analytical method)**

For the quantification of the endo-1,4-beta-xylanase activity in the feed additive, premixtures and feedingstuffs:

- colorimetric method measuring water soluble dye released by action of endo-1,4-β-xylanase from azurine cross-linked wheat arabinoxylan substrates

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of endo-1,4-β-xylanase 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_FWD. APPL. 1831-0007-2017
[2] *Application, Proposal for Register Entry, Annex A
[3] *Technical Dossier, Section II, 2.1.4 Purity
[6] *Technical Dossier, Section II, 2.1.3 Qualitative and quantitative composition
[7] *Technical Dossier, Section II, 2.5.1 Proposed mode of use in animal nutrition
[12] *Technical Dossier, Section II, Annexes, Reference_II.54
[14] *Technical Dossier, Section II, Annexes, Reference_II.30
[16] HostaX_Precision data as recalculated by the EURL_AGES.xls
*Refers to Dossier no: FAD-2017-0010

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

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Fødevarestyrelsens Laboratorie Ringsted (kemisk og mikrobiologisk), Ringsted (DK)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)