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European Union Reference Laboratory for Feed Additives

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

Addendum to the EURL report
FAD-2016-0079 Endo-1,4-beta-xylanase (4a62)
(JRC.F.5/CvH/MGH/ACS/ARES(2017)359427)

Upon request from EFSA [1], the EURL evaluated the supplementary information provided in the frame of the FAD-2016-0079 dossier [2] for the quantification of the activity of *endo-1,4-beta-xylanase* in the *liquid* form of the *feed additive*, while the original EURL report related to this dossier focused on the *solid* form of the same feed additive. The Applicant submitted a single-laboratory validated and further verified viscometric method [3].

The *endo-1,4-beta-xylanase* catalyses the hydrolysis of xylosidic bonds in the wheat arabinoxylan substrate to yield *xylose*, and reduces consequently the viscosity of sample solution. In the frame of the validation and verification studies, the Applicant reported precisions ranging from 2.4 to 4.5 % for the liquid form of the *feed additive* [3]. Similar performance characteristics were reported when the method was applied to the solid form of the *feed additive* [2].

Based on the performance characteristics available the EURL recommends for official control the validated and further verified viscometric method for the quantification of *endo-1,4-beta-xylanase* in the liquid form of the *feed additive*. Moreover, the principle of the methods for the solid and liquid forms of the feed additive is identical. In consequence, also the ***recommended text for the register entry*** included in the original report [2] applies to this amendment.

References


- [1] Supplementary Information – EFSA request cf. liquid formulation, Ares(2018)5803066
- [2] EURL Evaluation Report – JRC.F.5/CvH/MGH/ACS/ARES(2017) 359427
- [3] FAD-2016-0079 Supplementary information_Liquid form_Annexes 10-12

Addendum

- Prepared by María José González de la Huebra
 - Reviewed and approved by Christoph von Holst (EURL-FA) Geel, 13/11/2018
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 Ref. Ares(2017)3359427 - 04/07/2017

JRC F.5/CvH/MGH /ACS/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**

Endo-1,4-beta-xylanase (4a62)
(FAD-2016-0079; CRL/160045)

**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-2016-0079 - CRL/160045**

Name of Feed Additive: ***Endo-1,4-beta-xylanase produced by
Aspergillus niger (CBS 109.713) (4a62)***

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

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

Report prepared by: **María José González de la Huebra**

Report checked by: **Piotr Robouch (EURL-FA)**
Date: **03/07/2017**

Report approved by: **Christoph von Holst**
Date: **03/07/2017**

EXECUTIVE SUMMARY

Natugrain[®] *Wheat TS* is the trade name of a *feed additive* containing as active substance *endo-1,4-beta-xylanase* (EC 3.2.1.8) produced from *Aspergillus niger* (CBS 109.173). This *feed additive* is currently authorized by different Commission Regulations under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003 (*feed additive* identification number 4a62). In the current application a renewal of the *feed additive* authorisation under article 14 of the Regulation (EC) No 1831/2003 is requested for different avian species.

The *endo-1,4-beta-xylanase* activity is expressed in TXU units, where "one TXU is the amount of enzyme, which liberates five micromoles per minute of reducing sugars, expressed as xylose equivalents, from wheat arabinoxylan at pH 3.5 and 40 °C". The *feed additive endo-1,4-beta-xylanase (4a62)* is intended to be marketed as a fine yellow-brown powder with a minimum activity of 5600 TXU/g. *Endo-1,4-beta-xylanase (4a62)* is intended to be incorporated directly or through *premixtures* at a minimum *endo-1,4-beta-xylanase* activity in *feedingstuffs* of 280 or 560 TXU/kg depending on the target species.

For the quantification of the activity of *endo-1,4-beta-xylanase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted a single-laboratory validated and further verified viscometric method. External calibration is performed using an *endo-1,4-beta-xylanase* standard with a known enzyme activity expressed in TXU, determined by colorimetry under the conditions specified in the TXU definition (i.e. pH 3.5 and 40 °C). Based on the performance characteristics available the EURL recommends for official control this method for the quantification of the total *endo-1,4-beta-xylanase* activity in these three matrices.

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 (4a62), *Natugrain*[®] *Wheat TS*, zootechnical, digestibility enhancers, turkeys for fattening and reared for breeding, chickens for fattening, ornamental birds, minor avian species except ducks and laying birds

1. BACKGROUND

Natugrain[®] *Wheat TS* is the trade name of a *feed additive* containing as active substances *endo-1,4-beta-xylanase* (EC 3.2.1.8) produced from *Aspergillus niger* (CBS 109.173) [1], currently authorized for turkeys for fattening [2], chickens for fattening and ducks [3][4], turkeys reared for breeding, minor avian species for fattening and reared for breeding and for ornamental birds [5] under the category/functional 4(a) "zootechnical additives"/"digestibility enhancers" according to the classification system of Annex I of Regulation (EC) No 1831/2003 (*feed additive* identification number 4a62) [1][6]. In the current application a renewal of the *feed additive* authorisation under article 14 of the Regulation (EC) No 1831/2003 is requested for turkeys for fattening and reared for breeding, chickens for fattening, ornamental birds, minor avian species except ducks and laying birds [1][6].

The *endo-1,4-beta-xylanase* activity is expressed in TXU units, where "one TXU is the amount of enzyme, which liberates five micromoles per minute of reducing sugars, expressed as xylose equivalents, from wheat arabinoxylan at pH 3.5 and 40 °C" [1].

Endo-1,4-beta-xylanase (4a62) is intended to be marketed as a fine yellow-brown powder with a minimum activity of 5600 TXU/g [7].

The *feed additive* is intended to be incorporated directly or through *premixtures* at a activity of *endo-1,4-beta-xylanase* minimum in *feedingstuffs* of 280 or 560 TXU/kg, depending on the target specie [1][9].

Note: The analytical methods for the quantification of *endo-1,4-beta-xylanase (4a62)* in the relevant matrices were already evaluated and recommended by the EURL in the frame of previous dossiers [8].

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-beta-xylanase (4a62)* 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 [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 activity of *endo-1,4-beta-xylanase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the same single-laboratory validated [11] and further verified [12] viscometric method, referred in the previous Commission Regulations [2][3][4][5] in which the *endo-1,4-beta-xylanase* catalyses the hydrolysis of xylosidic bonds in the wheat arabinoxylan substrate to yield *xylose*, and reduces consequently the viscosity of sample solution. The decrease in viscosity of the sample solution is determined using a falling ball viscometer at pH 3.5 and 55 °C. If present in the samples, other enzymes lowering the viscosity of the substrate solution under the given test conditions would lead to an overestimated *endo-1,4-beta-xylanase* activity [11].

The *feed additive*, *premixtures* and *feedingstuffs* are weighed and extracted at room temperature with citric acid buffer. The obtained extracts are diluted appropriately to fall within the measuring activity range. The substrate (wheat arabinoxylan) is then mixed with the corresponding dilutions of the *feed additive*, *premixtures* and *feedingstuffs* extracts and incubated before performing the measurements. The calibration solutions are submitted to the same analytical process. The *xylanase* activity of the unknown samples is then quantified against a calibration curve prepared in citric buffer (external calibration) from a reference *endo-1,4-beta-xylanase* standard with known activity expressed in TXU units and available from the Applicant upon request [13] [14].

The activity of the *endo-1,4-beta-xylanase* enzyme standard expressed in TXU is determined by the Applicant in a separate experiment. The enzyme is subjected to the conditions specified in the definition of the TXU unit, where arabinoxylan is used as substrate, while pH and temperatures are set to 3.5 and 40 °C, respectively. The enzyme reference activity is determined against the formed xylose quantified by external calibration [15].

Table 1: Performance characteristics for the determination of *endo-1,4-beta-xylanase* activity in the *feed additive* (FA) *premixtures* (PM) and *feedingstuffs* (FS) by viscometry, obtained in the frame of the validation (Val) and verification (Ver) studies.

Matrices	RSD _r (%)		RSD _{ip} (%)		R _{Rec} (%)
	Val [11]	Ver [12]	Val [11]	Ver [12]	Ver [12]
FA	2.9 - 3.9	1.9 - 4.9	7.5	3.8	97
PM	2.3 - 6.7	3.3 - 7.0	9.9	5.8	98
FS	4.6 - 7.6	5.9 - 7.0	8.3	6.2	97

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*; R_{Rec}: *Recovery rate*

Based on the performance characteristics available the EURL recommends for official control the single-laboratory validated and further verified viscometric method for the quantification of the total *endo-1,4-beta-xylanase* activity in the *feed additive*, *premixtures* and *feedingstuffs*.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the validated and further verified viscometric method for the quantification of *endo-1,4-beta-xylanase* in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

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

- viscometric method based on decrease in viscosimetry produced by the action of *endo-1,4-beta-xylanase* on the xylan containing substrate (wheat arabinoxylan)-

One *endo-1,4-beta-xylanase* unit (TXU) is the amount of enzyme, which liberates five micromoles per minute of reducing sugars, expressed as xylose equivalents, from wheat arabinoxylan at pH 3.5 and 40 °C.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *endo-1,4-beta-xylanase (4a62)* 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] Commission Regulation (EC) No 1380/2007 of 26 November 2007 concerning the authorisation of endo-1,4-beta-xylanase (Natugrain Wheat TS) as feed additive
- [3] Commission Regulation (EC) No 1096/2009 of 16 November 2009 concerning the authorisation of an enzyme preparation of endo-1,4-beta-xylanase produced by *Aspergillus niger* (CBS 109.713) as a feed additive for chickens for fattening and the authorisation of a new use of this preparation as a feed additive for ducks (holder of authorisation BASF SE) and amending Regulation (EC) No 1458/2005
- [4] Commission Regulation (EC) No 1019/2012 of 6 November 2012 amending Commission Regulation (EC) No 1096/2009 as regards the minimum content of endo-1,4-beta-xylanase produced by *Aspergillus niger* (CBS 109.713) as a feed additive for chickens for fattening and for ducks (holder of authorisation BASF SE)
- [5] Commission Implementing Regulation (EU) No 843/2012 of 18 September 2012 concerning the authorisation of endo-1,4-beta-xylanase produced by *Aspergillus niger* (CBS 109.713) as a feed additive for turkeys reared for laying or breeding and ornamental birds (holder of authorisation BASF SE)
- [6] *Application, Reference SANTE_E5_FWD. APPL. 1831-0057-2016
- [7] *Technical dossier, Section II Identity, characterisation and conditions of use of the additive; methods of analysis – 2.1.3 Qualitative and quantitative composition
- [8] EURL Evaluation Reports:
<https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2006-0019.pdf>
<https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2008-0023.pdf>
- [9] *Technical dossier, Section II Identity, characterisation and conditions of use of the additive; methods of analysis – 2.5 Conditions of use
- [10] 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
- [11] *Technical dossier, Section II, Annexes II.77, II.79, II.85 & II.88
- [12] *Technical dossier, Section II, Annexes II.80, II.86 & II.89
- [13] Supplementary Information – Definition of enzymatic activity unit (TXU) for Natugrain Wheat TS cf. FAD-2016-0079, Ares (2017)2733816
- [14] *Technical dossier, Section II, Annexes II.76, II.78, II.84 & II.87
- [15] Supplementary Information – Absolute method

*Refers to Dossier no: FAD-2016-0079

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:

- Fødevarestyrelsens Laboratorie Ringsted (DK)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)